Regulation of IgE antibody responses in mice
by Judith Estelle Klein Manning

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY In Microbiology
Montana State University

Abstract:
The validity of the heterologous (rat) passive cutaneous anaphylaxis (PCA) test to assess mouse IgE responses was confirmed by comparing the 72 hr homologous PCA test with the 24 hr heterologous PCA test, and by the diminution in titer when undiluted serum was heated at 56°C for 90 min. Earlier work, showing that dilution of the serum prior to heating abrogated the effect of heating on the skin sensitizing activity of IgE, was confirmed. Treatment with an anti-helminthic agent improved the homologous, 72 hr PCA reactions in certain mice probably due to a decline In endogenous anti-worm IgE levels as a result of the elimination of pinworms.- Neonatally initiated anti-μ treatment suppressed subsequent IgE responses to Infection with N. brasiliensis showing that IgE forming cells are derived from the same precursors I cell(s) as are IgM, IgG and IgA forming cells.

The induction and regulation of IgE responses in Balb/c mice were studied, using the 24 hr heterologous PCA test. Immunization with one intraperitonea 1 (IP) injection of either 1 ù, 10 μg, 100 μg or 1000 μg doses of ovalbumin (OVA), each with 1 mg alum, Induced high PCA and passive hemagglutination (PHA) antibody titers that persisted for at least 56 days. At 100 μg and 1000 μg doses, a lag in the peak IgE response was seen. When B_. pertussis vaccine was used instead of alum, a PHA antibody was Induced but IgE antibody could not be detected. Immunization by one IP injection of DNPn c-OVA + I mg alum Induced a transient anti-DNP IgE response anda persistent anti-OVA IgE response. In contrast, Immunization with DNPgif-OVA induced a persistent anti-DNP IgE response but no ant I-OVA IgE could be detected. Immunization with multiple, IP injections of normal rabbit serum and OVA, without adjuvant, Induced an IgE antibody response.

When mice, primed with one IP Injection of 10μg OVA + I mg alum, were given 3, intravenous injections of 100 μg OVA on days 3, 5, and 7 their PCA antibody titers and the number of IgE forming cells In the spleen declined to undetectable levels by day 16, whereas their PHA antibody titers did not change. Secondary IgE responses were also suppressed by this treatment. The route of administration of this treatment was shown to be an important factor In producing this effect. This suppression could be transferred to normal animals by spleen cells from suppressed animals, suggesting that this treatment Induced suppressor cells.

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ABSTRACT

The validity of the heterologous (rat) passive cutaneous anaphylaxis (PCA) test to assess mouse IgE responses was confirmed by comparing the 72 hr homologous PCA test with the 24 hr heterologous PCA test, and by the diminution in titer when undiluted serum was heated at 56°C for 90 min. Earlier work, showing that dilution of the serum prior to heating abrogated the effect of heating on the skin sensitizing activity of IgE, was confirmed. Treatment with an anti-helminthic agent improved the homologous, 72 hr PCA reactions in certain mice probably due to a decline in endogenous anti-worm IgE levels as a result of the elimination of pinworms.

Neonatally initiated anti-μ treatment suppressed subsequent IgE responses to infection with N. brasiliensis showing that IgE forming cells are derived from the same precursoral cell(s) as are IgM, IgG and IgA forming cells.

The induction and regulation of IgE responses in Balb/c mice were studied, using the 24 hr heterologous PCA test. Immunization with one intraperitoneal (IP) injection of either 1 μg, 10 μg, 100 μg or 1000 μg doses of ovalbumin (OVA), each with 1 mg alum, induced high PCA and passive hemagglutination (PHA) antibody titers that persisted for at least 56 days. At 100 μg and 1000 μg doses, a lag in the peak IgE response was seen. When B. pertussis vaccine was used instead of alum, a PHA antibody was induced but IgE antibody could not be detected. Immunization by one IP injection of $DNP_{0.5}$-OVA + 1 mg alum induced a transient anti-DNP IgE response and a persistent anti-OVA IgE response. In contrast, immunization with DNP_{24}-OVA induced a persistent anti-DNP IgE response but no anti-OVA IgE could be detected. Immunization with multiple, IP injections of normal rabbit serum and OVA, without adjuvant, induced an IgE antibody response.

When mice, primed with one IP injection of 10 μg OVA + 1 mg alum, were given 3, intravenous injections of 100 μg OVA on days 3, 5, and 7 their PCA antibody titers and the number of IgE forming cells in the spleen declined to undetectable levels by day 16, whereas their PHA antibody titers did not change. Secondary IgE responses were also suppressed by this treatment. The route of administration of this treatment was shown to be an important factor in producing this effect. This suppression could be transferred to normal animals by spleen cells from suppressed animals, suggesting that this treatment induced suppressor cells.

INTRODUCTION

In humans, the "skin sensitizing factor" present in the serum of hay fever patients has been clearly identified as belonging to a distinct immunoglobulin class, designated as IgE for its ability to bind purified ragweed antigen (antigen E) in radioimmunoelectrophoresis (1). Other names for this antibody include: reagin, homocytotropic antibody, and P-K antibody.

IgE differs from other known immunoglobulins in physicochemical, antigenic and biological properties. It contains 12% carbohydrate, has a sedimentation coefficient of 8.0S and a molecular weight of approximately 190,000. It is present in serum at much lower concentrations than other immunoglobulins, with an average concentration of 0.3 μg/ml. Elevated serum levels are present in individuals who are atopic or infected with worms. Epsilon heavy chains have unique antigenic determinants and do not possess any of the major antigenic determinants present in the other four known immunoglobulin classes (1).

The most distinctive property of IgE is its ability to bind to mast cells for up to several weeks at least, with the release of histamine and other pharmacologically active substances from these cells upon interaction of the cell bound IgE with antigen (1, 2). This property is the basis for most of the assays for this antibody in animals and, until recently, in humans. The effects of histamine

and other pharmacologically active substances are the cause of the symptoms experienced by allergic individuals. This cell binding activity can be destroyed by heating at 56°C for 2-4 hours (1, 2).

The dog is the only other species in which spontaneous allergic symptoms similar to those found in hay fever patients have been observed. A skin sensitizing factor similar to human IgE has been detected in serum from these animals (3). However, heat labile, homocytotropic antibodies which remain bound to skin mast cells for long periods of time (i.e., 72 hours) can be deliberately induced in many species and are considered the animal counterpart to human IgE (1, 2, 4). These antibodies have been detected in many other mammals including monkeys, dogs, mice, rats, rabbits, cattle, sheep, pigs and guinea pigs (4), and in a marsupial, the quokka (5). The physicochemical properties of these homocytotropic antibodies are similar to human IgE (1, 2, 4) and in some cases, cross-react immunologically with anti-human IgE (6, 7).

The deliberate induction of IgE (contrasted with the "atopic" condition) requires a suitable combination of antigen, dose of antigen, adjuvant, immunization schedule and species (4). It is generally agreed that for most antigens some adjuvant is required for IgE induction. Adjuvants commonly used to induce IgE responses are aluminum hydroxide gel (alum) and vaccines or soluble extracts of

Bordetella pertussis (4). Bacterial lipopolysaccharides (LPS) (4) and concanavalin A (con A) (8) have also been used, as well as Freund's complete adjuvant, but the latter is not considered a good adjuvant for IgE (4). Experimental observations on the various adjuvant-species interactions suggest that the mechanisms regulating IgE production may differ both for species and for adjuvant (4).

One apparent exception to this requirement for adjuvant is the induction of anti-helminthic IgE. Some investigators believe that live worm infection may provide adjuvant action because worm extracts, by themselves, cannot induce the IgE response (4). Another possible exception is the induction of IgE antibodies to heterologous serum by immunization with one intraperitoneal (IP) injection of heterologous serum in the form of anti-lymphocyte serum (9).

Experimental evidence has firmly established that collaboration between bone-marrow derived (B) and thymus-derived (T) lymphocytes is required for the induction of certain antibody responses (10). The importance of a third cell type, the macrophage, has also been recognized (11). Several lines of evidence demonstrate that similar cellular events are also necessary for IgE production as outlined below.

1) Using hapten-carrier systems, it has been shown that T cells are primed with carrier and then interact with B cells to form hapten-specific IgE in both adoptive transfer experiments (12-15) and in

vitro experiments (16).

2) Rats thymectomized within 24 hours of birth cannot produce IgE antibodies (17).

3) Lethally irradiated rats reconstituted with thymus and bone marrow cells produce a low level of IgE whereas those receiving bone marrow alone do not (18).

4) Congenitally athymic (nu/nu) mice cannot produce detectable levels of IgE to ovalbumin unless reconstituted with thymocytes (19) or thymus glands (20).

Some investigators have suggested a heterogenous population of helper T cells; one specific for IgE and one specific for IgG (16). Others suggest one population of helper T cells for both immunoglobulin classes with a differential susceptibility to T cell regulatory influences of the respective classes of antibody forming B cells (21). More recently a critical role for adherent cells in the induction of helper T cells for IgE production has been shown (22).

Two different patterns of IgE antibody production have been recognized. One is transient, non-boostable and usually the result of immunization with a high dose of antigen. This pattern has been shown in several species (4). The other is sustained for relatively long periods of time and boostable. This pattern is characteristic of IgE production to pollen antigens in humans with hay fever (23),

to helminth antigens as a result of parasite infection (24), and in mice immunized with a low dose of antigen adsorbed to alum (25).

Interest in the management of clinical allergy has prompted studies of regulatory mechanisms in IgE antibody formation. In individuals with hay fever, it has been shown that serum IgE antibody titers persist and are enhanced after contact with antigen during hay fever season (23). Hyposensitization treatment of allergy consists of multiple injections of minute doses of allergen over long periods of time. As a result of this treatment IgG antibody levels are increased and secondary IgE titers are eventually suppressed. It was thought that this treatment induced a blocking antibody, presumably IgG, that competed with IgE for binding of allergen. However, analysis of titers of blocking antibody in certain treated patients failed to support this hypothesis (23). This evidence coupled with the fact that IgG is not present in very great quantities in respiratory secretions, the site where they would be most effective in competing for antigen, suggested that an increase in blocking antibody may not be responsible for the effect of hyposensitization on clinical symptoms. An alternative hypothesis was suggested, namely, that a change in the memory cell population, particularly in T cells, occurred with the subsequent suppression of the secondary IgE response (23).

The transient pattern of IgE antibody formation in certain laboratory animals provided one means of studying the suppression of this class of antibody. Using the rat as an experimental animal and a unique immunization schedule to induce IgE, the terminating event in the transient pattern of response was shown to involve regulation at the T cell level. In this model, IgE responses to a dinitrophenyl conjugate of Ascaris suum extract (DNP-Asc) were induced by a high dose of DNP-Asc (1 mg) injected with a B. pertussis vaccine via the footpads, on day 0, followed by an intramuscular injection of 0.5 mg of DNP-Asc on day 5 (26). IgE titers peaked by day 14 and then declined by day 21. The lines of evidence for the regulation of IgE by T cells in this system include enhancement and maintenance of high IgE levels beyond day 21 by anti thymocyte serum (ATS) (27); adult thymectomy (17), splenectomy (17) and x-irradiation (28). In addition, it was shown that the IgE responses prolonged by these means could be suppressed by transfer of splenocytes or thymocytes from animals hyperimmunized with carrier antigen (29). Other classes of antibody were not similarly affected by these treatments.

Suppression of IgE production by passive administration of antigen specific IgG antibody has also been demonstrated in the rat (26) and in the rabbit (30). It is interesting to note that in the rabbit, passive administration of antigen-specific IgM antibody en-

hanced IgE formation (31).

A somewhat different experimental model for IgE responses exists in the mouse. When mice are immunized with a low dose of antigen in combination with alum they give a high and persistent IgE response (25). This model most closely resembles the pattern of IgE production in hay fever patients. Several examples of regulation of IgE antibody formation in this model have been demonstrated.

The ability of mice to form IgE antibody, as well as IgG antibody, to low doses of antigen (0.1 μg-1 μg) in alum appears to be under genetic control, dependent on H-2 type (32) and comparable to the type of immune response regulation observed with the synthetic, branched polypeptides (TG)-AL and (HG)-AL (33). Strain differences to higher doses of antigen (100 μg) were not as apparent (32). When hapten-protein conjugates were used, this strain difference was shown to be related to carrier protein, suggesting that the genetic control occurred at the T cell level (32). Another type of genetic control, unrelated to H-2 type and specific for IgE has been observed (32, 34). Thus, the SJL strain of mouse produces high titers of IgG antibody against many different antigens but low levels of IgE, even with high doses of antigen. The IgE titers of these mice can be enhanced by using Con A as adjuvant instead of alum (35). Additionally, it has been shown that the low reagin production is due to the

presence of non-specific suppressor T cells (36).

Another means of regulating IgE antibody in the mouse system is by amount of antigen. Whereas low doses of alum-adsorbed antigen induce a persistent response, high doses of alum-adsorbed antigen induce a transient response (25). Another type of regulation has been observed in mice immunized with a low dose of alum-adsorbed antigen. Maia et al. (37) reported the selective depression of IgE antibody formation to ovalbumin in DBA/1J mice. If, after being immunized with an optimal dose of ovalbumin (0.1 μg-1.0 μg) and alum, (IP), mice were given intravenous (IV) injections of 100 μg of soluble ovalbumin on days 3, 5, 7, their reaginic response was near normal on days 8, 9, 10 but had declined by day 16. Control animals continued to make reaginic antibody. The IgG response, as measured by the 2 hour homologous PCA test, was not affected. This protocol is similar to hyposensitization therapy in hay fever patients. Tada (4) has suggested that this phenomenon may represent an example of tolerance in IgE forming B cells. Bach and Brashler (38), reporting a similar phenomenon produced by multiple IV injections of acetylated ovalalbumin, speculate that their system may represent the formation of suppressor T cells. More recently it has been demonstrated that this type of suppression can be transferred to normal animals via splenic T lymphocytes (39). In addition, it was subsequently shown that these suppressor cells could be generated <u>in vitro</u> only

if adherent cells were absent from the culture (22).

STATEMENT OF THESIS

The purpose of this study was to examine the regulation of IgE antibody production in mice and to relate this to cellular events. The study was focused primarily on the Balb/c strain in order to avoid the complications arising from strain differences in regulation of IgE synthesis.

The experimental approach consisted of:

1) An examination of the relationship of IgE-producing cells to other classes of antibody-producing cells by testing the effect of neonatally initiated anti-μ on subsequent IgE antibody responses.

2) A comparison of the various ways in which IgE responses can be induced and regulated.

3) An attempt to elucidate the mechanism(s) responsible for the selective suppression of IgE responses by three intravenous injections of high doses of soluble antigen.

MATERIALS AND METHODS

Animals

Balb/c mice were raised in our own laboratory facilities. C3H/HeJ mice were obtained from Jackson Laboratories, Bar Harbor, Maine. ICR mice were obtained from the Research Animals Resources Center, University of Wisconsin, Madison. CF_1 mice were purchased from Carworth Farms, Portage, Michigan.

Outbred albino rats were purchased from ARS Sprague-Dawley or Holtzman, Co., Madison, Wisconsin.

Antigens

The following antigens were obtained from Calbiochem (San Diego, California): Ovalbumin, (OVA) 5X crystallized, lot #387039 and the following dinitrophenylated (DNP-) proteins: DNP-ovalbumin (DNP_{24}-OVA), DNP-bovine serum albumin (DNP_{38}-BSA), DNP-Keyhole limpet hemocyanin (DNP_{570}-KLH). Subscripts refer to the average number of groups per molecule of protein. In the case of KLH, this was based on a molecular weight of 2×10^6.

Other antigens and their commercial sources are: normal rabbit serum (NRS), Colorado Serum Co.; ovine albumin, Sigma; ovine gamma globulin, Pentex.

Ragweed extract was a gift from Dr. E. Rau, Endocrine Laboratories, Madison, Wisconsin.

Nippostrongylus brasiliensis antigen was prepared according to the method of Ogilvie (40).

Lightly dinitrophenylated ovalbumin ($DNP_{0.5}$-OVA) was prepared by the method of Eisen (41) as modified by Ishizaka and Okudaira (42). Briefly, 50 mg each of dinitrobenzene sulfonic acid, 2X recrystallized, and of sodium carbonate were dissolved in 6 ml distilled water. To this, 100 mg of OVA were added and the mixture stirred for 3-1/2 hr at room temperature. The resulting conjugate was dialysed against saline, at 4°C for several days (changed at least 5X). The number of DNP groups per molecule of OVA was determined spectrophotometrically (43) by measuring absorbance at 360 nm for DNP and 280 nm for OVA. The protein concentration was estimated by lowering the observed OD_{280} reading by 38.5% to correct for the absorption at 280 nm by DNP (43).

Adjuvants

Preparation of $Al(OH)_3$ gel (Alum): Fifteen g of alum (purchased from a local drugstore) was dissolved in 180 ml of distilled water. To this solution, 75 ml of 1N NaOH was added dropwise with continuous mixing. The resulting precipitate was washed 6-8X with distilled water. Dry weight determinations were done on the final solution.

Bordetella pertussis vaccine was purchased from Eli Lilly,

Co., Indianapolis, Indiana (control #8AL31A).

Immunizations

The standard immunization used to induce a good IgE response was 10 μg OVA mixed with 1 mg alum given IP on day 0. The OVA was freshly prepared each time, by dissolving 4 mg OVA in 100 ml saline. An equal volume of this solution was mixed with an equal volume of alum (4 mg/ml), and 0.5 ml of this mixture was injected. For those experiments in which a different antigen was used or the amount of antigen varied, a similar protocol was followed with the appropriate adjustments.

In some experiments, animals received, in addition to the standard immunization, three IV injections of antigen without adjuvant on days 3, 5, 7. In these experiments, the term sensitized is used for those animals receiving the standard immunization; the term sensitized-desensitized is used for those receiving the additional treatment.

Other immunization protocols are described in the text.

Serum collection

At the appropriate times after immunization, blood was collected from the tail into plastic microcentrifuge tubes to which 1 drop of saline had been added. The clotted blood was rimmed and left for several hours or overnight at 4°C. The serum was collect-

ed after centrifugation and stored in 1/4 or 1/2 dram vials at -20°C or -70°C. Serum stored in this manner displayed equivalent PCA titers 9 months after collection.

Assays

IgE responses were assayed by the passive cutaneous anaphylaxis (PCA) test performed, for most experiments, in rats (44, 45). For each serum, or serum pool, a 0.10 ml aliquot of each of a series of twofold dilutions was injected intradermally (ID) into the shaved backs of test rats. Approximately 40 injections per rat were routinely made. The rats were challenged 24 hr later by intravenous injection of 5 mg antigen dissolved in 0.5 ml 1% Evan's blue. When assaying for PCA responses against NRS, the challenge antigen consisted of 2 ml of NRS in which 5 mg Evan's blue dye was dissolved. Similarly, for PCA responses against Nippostrongylus antigens, 5 mg of Evan's blue dye was dissolved in 1-2 ml of a saline solution containing 2,000 worm equivalents. Thirty minutes after IV challenge, the rats were sacrificed and the reactions read on the underside of the skin. A positive reaction was indicated by a discrete circle of blue.

For both the ID and IV injections, the rats were sedated by an intramuscular injection of 0.04-0.06 ml Innovar-vet (Pitman-Moore Co.).

When the PCA test was performed in mice, 0.05 ml of a serum dilution was injected, ID, into the shaved backs of mice. The mice were challenged IV, 72 hr later, with 0.2 ml 1% Evan's blue dye, in saline, containing 1-2 mg of antigen. Only one ID injection per mouse was made.

IgE antibody-producing cells were measured by the heterologous adoptive cutaneous anaphylaxis (HACA) test (46). This procedure is similar to the PCA assay except that 0.1 ml aliquots of the appropriate cell suspensions were injected into the shaved backs of rats.

Antibodies other than those of the IgE class were assayed by passive hemagglutination (PHA) using erythrocytes coated with OVA. Two different methods for coating erythrocytes were used. In one method (47), OVA was coupled to guinea pig erythrocytes with carbodiimide. To 3 ml of PBS in which 30 mg of OVA was dissolved was added 0.05 ml of washed packed guinea pig erythrocytes. To this was added 50 mg 1-ethyl-3 (3-dimethylaminopropyl)-carbodiimide hydrochloride (Sigma) dissolved in 0.5 ml PBS. This mixture was left at room temperature for 1 hr with occasional mixing. The cells were then washed 3X and resuspended in modified Alsevier's solution (48) to approximately 0.5%.

In the other method (49), OVA was coupled to sheep erythrocytes with chromium chloride ($CrCl_3$). Stock solutions of $CrCl_3$ (10 mg/ml in distilled water) and OVA (1 mg/ml in saline) were pre-

pared. For the coupling procedure, the stock $CrCl_3$ solution was diluted 1/20 in saline. To 0.2 ml of $CrCl_3$ solution was added 0.2 ml of OVA solution and 0.2 ml washed packed sheep erythrocytes. This mixture was left at room temperature for 5 minutes, and then the cells were washed 4X in saline. After the last wash the packed cells were diluted 1/60 in modified Alsevier's solution and stored. For use in assays a further 1/10 dilution of the cells was made (final dilution = 1/600).

PHA titers were determined by microtiter using plastic V-bottom microtiter plates, 25 μl diluting loops and 25 μl pipette droppers. Modified Alsevier's solution was used as a diluent. All titers are expressed as the reciprocal of the last dilution to give a positive result.

Anti-μ treatment

The anti-μ antiserum was prepared by Dr. Dean D. Manning, University of Wisconsin, Madison, as described previously (50). Within 14 hr of birth, each of three litters was divided into three groups. One group in each litter was injected IP with 0.05 ml of anti-μ serum, the other two receiving an identical injection of NRS or phosphate-buffered saline (PBS); further injections of 0.05, 0.07, 0.07, 0.08, and 0.08 ml were made on days 2, 4, 6, 8, and 10, respectively. Thereafter all mice received a 0.10 ml injection of

PBS or diluted NRS or anti-μ serum (both diluted 1:2.5 in PBS) every Monday, Wednesday, and Friday until termination of the experiment.

Worm infection

At approximately 40 days of age all mice received a subcutaneous injection of 300 third-stage (infectious) larvae of a mouse-adapted strain of N. brasiliensis (51). 2 wk later all mice were reinfected with 300 such larvae and 13 days thereafter were exsanguinated retro-orbitally.

RESULTS

Induction of homocytotropic antibodies in mice

Previous studies in this laboratory have established that 10 μg OVA + 10 mg alum given IP to Balb/c mice is very effective for induction of 72 hr PCA antibody using the homologous PCA assay (20). This was confirmed and it was shown that 1 mg of alum was just as effective as 10 mg. In addition, C3H/HeJ mice gave a PCA titer, at 10 days after immunization, that was similar to Balb/c mice.

The heterologous (rat) PCA assay for mouse IgE

Although IgE is considered a homocytotropic antibody, it was shown that human IgE could bind to monkey mast cells (52). Similarly, various lines of evidence have shown that mouse IgE, but not IgG_1, will bind to rat mast cells (44, 45, 53, 54). Since this binding is selective for mouse IgE, when rats, instead of mice, are used as the recipients for the passive transfer of mouse antiserum in the PCA assay a latent period of 2-72 hours can be employed (45).

A comparison between the homologous and heterologous (rat) PCA assays is shown in Table I. Similar PCA titers were observed with the two assays, although the titer in the homologous assay is one dilution lower.

To further test the ability of the rat PCA assay to measure mouse IgE, I examined the heat sensitivity of the PCA antibody. It had been reported previously (55) that diluting serum prior to heat

treatment at 56°C for 30 minutes would protect the IgE skin sensitizing activity from heat destruction. Since these workers were using the homologous PCA assay I retested this observation using the heterologous (rat) PCA assay with a longer heating time.

The heat treatment was carried out as follows: Two 0.1 ml aliquots were removed from a serum pool from 10 mice. One aliquot was placed in a 10 x 75 mm test tube, corked tightly and the tube was then placed in a 56°C water bath for 90 minutes. The other aliquot was diluted 1:8 in PBS and divided into 2 portions. One of these was heated as above while the other was placed in the cold. After the incubation period, further dilutions of the samples were made and tested for PCA activity in rats. The results are shown in Table II. The PCA titer was diminished from 256 to <4 for pool 1 and from 2048 to 4 for pool 2 by heating serum before dilution while serum diluted prior to heating showed only a slight reduction in titer. These results confirm that the antibody measured by the rat PCA assay is heat labile and that dilution of the antibody prior to heat treatment protects it from destruction.

Effect of an anti-helminth agent on the homologous PCA reaction in ICR mice

A comparison between various outbred mice as recipients for the homologous PCA test produced an interesting finding. It was noted

that ICR mice obtained from the Research Animals Resources Center, University of Wisconsin, Madison, consistently gave very poor or negative PCA reactions with antiserum that produced strong reactions in CF_1 mice obtained commercially. Jarrett et al. (56) have reported that Nippostrongylus brasiliensis infection in rats had an inhibitory effect on subsequent homologous PCA assays. I hypothesized that a similar effect due to pinworm infection might be responsible for the effect on PCA reactions seen in ICR mice.

A preliminary test of this hypothesis was made in collaboration with Dr. Ralph Anslow, University of Wisconsin. Since the ICR mice were found to have a high pinworm burden, it was decided to administer an anti-helminth agent to these mice and test the effect of such treatment on their reactivity in the PCA assay.

A group of retired female breeder mice was separated from the main colony and divided into 2 groups. A solution of Dyrex R (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa) at a concentration of 2.5 gm per liter of water was administered as the sole source of drinking water for 14 days to one group while the other group was given plain water. Approximately two weeks after the treatment period several mice from each group were sacrificed and their intestines examined for pinworms. The treated group was apparently free of worms, whereas the untreated group had worms. Two months

later, the mice were used as recipients for the homologous PCA test. The results shown in Table II indicate that there is a definite difference in the quality and intensity of the PCA lesion between the two groups. The PCA lesions of the treated group were larger, darker blue and more discrete than those of the untreated group. When the intestines of these mice were examined, pinworms could not be detected in either group. Although in this case, the PCA reactivity could not be related to pinworm burden at the time of the assay there was a strong correlation between anti-helminth treatment and PCA reactions. This strongly suggests that the poor reactivity of the ICR mice was due to a worm infection.

Suppression of IgE antibody formation by treatment with anti-μ antiserum

It was of interest to know whether the ontogenic development of IgE forming cells is related to IgM bearing precursoral cells, as are the cells forming other classes of immunoglobulins. One way of studying this relationship is by suppression of immunoglobulin formation by neonatally initiated anti-μ treatment. Such treatment has been shown to suppress the formation of IgM, IgG_1, IgG_2, and IgA in mice (57). Kishimoto and Ishizaka (58) showed that anti-μ antibodies cannot suppress secondary IgE responses of rabbit cells *in vitro*. Therefore, it was decided to test the effect of *in*

TABLE I

A comparison between the homologous and heterologous passive cutaneous anaphylaxis (PCA) tests for mouse IgE antibody against ovalbumin

Mouse antisera[a]	PCA titers	
	Mouse	Rat
Pool 1	128	256
Pool 2	128	256

[a]Titers were done on a serum pool from 3-5 mice. Mice were immunized with 10 μg ovalbumin + 1 mg alum IP on day 0 and bled 10 days later.

TABLE II

Effect of heat[a] on passive cutaneous anaphylaxis (PCA)[b] titers of mouse antiserum

Serum pool[c] from 10 mice	1:8 dilution unheated	Undiluted serum heated	1:8 dilution heated
Pool 1	256	<4	128
Pool 2	2048	4	1024

[a]56°C for 90 minutes.

[b]PCA test performed in rats.

[c]Mice were immunized with 10 μg OVA + 1 mg alum, IP.