

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
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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 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.

TABLE III

Effect of an anti-helminth agent on the homologous passive cutaneous anaphylaxis (PCA) reaction

	PCA activity[a]		
	Intensity	Diameter	Description
Control[b]	2+	15 x 15	discrete
	±	unmeasurable	barely visible
	1+	12 x 10	discrete
	±	10 x 10	diffuse, splotchy
	±	12 x 15	diffuse, splotchy
Treated[b]	2+	12 x 15	diffuse
	4+	20 x 15	discrete
	3+	20 x 25	discrete
	3+	20 x 20	discrete
	3+	20 x 25	discrete

[a]Antiserum used for passive transfer was a pool of serum obtained from mice immunized with 10 μg ovalbumin + 1 mg alum.

[b]Treated animals were given Dyrex R in drinking water for 14 days. Control group was left untreated. Approximately 2 months later the mice were tested for ability to show PCA reactions.

vivo, anti-μ treatment on the ability of mice to give a subsequent IgE antibody response to N. brasiliensis infection (59). Early attempts to utilize the ovalbumin-alum model were frustrated by the frequent suppression of anti-ovalbumin responses by multiple injections of NRS (see below). While these studies were in progress, Dwyer et al. (60) reported that they failed to suppress IgE responses to immunization with ovalbumin and alum by neonatally initiated anti-μ treatment.

The results of this study are shown in Table IV. It is clear that animals receiving anti-μ treatment failed to produce detectable levels of PCA antibody to worms, whereas those receiving either PBS or NRS made high levels of specific PCA antibody. That all mice were indeed actively infected with worms was determined by worm egg counts in the feces (51). In addition, the achievement of severe panspecific humoral immunosuppression as a result of anti-μ treatment was confirmed by assay of serum immunoglobulin levels of IgM, IgG_1, IgG_2, and IgA (59).

Effect of multiple injections of NRS on subsequent IgE antibody formation to immunization with ovalbumin and alum

During the studies of the effect of anti-μ treatment on IgE antibody formation the preliminary observation was made, that in control animals receiving NRS treatment the IgE response, when test-

ed 10 days after one IP injection of OVA and alum, was suppressed. This phenomenon was investigated further. The results from 2 different litters given multiple injections of NRS from birth are shown in Table V. In all animals receiving PBS treatment, the PCA and PHA responses were high, whereas in the NRS treated animals most of the responses for both PCA and PHA antibody were either undetectable or significantly lowered.

However, the suppressive effect was not always present to the same degree. One animal in the NRS treated group showed a PCA titer of 128 and PHA titer of 64 which can probably be considered normal and one animal showed a PCA titer of 64 and PHA titer of 16 which is only slightly lower than that obtained normally but which probably represents a significant suppression. The other responses in this group clearly are suppressed.

Thus, it was shown that NRS treatment did have a suppressive effect on the IgE antibody response, but this effect was variable in its expression and was not selective for IgE; suppression of PHA responses paralleled that of IgE. Similar results have been observed by another investigator (G. M. Lang, personal communication).

Kinetics of the PCA and PHA responses of mice immunized with 10 μg OVA + 1 mg alum

Vaz et al. (25) showed that one IP injection of a low dose of

TABLE IV

IgE anti-worm responses in mice treated from birth with anti-μ antiserum

Anti-worm PCA titers[a]		
PBS treated[b]	NRS treated[b]	Anti-μ treated[b]
512	128	<4
512	128	<4
32	128	0
256	256	0
256	512	0
256	32	0
	512	0
	256	

[a]PCA = passive cutaneous anaphylaxis assay performed in rats.

[b]Mice received either PBS, NRS or anti-μ, IP, according to the following schedule: Days 0, 2-0.05 ml; days 4, 6-0.07 ml; days 8, 10-0.08 ml; thereafter, every Monday, Wednesday and Friday-0.10 ml. At approximately 40 days all mice received 300 third-stage (infectious) larvae of N. brasiliensis. 2 wk later all mice were reinfected with the same dose of larvae and bled 13 days later.

TABLE V

Effect of neonatally initiated normal rabbit serum (NRS) treatment on anti-ovalbumin responses in mice

Treatment[a]	PCA[b]	PHA[b]
PBS	512	512
	512	256
	512	128
	256	512
	256	512
NRS	<4	2
	<4	2
	<4	4
	16	2
	64	16
	128	64

[a]Mice received either NRS or PBS, IP, according to the following schedule: Days 0, 2-0.05 ml; day 5-0.07 ml; day 8-0.08 or 0.10 ml; days 13, 18, 23, 28, 35, 37, 42-0.10 ml. Day 0 = day of birth.

[b]Mice were prebled on day 36; immunized on day 37 with 10 μg OVA + 10 mg alum, IP; bled on day 47. Results represent individual serum titers, of 2 different litters, on day 47.

antigen with alum was a reliable method of inducing high and persistent IgE titers in mice. This was confirmed for Balb/c mice immunized with 10 μg OVA + 1 mg alum. From the results shown in Table VI, it can be seen that PCA antibody is present at peak levels at day 10 and is maintained at that level for at least 5 months. A PHA antibody is also induced and maintained.

Dose response studies

Vaz et al. (25) have also reported that, in certain strains of mice, immunization with a 100 μg dose of OVA + 10-20 mg of alum exerted a suppressive effect on IgE responses. In contrast to the high and persistent IgE response displayed by mice immunized with low doses (0.1-1 μg) of alum-adsorbed OVA, mice immunized with a high dose (100 μg) of alum-adsorbed OVA showed a somewhat lower homologous PCA titer that declined, to very low or undetectable levels, by day 21.

Since these investigators did not use Balb/c mice in their study, I tested the effect of one injection of different doses of OVA on the antibody responses of this strain to see if a similar type of regulation could be demonstrated. In these studies, the amount of alum was kept constant (1 mg) and the PCA titers were assayed by the heterologous (rat) PCA test.

The results of the PCA and PHA titers are shown in Tables VII

and VIII, respectively. No evidence of regulation of IgE, at day 21, is seen at high doses of antigen. Over the dosage range of 1-1000 μg, all groups show a high titer at days 21 and 28. There is a slight lag in the peak IgE response, with higher doses of antigen, but this effect is gone by day 21. A similar lag has been observed with high doses of certain other antigens and may represent a transient neutralization of IgE antibody by excess antigen. A lag in peak PHA responses was not seen.

A second experiment (results not shown) confirmed the persistence of the IgE response, even at high doses of antigen; at day 56 after mice were immunized with 10 or 1000 μg OVA + 1 mg alum, the PCA titers obtained were similar, at both dosage levels, and to the respective responses on day 14. It is interesting to note that by day 21 there was very little difference between the titers obtained, in both the PCA and PHA assays, when 10-1000 μg of antigen was used for immunization.

IgE responses to DNP-conjugates of ovalbumin

Studies in the rat, on IgE antibody responses to dinitrophenylated Ascaris extracts (DNP-Asc) showed that the level of anti-DNP IgE was inversely related to the number of DNP groups linked to the carrier (61). Thus, immunization with 4×10^{-4} moles DNP/mg carrier produced an average anti-DNP PCA titer of 13, whereas 1×10^{-4}

TABLE VI

Long term maintenance of immune responses to alum adsorbed ovalbumin[a] in Balb/c mice

Type of antibody	Day 10	Day 21	Day 56	Day 150
PCA[b]	256[c]	512	512	128
PHA[b]	128	512	1024	1024

[a]Mice were immunized with one IP injection of 10 μg ovalbumin + 1 mg alum.

[b]PCA = passive cutaneous anaphylaxis.
PHA = passive hemagglutination.

[c]Each result was obtained from a serum pool from 5 mice.

TABLE VII

IgE responses to various doses of ovalbumin adsorbed to alum

Dose of ovalbumin[a]	Day 10	Day 14	Day 21	Day 28
1 μg	256[b]	256	128	256
10 μg	512	512	512	1024
100 μg	8	64	512	1024
1000 μg	<8	256	512	1024

[a]Mice were given one IP injection of 0.25 ml containing the indicated amount of ovalbumin in saline mixed with an equal volume of a solution containing 1 mg alum.

[b]Each result was obtained from a serum pool from 4 mice.

TABLE VIII

Passive hemagglutination antibody responses to various doses of ovalbumin adsorbed to alum

Dose of ovalbumin[a]	Day 10	Day 14	Day 21	Day 28
1 μg	64[b]	256	512	256
10 μg	512	1024	1024	1024
100 μg	512	512	1024	2048
1000 μg	256	512	512	2048

[a]Mice were given one IP injection of 0.25 ml containing the indicated amount of ovalbumin in saline mixed with an equal volume of a solution containing 1 mg of alum.

[b]Each result was obtained from a serum pool from 4 mice.

moles DNP/mg carrier gave a titer of 103. The anti-carrier responses followed the same pattern.

Certain studies on anti-hapten and anti-carrier IgE responses in the mouse have revealed a dichotomy in these responses. Lee and Sehon (62) reported that when mice were immunized with 1 μg of heavily dinitrophenylated OVA (DNP_{21}-OVA) with alum the PCA antibody response produced could be elicited in the PCA recipient by challenge with DNP_{21}-OVA but not with OVA.

Ishizaka and Okudaira (42) showed a dosage effect, related to the carrier, on anti-hapten IgE responses using a lightly dinitrophenylated conjugate of OVA ($DNP_{3.6}$-OVA) for immunization. In $DBA/_1J$, mice which produce high and persistent IgE responses to minute doses (0.05-0.2 μg) of OVA, they showed that immunization with low doses of $DNP_{3.6}$-OVA produced high and persistent anti-DNP responses, whereas higher doses (10 μg) produced much lower titers, which were transient.

Anti-OVA PCA responses did not parallel the anti-DNP responses; anti-OVA antibody formation was persistent although the level of antibody was lower at higher doses (42). In addition, priming with carrier, at all doses, resulted in a secondary anti-carrier response to immunization with $DNP_{3.6}$-OVA whereas the anti-DNP response was enhanced or accelerated when animals were primed with a low dose of

carrier but suppressed when they were primed with a high dose (42).

The anti-hapten and anti-carrier IgE responses of Balb/c mice to immunization with either $DNP_{0.5}$-OVA or DNP_{24}-OVA were compared using the heterologous (rat) PCA assay. When mice were immunized with $DNP_{0.5}$-OVA and 1 mg alum (Table IX) their anti-DNP responses were transient and inversely related to dose. The anti-OVA responses were high and persistent, similar to that obtained with unconjugated OVA, except that 1 μg $DNP_{0.5}$-OVA produced a 4-fold higher titer than 100 μg.

When mice were immunized with DNP_{24}-OVA and 1 mg alum (Table X) the observation of Lee and Sehon (62) was confirmed and extended. Animals immunized with 1-100 μg of antigen produced both anti-DNP_{24}-OVA and anti-DNP responses that were high and persistent. However, animals immunized with 1000 μg of antigen show no detectable antibody. Anti-OVA antibody could not be detected in any of the experimental groups.

When a 1/8 dilution of an anti-OVA serum that had displayed a titer of 256 upon challenge with ovalbumin, was passively transferred to a rat, the subsequent challenge with 5 mg DNP_{24}-OVA was able to elicit a PCA response for that antiserum. This indicates that at least some of the B cell antigenic determinants for OVA were present on the DNP_{24}-OVA molecule.

Immune response to various doses of ovalbumin using B. pertussis as adjuvant

Balb/c mice were given one IP injection of 0.25 ml of a solution containing 10, 100, or 1000 μg OVA + 0.25 ml of B. pertussis vaccine equivalent to 10^{10} organisms. The results are shown in Table XI. Mice so immunized produced a PHA antibody, but apparently no PCA antibody.

PCA antibody responses to other antigens using alum as adjuvant

Other alum-precipitated substances which were able to induce PCA antibodies, at days 14 and 28, by immunization with one IP injection were: 1) ragweed antigen at doses of 1 μg, 10 μg, and 100 μg, and 2) NRS and normal sheep serum when 0.25 ml of either undiluted or diluted 1:5 in saline was used. When undiluted serum was used, a lag in the peak response was seen similar to that observed with high doses of OVA. Immunization with 10 μg, 100 μg, or 1000 μg doses of ovine albumin or OGG precipitated with alum did not produce detectable levels of PCA antibody.

Induction of IgE responses by multiple injections of antigen without adjuvant

It is generally accepted that in order to induce IgE responses in laboratory animals an "adjuvant" is required. An exception to this is the induction of specific IgE antibody as a result of worm

TABLE IX

IgE anti-hapten and anti-carrier responses of Balb/c mice immunized with lightly dinitrophenylated ovalbumin ($DNP_{0.5}$-OVA)

Dose of antigen[a]	PCA titers[b] Anti-DNP Day 14	Anti-DNP Day 36	Anti-ovalbumin Day 14	Anti-ovalbumin Day 36
1 μg	512[c]	<8	2048	512
10 μg	64	<8	512	256
100 μg	<8	<8	64	128

[a]Mice were given one IP injection of 0.25 ml of the indicated dose of $DNP_{0.5}$-OVA in saline mixed with an equal volume of a solution containing 1 mg alum.

[b]PCA (passive cutaneous anaphylaxis) tests were performed in rats which were challenged IV with 5 mg of either DNP-BSA or ovalbumin.

[c]Each result was obtained from a serum pool from 3 mice.

TABLE X

IgE anti hapten-carrier conjugate, anti-hapten and anti-carrier responses of Balb/c mice immunized with heavily dinitrophenylated ovalbumin (DNP_{24}-OVA)

Dose of antigen[a]	PCA titers[b]					
	Anti DNP_{24}-OVA		Anti-DNP		Anti ovalbumin	
	Day 14	Day 56	Day 14	Day 56	Day 14	Day 56
1 μg	128[c]	128	256	256	<8	<8
10 μg	256	128	256	256	<8	<8
100 μg	256	256	≥8	128	<8	<8
1000 μg	<2	<4	<4	<4	<8	<8

[a]Mice were given one IP injection of 0.25 ml of the indicated dose of $DNP_{0.5}$-OVA in saline mixed with an equal volume of a solution containing 1 mg alum.

[b]PCA (passive cutaneous anaphylaxis) tests were performed in rats which were challenged IV with 5 mg of either DNP_{24}-OVA, DNP-BSA or ovalbumin.

[c]Each result was obtained from a serum pool from 4 mice.

TABLE XI

Anti-ovalbumin responses in mice immunized with various doses of ovalbumin and *Bordetella pertussis*

Dose of ovalbumin[a]	Day 10		Day 14		Day 21		Day 28	
	PCA	PHA	PCA	PHA	PCA	PHA	PCA	PHA
10 μg	<4[b]	16	<4	ND[c]	<4	16	<4	ND
100 μg	<4	32	<4	ND	<4	16	<4	ND
1000 μg	<4	128	<4	ND	<4	128	<4	ND

[a]Mice were given one IP injection of 0.25 ml ovalbumin in saline mixed with an equal volume *B. pertussis* vaccine equivalent to approximately 10^{10} organisms.

[b]Results were obtained from a serum pool from 3 mice.

[c]Not done.

infection (40). One result of live worm infection may be the repeated release of low doses of worm antigens during the various stages of the infection. Similar antigenization may occur in atopic people. I therefore considered the possibility that repeated immunization with antigen alone might overcome the requirement for adjuvant in the induction of IgE responses.

Two different immunization protocols were used. The first was started on the day of birth and given approximately every 2-3 days as outlined in Table XII. From the results, it is apparent that a heat labile, PCA antibody response could be elicited by neonatally initiated, multiple, IP, injections of NRS. Relatively high levels of precipitation antibody were also formed. Heat treatment diminished the PCA titer, but not the precipitin titer. However, there seemed to be a portion of the PCA antibody that is resistant to heating.

A similar response was obtained when mice received neonatally initiated multiple injections of low doses of ovalbumin (0.02-10 μg) at approximately weekly intervals. A pool of serum from 7 mice gave an anti-ovalbumin titer of 64 at day 48, 7 days after the last injection.

In the second protocol the immunizations were initiated in adult animals. A total of six injections of 0.25 ml was adminis-

tered IP at various intervals. The mice were assayed at day 18, 4 days after the last injection. The results, shown in Table XIII were similar to those obtained with neonatally initiated injections. A pool of serum from group I was tested for heat sensitivity; the untreated pool gave a titer of 128, whereas the heated pool gave a titer of <16. It was originally thought that by giving an additional immunization of OVA + alum, to group II, to stimulate anti-ovalbumin responses, the anti-NRS response might have been potentiated. However, within the time course of this experiment this did not occur and in fact, there appears to be a slight suppression.

These results clearly indicate that, in mice, multiple injections of antigen, without an adjuvant, can elicit PCA responses that most probably are of the IgE class.

The following experiments were designed to examine, in more detail, the phenomenon reported by Maia et al. (37) in which multiple high doses of antigen, without alum, given IV, resulted in the selective suppression of IgE responses.

Confirmation of previous findings

Balb/c mice were sensitized on day 0 with one IP injection of 10 μg OVA + 1 mg alum followed by a desensitizing treatment, on days 3, 5, 7 which consisted of the intravenous injection of either saline (control animals), 100 μg OVA without alum or 1000 μg OVA

TABLE XII

Immune responses of mice immunized by neonatally initiated, multiple, injections of normal rabbit serum (NRS) without adjuvant[a]

Mouse no.	Anti-NRS responses: PCA[b] Untreated	PCA[b] Heated[c]	Precipitin Unheated	Precipitin Heated[c]
1	64	8	32	32
2	128	ND[d]	64	ND
3	64	16	64	64
4	64	16	32	32
5	32	4	64	64
6	16	8	64	64

[a]Mice received NRS, IP, according to the following schedule: Days 0, 2-0.05 ml; day 5-0.07 ml; day 8-0.08 or 0.10 ml; days 13, 18, 23, 28, 35, 37, 42-0.10 ml (day 0 = day or birth). They were bled on day 36.

[b]PCA = passive cutaneous anaphylaxis performed in rats.

[c]Undiluted serum samples were heated at 56°C for 90 minutes.

[d]Not done.

TABLE XIII

IgE responses of mice immunized by adult initiated multiple injections of normal rabbit serum (NRS) without adjuvant

	Anti-NRS PCA responses[a]
Group I[b]	128
	128
	16
	64
Group II[b, c]	32
	8
	32
	64

[a]PCA = passive cutaneous anaphylaxis performed in rats. Results are from sera collected on day 18.

[b]Mice in both groups received 0.25 ml NRS, IP, on days 0, 2, 4, 7, 11 and 14 (day 0 = day of first injection).

[c]Mice in group II received, in addition, one IP injection of 10 μg OVA + 1 mg alum on day 9.

without alum each day. These 2 dosages were tested because I was using 10-100X greater sensitizing dose for Balb/c mice than had been used in the original study. As can be seen from the results in Table XIV, treatment with both 100 μg and 1000 μg OVA resulted in the suppression of the PCA response by day 21, while the group receiving saline had high titers. With the 100 μg dose, the IgE response is at near normal levels on day 10, whereas with the 1000 μg dose the response is much lower, probably indicating that the suppression was expressed a little sooner with the higher dose. The PHA response did not appear to be similarly affected by the treatment.

Subsequent experiments with a similar design were carried out. 100-200 μg OVA for the desensitizing treatment was routinely used.

From the results of these experiments it can be concluded that desensitization is a repeatable, valid experimental finding. One hundred or 200 μg is equally effective in inducing desensitization. By making comparisons between different experiments, it appeared that termination of the IgE response occurred by day 16 and remained at least through day 24. Also, the expression of desensitization appeared to be progressive. At day 9 the PCA titers were normal, at day 10 some mice began to show a lower PCA titer and by day 11 most animals showed significant suppression.

Maintenance of desensitization

When serum from the animals discussed above was tested at 77 days after sensitization, the PCA responses of the groups receiving either 100 μg or 1000 μg OVA were still <8 while the control group was still making PCA antibody. This suggested that the phenomenon was long lived. In another experiment, in which individual responses were tested, it was seen that the desensitized state was not uniformly maintained in animals treated with 100 μg OVA. Typical results are shown in Table XV. It can be seen that in some animals, which received multiple, IV injections of 100 μg OVA, PCA antibody was detectable on day 52 even though their response had previously dropped to undetectable levels by day 16. This has been observed in other experiments.

Effect of desensitization on IgE antibody-secreting cells

In order to determine whether the suppression of IgE antibody responses induced by treatment with multiple high doses of antigen was due to suppression of antibody secretion and/or formation or to neutralization of antibody by excess antigen, the HACA test (34) was employed. For this test, animals were sacrificed at various times after sensitization and single cell suspensions of their spleens, thymuses and bone marrows prepared in PBS. The cell preparations were washed 2X with PBS and resuspended to an appropriate

concentration. Viable cell counts by Trypan blue exclusion were made. Two-fold dilutions of this preparation were made and 0.1 ml aliquots were injected into the skin of rats which were challenged 24 hr later with Evan's blue dye and antigen.

The results using cells from sensitized animals are shown in Table XVI. IgE secreting cells were present in the spleens and bone marrows, but not the thymuses of sensitized mice at days 17 and 32. This confirmed previous work (34). The results, using cells from desensitized animals, are shown in Table XVI. It can be seen that at the cell concentrations tested, no IgE secreting cells were detected in either spleen or bone marrow. Considering that the number of cells which gave a negative reaction was 100-fold greater for spleen cells and 5-10 fold greater for bone marrow cells than the least number of cells, from the sensitized group, that gave a positive reaction, these results strongly suggested that the suppression of IgE responses, due to intravenous injection of antigen, was the result of a lack of IgE antibody-secreting cells and not to neutralization of antibody by excess antigen.

Effect of giving multiple, high doses of antigen without alum by the IP route

From the results shown in Table XVII, it can be seen that when 3 injections of high doses of antigen are given IP instead of IV,

the IgE antibody responses are only slightly diminished when compared with the responses of animals receiving the high doses IV. This is true when either 100 μg or 1000 μg was used for the IP injections. These results indicate that the route of administration of the desensitizing treatment was very important. Thus, the extent of suppression by IP injection was not nearly as great as that induced by a low dose of antigen given intravenously (see below).

Effect of giving an optimally immunogenic dose of antigen as a desensitization treatment

Table XVIII shows the responses of animals given 10 μg OVA IV, compared to 100 μg OVA, IV. It can be seen that 10 μg was able to induce a significant suppression of PCA titers. This supports the hypothesis that an important requirement for desensitization is the route of injection.

A comparison of multiple and single treatments for desensitization

Table XIX shows the effect of giving one IV injection of 300 μg of OVA on either day 3, day 5, or day 7. It can be seen that one single injection induced a significant suppression in PCA titers but not as great as that obtained with multiple injections. There didn't seem to be much difference among single injections given on different days.

TABLE XIV

Effect of intravenous, multiple injections of soluble antigen on the anti-ovalbumin responses of mice sensitized previously with an i.p. injection of antigen + alum

Treatment	Day 10		Day 21	
Day 0 - Days 3, 5, 7	PCA	PHA	PCA	PHA
Sensitized-saline[a]	512[d]	64	256	512
Sensitized-desensitized[b] 100 μg	128	512	<4	256
Sensitized-desensitized[c] 1000 μg	32	512	<4	512

[a]Mice were sensitized on Day 0 by an intraperitoneal injection of 0.25 ml ovalbumin in saline mixed with an equal volume of a solution containing 1 mg alum. On days 3, 5, 7 they were given an intravenous injection of saline.

[b]Mice were treated as above except they were given 100 μg soluble ovalbumin instead of saline on days 3, 5, 7.

[c]Mice were treated as above, except they were given 1000 μg soluble ovalbumin on days 3, 5, 7.

[d]Results were obtained from a serum pool from 3 mice.

TABLE XV

Effect of intravenous, multiple injections of soluble ovalbumin on IgE responses

Treatment	Day 10	Day 16	Day 52
Sensitized[a]	256	64	128
	1024	1024	256
	1024	512	128
	1024	512	256
	512	512	256
	512	512	128
	1024	512	512
	256	256	256
	512	512	256
	256	256	256
Sensitized-desensitized[b]	128	<8	64
	128	<8	64
	64	<8	32
	128	<8	32
	256	<8	32
	64	<8	32
	128	<8	16
	32	<8	<8
	32	<8	<8
	16	<8	<8
	8	<8	<8

[a]Mice were sensitized on day 0 by an i.p. injection of 0.25 ml ovalbumin in saline mixed with an equal volume of a solution containing 1 mg alum.

[b]Mice were treated as above, except they were given three i.v. injections of 100 μg egg albumin without alum on days 3, 5, 7.

TABLE XVI

Heterologous adoptive cutaneous anaphylaxis reactions in sensitized and sensitized-desensitized mice

	No. of cells required for a positive reaction	
	Day 17	Day 32
Sensitized[a]		
Spleen	7.5×10^5	4.6×10^5
Bone marrow	6.0×10^6	2.5×10^6
Thymus	$>5 \times 10^7$	$>3 \times 10^7$
Sensitized-desensitized[b]		
Spleen	$>5.2 \times 10^7$	$>6.2 \times 10^7$
Bone marrow	$>3.2 \times 10^7$	$>3.5 \times 10^7$
Thymus	$>4.5 \times 10^7$	$>2 \times 10^7$

[a]Mice were immunized with one IP injection of 10 μg OVA + 1 mg alum on day 0.

[b]Mice were immunized with one IP injection of 10 μg OVA + 1 mg alum on day 0 and subsequently given three 100 μg doses of OVA without alum on days 3, 5, 7.

TABLE XVII

Effect of multiple, intraperitoneal injections of soluble antigen on anti-ovalbumin responses at day 16

Treatment[a]	PCA	PHA
Sensitization + saline	256	1024
	128	2048
	256	2048
Sensitization + 100 μg IV	<4	2048
	<4	1024
	4	4096
Sensitization + 100 μg IP	128	1024
	64	4096
	64	4096
Sensitization + 1000 μg IP	256	4096
	64	4096
	32	2048

[a]On day 0, all mice were given one IP injection of 10 μg OVA + 1 mg alum. On days 3, 5, 7 they were treated as indicated.

TABLE XVIII

Effect of giving multiple intravenous injection of low doses of soluble antigen on desensitization

Treatment[a]		Anti-ovalbumin responses[b] PCA[c]	PHA[d]
Sensitization + saline	Exp. 1	512	ND
		256	ND
		256	ND
	Exp. 2	256	1024
		128	2048
		256	2048
Sensitization + 100 μg IV	Exp. 1	<4	ND
		<4	ND
		<4	ND
	Exp. 2	<4	2048
		<4	1024
		<4	4096
Sensitization + 10 μg IV	Exp. 1	64	ND
		32	ND
		16	ND
	Exp. 2	8	4096
		16	4096
		32	4096

[a]All mice were sensitized on Day 0 with one IP injection of 10 μg OVA + 1 mg alum. On days 3, 5, 7 they were given intravenous injections of either saline, 100 μg OVA or 10 μg OVA.

[b]Results are from individual mice bled on day 16 (Exp. 1) or 19 (Exp. 2).

[c]PCA = passive cutaneous anaphylaxis.

[d]PHA = passive hemagglutination.

TABLE XIX

A comparison of the effect of multiple injections and a single injection of ovalbumin on desensitization

Treatment[a]	Day(s) of treatment	PCA responses on day 16
Saline	3, 5, 7	512, 128, 256
100 μg OVA	3, 5, 7	<4, <4, <4
300 μg OVA	3	64, 16, 16
300 μg OVA	5	32, 64, ND
300 μg OVA	7	128, 32, 8

[a]All treatments were given IV. On day 0, all mice were given one IP injection of 10 μg OVA + 1 mg alum.

Effect of giving other proteins or hapten-protein conjugates intravenously

In order to determine whether the suppression observed in sensitized mice that were given three subsequent IV injections of 100 μg OVA might possibly be due to a non-specific effect, perhaps similar to that observed with multiple injections of NRS, other substances were injected IV in a similar protocol. The results, shown in Table XX, showed that neither 100 μg sheep albumin nor 100 μg DNP-KLH had any suppressive effect on the PCA responses of mice previously sensitized with 10 μg OVA + 1 mg alum. The results were equivocal when 100 μg DNP_{24}-OVA was given. One mouse out of three showed a significant suppression of PCA activity, whereas the mean PCA value for the other two was only one tube lower than that for the animals given no treatment. However, even DNP_{24}-OVA was not able to suppress the PCA responses to the extent that 100 μg OVA could.

Effect of desensitization treatment on secondary IgE responses

The effect of desensitization on a subsequent IgE secondary response was tested by giving a second IP injection of 10 μg OVA + 1 mg alum to sensitized and sensitized-desensitized mice. The second immunization was administered at two different intervals after the primary injection. The results are shown in Table XXI. When a

TABLE XX

Effect of administering other proteins or hapten-protein conjugates intravenously on the anti-ovalbumin PCA responses of mice previously sensitized with alum-adsorbed ovalbumin

Treatment[a] on days 3, 5, 7	PCA responses[b] on day 16-21
Experiment 1	
Saline	256
100 μg OVA	<8
100 μg sheep albumin	256
Experiment 2	
Saline	128, 512, 256
100 μg OVA	<4, <4, <4
100 μg DNP_{24}-OVA	128, 128, 32
100 μg DNP-KLH	512, 512, 512

[a]On day 0, all mice received 10 μg OVA + 1 mg alum, IP. On days 3, 5, 7 they received, intravenously, the indicated substances.

[b]Results for experiment 1 are from a serum pool from 3 mice collected on day 16. Results for experiment 2 are from individual serum samples collected on day 21.

secondary immunization was given to sensitized animals on day 29 (Group I) there was an 8-fold increase in PCA titer at first, which decreases one dilution later on. When the second challenge was administered on day 50 (Group III) there was a 4-fold increase in titer. I do not consider that these increases are different from each other, however, they do represent significant increases when compared to the group that did not receive a second immunization (Group II). On the other hand, the pattern of secondary responses was somewhat different in sensitized-desensitized mice. In Group IV, which received a second immunization on day 29, the PCA increased by only one dilution, whereas in Group VI, which received a second immunization on day 50, the PCA titer increased 32-fold. However, the titer was still significantly lower than the respective response in the sensitized group. In Group V, which did not receive any secondary immunization, the PCA titer increased slightly between days 29 and 50.

Thus, it appears that, in desensitized animals, the extent of increase in secondary PCA titers depends on when the second immunization was given in relation to the initial sensitization and desensitization treatment. The secondary response was higher, when the time interval was increased between desensitization treatment and secondary challenge. This was, apparently, not true for animals

which had been sensitized only.

The PHA responses did not seem to be similarly influenced (data not shown). All groups receiving a secondary challenge showed a significant increase in titer and the extent of that increase was similar in all cases.

Suppressive effect of spleen cells from sensitized-desensitized animals

Experiments were undertaken to see if the transfer of spleen cells from sensitized-desensitized animals to normal animals would suppress the subsequent IgE response of the recipient. Two separate experiments were set up. In experiment 1, 2 groups of donors were sensitized on day 0 with 10 μg OVA + 1 mg alum. On days 3, 5, 7 one group received an intravenous injection of 100 μg OVA alone. At day 18, the animals were bled and their sera assayed for PCA activity. At day 19, 4 animals from the sensitized-desensitized group which had undetectable levels of PCA antibody (<4) and 4 animals from the sensitized group (PCA $\geq$ 64) were sacrificed and single cell suspensions of their spleens were prepared in PBS containing 1% fetal calf serum. After 1 wash in this medium, the cells from each group were pooled and a viable count was made. Approximately 2.5×10^8 cells in a volume of 0.35-0.50 ml, from each group, were injected IV into normal recipients which were immunized the follow-

TABLE XXI

Secondary responses of mice desensitized with multiple, high doses of ovalbumin given intravenously

Group	Treatment	Day of second immunization[c]	PCA anti-ovalbumin responses			
			Day 29	Day 36	Day 50	Day 57
I	Sensitization[a]	29	256[d]	2048	1024	1024
II	"	NONE	256	512	512	512
III	"	50	512	512	512	2048
IV	Sensitization-desensitization[b]	29	64	128	128	128
V	"	NONE	<4	8	16	16
VI	"	50	<4	16	16	512

[a]All mice received 10 μg OVA + 1 mg alum, IP, on day 0.

[b]All mice received 10 μg OVA + 1 mg alum, IP, on day 0 and 100 μg OVA, without alum, IV, on days 3, 5, 7.

[c]Mice received either saline or 100 μg OVA + 1 mg alum, IP, on the day indicated.

[d]Results are from a serum pool from 3 mice.

ing day with 10 μg OVA + 1 mg alum. The anti-OVA IgE antibody responses of the recipients are shown in Table XXII. It can be seen that the PCA responses of animals receiving desensitized cells are significantly lower than those of animals receiving sensitized cells. When the means of the $\log_2$ values for these two groups were analyzed by Student's t test, the differences between the two groups were statistically significant ($p < 0.05$).

In the second experiment, 3 groups of donors were used. All groups were primed on day 0 with 10 μg OVA + 1 mg alum. On days 3, 5, 7 one group received IV injections of 100 μg OVA and another group received IV injections of 1000 μg OVA. The third group received no treatment. At day 19, the spleens and thymuses of all 3 groups were prepared in a manner similar to that described above. For each group of donors, a spleen cell suspension containing 2.5×10^8 or 2.5×10^7 cells in 0.5 ml was prepared and injected, IV, into normal recipients. Additionally, another group of recipients received an IV injection of 2×10^8 thymocytes from the same group of donors. The anti-OVA PCA responses of recipients receiving spleen cells are shown in Table XXII (Experiment 2). The PCA responses of animals receiving 2.5×10^8 spleen cells from animals treated with 1000 μg OVA were 8-fold lower than those of animals receiving the same number of sensitized cells. When 10-fold less

cells from the same donors were transferred, the PCA responses were 4-fold lower than those of sensitized animals, however there were only 2 animals left in this experimental group.

The PCA responses of the 2 mice receiving 2.5×10^8 spleen cells from donors desensitized with 100 μg doses of OVA were similar to those of experiment 1 and significantly lower than the responses of mice receiving sensitized cells. When 10-fold less cells from this group were transferred, the suppressive effect was not seen.

When 2×10^8 thymocytes from each of the three groups were transferred the PCA responses of serum pools from 3 recipients for each group, were identical (PCA titer = 256 in all 3 cases).

These results showed that the suppression of IgE by 3, IV injections of antigen was transferrable to normal mice by spleen cells, but not by thymocytes.

TABLE XXII

Suppressive effects of spleen cells from desensitized animals on IgE anti-ovalbumin responses when transferred to normal, syngeneic mice

Treatment of donors of spleen cells[a]	No. cells transferred	PCA responses of recipients[b] Day 14-15
Experiment 1		
Sensitized	2.5×10^8	256, 512, 256
Sensitized-desensitized-100 μg	2.5×10^8	32, 128, 64
Experiment 2		
Sensitized	2.5×10^8	512, 512, 512
	2.5×10^7	512, 512, 512
Sensitized-desensitized-100 μg	2.5×10^8	128, 64, ND
	2.5×10^7	256, 256, 512
Sensitized-desensitized-1000 μg	2.5×10^8	64, 64, 64
	2.5×10^7	128, 128, 128

[a]All mice were given 10 μg OVA + 1 mg alum, IP, on day 0. In the sensitized-desensitized groups, mice were given 3, IV injections of the indicated doses of OVA on days 3, 5, 7.

[b]One day after cell transfer, all recipients received 10 μg OVA + 1 mg alum, IP.

DISCUSSION

The studies on the heterologous (rat) PCA test confirmed previous findings and helped establish the validity of this assay for mouse IgE. In addition, the inhibitory effect of dilution on the heat sensitivity of the mast cell binding ability of PCA antibody was confirmed and extended. In the study by Clausen et al. (55) the PCA antibody response was induced by immunization with 125 μg OVA plus a saline extract of B. pertussis and assayed by the homologous PCA. My studies extended this observation to PCA antibody induced by immunization with alum-adsorbed antigen and assayed by the heterologous PCA assay. Also, since Clausen et al. (55) employed a 30 min heating time, it might be argued that by diluting out the antibody, a longer heating time would be required to achieve the same effect. My studies showed that even when the heating time was extended to 90 min the effect was still seen.

A recent paper (63) has reported that there are 2 antibodies in mouse antiserum, active in the heterologous (rat) PCA assay when a 2 hr latent period was used, one heat-stable and one heat-labile. (The heat-stable antibody did not react with either anti-IgG_1 or anti-IgG_2.) This conclusion was based on 1) a lack of correlation of 48 hr homocytotropic antibody activity with 2 hr heterocytotropic (rat) activity, 2) the presence of a heat resistant antibody that is more active in the heterologous assay and 3) the presence of heterocyto-

tropic (rat) antibodies in mouse antiserum known not to contain PCA antibody active in the 48 hr homologous assay. It should be noted that in these studies, the heat treatment was applied to a 1:5 dilution of serum which could account for the presence of the "heat-resistant" antibody in some of the antisera tested. The differences between the 48 hr homocytotropic and 2 hr heterocytotropic antibody were not more than 2-4 fold. The apparent greater amount of heat resistant PCA antibody in the 2 hr heterologous assay when compared with the 48 hr homologous assay can be accounted for by the difference in time. It has been shown that although the half-life of IgE antibody in the skin was approximately 7-14 days (1, 64), the greatest rate of decrease occurred with the first 3-4 days (1). The presence of a PCA antibody in serum from SJL mice, that was active in the 2 hr heterologous but not the 48 hr homologous test and which appears to be totally heat resistant, is noteworthy. However, these investigators failed to compare 48 hr homologous assay results with 48 hr heterologous assay results. In all of my studies, a 24 hr latent period was employed. I believe that other studies (44, 45), as well as my own, have established the validity of the heterologous (rat) PCA assay to measure mouse IgE antibody.

The amelioration of the PCA lesion caused by IgE antibody, in ICR mice, due to treatment with an anti-helminthic agent has impor-

tant considerations for the use of the PCA assay. It is very likely that the poor PCA reactivity observed before treatment was due to a competitive inhibition or blocking of the binding of the passively transferred IgE by endogenous IgE produced as a result of worm infection. Ishizaka et al. (65) have shown, very convincingly, that when peritoneal mast cells were incubated with ^{125}I-labeled rat E myeloma protein, the number of IgE molecules combined with mast cells isolated from N. brasiliensis infected rat was about 10% of that bound to mast cells from normal rats.

The detection of pinworms in the experimental animals before treatment suggests strongly that this parasite was responsible for the effect seen. Although it has not been reported, it seems reasonable to assume that, based on other parasite systems (66), IgE formation occurs as a result of pinworm infection. It is felt that the observations reported here may represent an extreme case for the following reasons: 1) the animal colony from which the experimental animals were obtained was neither monitored nor treated for pinworms; 2) because retired female breed mice were used, the worm burden and hence, their IgE levels may have been exceptionally high due to the inhibitory effects of pregnancy and lactation on worm expulsion (67).

The observation that measurements of PCA activity may be in-

fluenced by pinworm infection is extremely important considering the ubiquity of pinworm infections in animal colonies. However, some commercial animal suppliers do monitor their colonies for pinworms and apply appropriate means to control the infections.

The suppression of IgE antibody formation by neonatally initiated treatment with anti-μ antiserum provides convincing evidence that IgE-forming cells, like those producing IgM, IgG_1, IgG_2 and IgA, arise from IgM bearing precursoral cells. It is possible, of course, that the anti-μ serum used in these studies contained undetected traces of anti-ε activity which might be responsible for the observed suppression. That situation seems unlikely, however, in view of the degree of purity of IgM used to induce formation of the antiserum and the subsequent extensive absorption of this antiserum with other immunoglobulins. It seems more likely that the effect is in fact due to anti-μ antibodies. It may be that suppression of IgE responsiveness, like IgA suppression, requires a relatively high level of suppressive treatment. This could account for the lack of IgE suppression observed by Dwyer et al. (60).

The suppression of anti-OVA responses by neonatally initiated NRS treatment proved to be variable in its expression and it is not known what influences this. It is clear however, that the effect is not restricted to the PCA response but extends to the PHA response

in a parallel manner. This effect may be due to some type of antigenic competition (68) or to a direct effect, perhaps cytotoxic, on the cells required for the generation of an immune response. Further study is needed.

The results of the studies on immunization of Balb/c mice with one IP injection of low doses (1 and 10 μg) of OVA and 1 mg alum confirmed that this procedure was a highly reliable means of inducing high and persistent levels of IgE antibody. The results, when higher doses of OVA (100 and 1000 μg) with 1 mg alum were used, showed that this procedure also induced a high and persistent IgE response. These results differed from that of Vaz et al. (25), who showed that high doses of OVA induced a low and transient IgE response. The differences observed, may be due to differences in the strains of mouse used and/or to differences in the amount of alum used. Vaz et al. (25) used SW-55, SWR/J and C57BL/6J mice and 10-20 mg alum with higher doses of OVA whereas I used Balb/c mice and 1 mg alum. From information in the Handbook of Experimental Immunology (69) it appears that approximately 50 μg of protein is the maximum amount that can be precipitated by 1 mg alum. Therefore, when I immunized with 100 or 1000 μg of OVA + 1 mg alum, the animals were probably receiving approximately 50 μg of alum precipitated antigen + 50 or 950 μg of non-precipitated antigen.

Presumably then, the alum precipitated antigen remained as a depot within the animal, whereas the rest of the antigen, the non-precipitated portion, acted as any other soluble antigen and became available to the circulation, creating a high circulating concentration of antigen, which was then metabolized and excreted normally. Therefore, the actual dose of antigen that persisted within the depot was probably about 50 μg, no matter how much more was given initially with 1 mg alum. This may not have been enough antigen to achieve the type of high dose regulation observed by Vaz et al. (25).

The differences observed between IgE anti-DNP responses produced in response to either lightly dinitrophenylated OVA or heavily dinitrophenylated OVA are interesting. Immunogenic doses of $DNP_{0.5}$-OVA induced a transient IgE anti-DNP response and the titers were inversely related to dose while immunogenic doses of DNP_{24}-OVA induced a high and persistent response.

This difference between the transient and persistent nature of the responses is probably best explained on the basis of studies by Klaus and Mitchell (69) in which they showed a distinct difference in the metabolism of DNP_5-BSA and DNP_{50}-BSA. DNP_{50}-BSA persisted, *in vivo*, for long periods of time, was retained mainly in the spleen and liver and was efficiently taken up by macrophages, *in vitro*,

but degraded very slowly. DNP_5-BSA, on the other hand was excreted rapidly, was more broadly distributed in various organs and was not taken up as efficiently by macrophages as DNP_{50}-BSA. Thus, it is thought that perhaps the IgE responses produced as a result of immunization with $DNP_{0.5}$-OVA are transient because this antigen is not retained *in vivo* for very long and therefore is not available to provide a constant stimulus for IgE B cells.

An additional explanation, the formation of suppressor T cells, may be considered. Recent work by Ishizaka and Adachi (22) showed that the *in vitro* culture of splenic lymphocytes, with antigen, in the absence of macrophages, resulted in the formation of T suppressor cells for IgE and IgG anti-hapten responses whereas culturing on antigen bearing macrophages resulted in the formation of T helper cells. Thus, suppressor T cells may be formed as a result of immunization with $DNP_{0.5}$-OVA but not with DNP_{24}-OVA because of the differences in the effect on macrophages between the two conjugates.

One other factor that might be influencing the responses to the DNP-conjugates may be related to the differences in the other classes of antibody induced by these antigens. Klaus and Cross (70) showed that DNP_5-BSA elicited mainly IgG antibody and immunological memory while DNP_{50}-BSA induced a primary IgM response, little IgG

antibody and poor memory. As mentioned above, antigen-specific IgM enhanced IgE formation in the rabbit (31) and antigen-specific IgG suppressed IgE formation in the rat (26). However, one must be careful in applying observations on IgE formation in one species to another (4). Also the regulatory effect of passively administered IgG antibody on IgE formation in mice has been shown to be different from that observed in the rat (71). It may be that all of these factors influence the IgE responses to the different DNP-OVA conjugates; however further work is required.

The dichotomy in anti-hapten and anti-carrier IgE responses observed by others (42, 58) was confirmed. Mice immunized with $DNP_{0.5}$-OVA produced high and persistent anti-OVA IgE responses. It may appear that this response contradicts the explanation advanced above for the transient nature of the anti-DNP response because it implies that antigen is retained in vivo and probably processed by macrophages. However, it seems probable that at such a low density of hapten conjugation, there are many OVA molecules which do not contain any DNP groups and hence would be processed as "native" alum precipitated OVA and produce a typical IgE response. The failure of DNP_{24}-OVA to induce an anti-OVA IgE response can best be explained by a masking of or a conformational change in the native OVA determinants.

The lack of a detectable PCA antibody response in mice immunized with OVA and B. pertussis vaccine was surprising because of previous work in which this adjuvant favored the production of IgE (4). However, B. pertussis vaccines are apparently unreliable for this purpose and only certain preparations are able to induce IgE formation to specific antigens (4).

The induction of IgE responses without adjuvant, by multiple injections of antigen has not been reported previously for laboratory animals. This is a significant observation because it may represent a model for IgE antibody induction that is more akin to the means by which allergic individuals become sensitized than the single injection of a low dose of alum-adsorbed antigen. Although this regimen induced high IgE responses, it also induced high precipitin responses, when NRS was used, which is not observed in the atopic condition. I believe that if much lower doses of antigen are used then the pattern of antibody responses with regard to immunoglobulin class would be altered such that the precipitin response would be considerably diminished and the IgE response enhanced.

I speculate that, in order to induce IgE antibody formation, repeated stimulation of the cells involved in IgE formation (either T or B or both) is required. This is so that a certain minimum

number of IgE producing cells are formed in order to attain high enough serum levels of IgE antibody that can be detected by the PCA assay. This could be achieved either by giving alum-adsorbed antigen or multiple injections of antigens.

Macrophage processing has been shown to be important in the formation of helper T cells for IgE responses (22). Although the mechanism by which alum-adsorbed antigen stimulates IgE formation is not known, Tada (4) speculates that small doses of alum-adsorbed antigen are phagocytized by macrophages and released in an extremely immunogenic form to stimulate certain populations of T or B cells. Presumably, alum-adsorbed antigens are taken up very efficiently by macrophages. It is not known to what extent soluble antigens, such as those used in multiple injections, in comparison to alum-adsorbed antigen, are processed by macrophages. It may be that by giving repeated injections of soluble antigen the requirement for macrophage involvement is overcome or alternatively, that macrophage processing of soluble antigen is stimulated by this regimen.

From the studies on the effect of multiple, high doses of antigen, given IV on days 3, 5, and 7 after prior immunization with 10 μg OVA + 1 mg alum, IP it can be concluded that this treatment results in the selective suppression of IgE responses to OVA and

that this effect can be transferred to normal animals by splenic cells.

While these studies were in progress, other workers reported that administration of 3, 100 μg, IV injections of urea denaturated OVA (UD-OVA) given on days 3, 5, and 7 after immunization with a low dose of alum-precipitated OVA, suppressed anti-OVA IgE and IgG responses and resulted in the formation of suppressor T cells (39). Their system was unique in that UD-OVA contained only the T-cell stimulating determinants of native OVA; the native OVA B-cell stimulating determinants were lost by the urea treatment (73).

In light of these studies, it seems reasonable to assume that 3, 100 μg, IV injections of native OVA also induces the formation of suppressor T cells, however the evidence for this is still indirect, supported mainly by the fact that suppression can be transferred by splenic cells.

One main difference between the UD-OVA and the native OVA used in my studies is the presence of B cell determinants on the native OVA. For this reason, other alternative hypotheses to explain the desensitization observed in my system might be considered.

One such hypothesis is that the multiple, high dose injections of soluble antigen induce a high affinity IgG antibody which then exerts a suppressive effect on the IgE response. It has been re-

ported that passively administered, antigen-specific IgG antibody from hyperimmune animals will exert a suppressive effect on IgE responses to the same antigen (71). The effect was transient however, and only lasted until the transferred antibody had been catabolized. Also, the suppression was most effective when the passive antibody was transferred within 24 hr of active immunization. The passively administered antibody could not terminate an existing IgE response or suppress a secondary IgE response.

These observations are inconsistent with the events seen when mice are desensitized with multiple injections of native OVA, because both a pre-existing and the secondary IgE response were shown to be suppressed by the treatment. Also, when a secondary challenge of antigen is given to sensitized animals, high levels of IgE antibody are maintained in the presence of extremely high levels of PHA antibody. The foregoing argues against the hypothesis that the observed suppression of IgE responses by administration of multiple IV injections of antigen is due to an IgG antibody mediated mechanism.

Another hypothesis that might be considered is the selective induction of IgE B cell tolerance. However, in the case of protein antigens, the induction of tolerance in presensitized B cells has proved to be difficult to accomplish (74). Some recent studies

have shown that tolerance in IgE B cells could be achieved, even after prior sensitization, by DNP coupled to non-immunogenic carriers (62, 75), and that the dose of tolerogen required for IgE tolerance induction was less than that required for IgG tolerance induction (75). It is not known whether this dosage effect applies to the induction of IgE B cell tolerance to protein antigens.

Although the induction of B cell tolerance cannot be ruled out, the fact that the suppression of IgE, which is induced by multiple, IV injections of antigen can be transferred to other animals by splenic cells makes this mechanism as the only explanation of desensitization highly unlikely and suggests very strongly the presence of a suppressor cell.

SUMMARY

The results of this thesis can be summarized as follows:

1) The suppression of IgE antibody responses by neonatally initiated anti-μ treatment showed that IgE-forming cells are derived from the same precursoral cell as are the cells forming other classes of immunoglobulins.

2) Various ways of inducing and regulating IgE responses in Balb/c mice were compared. Immunization with alum-adsorbed OVA over a wide dosage range induced a high and persistent IgE response. Failure to show a transient IgE response to high doses of antigen may be related to the inability of 1 mg alum to completely react with an excess of protein. Immunization with alum-adsorbed DNP-ovalbumin conjugates produced 2 different patterns of IgE responses related to the hapten density. This was thought to be related to the way in which these conjugates may be metabolized. Immunization with one injection of OVA with B. pertussis failed to induce IgE responses. A new means of inducing IgE responses by multiple IP injections of antigen was shown. Also, treatment with neonatally initiated multiple injections of NRS suppressed subsequent immune responses to immunization with alum-adsorbed OVA.

3) The selective suppression of IgE responses by 3, IV injections of high doses of soluble antigen given on days 3, 5, and 7 after prior sensitization with 10 μg OVA + 1 mg alum was shown to

be due, at least in part to the generation of suppressor cells in the spleen. Both antibody titers and the number of IgE producing cells were reduced to undetectable levels by this treatment. This phenomenon was antigen specific and very dependent on the route of administration. Whereas 100 μg of soluble antigen was very suppressive when given IV, the same or 10-fold higher doses were only slightly suppressive when given IP. However, an optimally immunogenic dose, 10 μg, given IV, induced significant suppression. Secondary responses were also affected by this treatment.

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