Regulatory effects of the thymus on the IgE response of mice
by Rhet Lucy Schneller

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Microbiology
Montana State University
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Abstract:
The regulatory effects of the thymus gland on the production of reaginic immunoglobulin E (IgE) were studied using congenitally athymic (nude) mice, phenotypically normal littermates (NLM) of nude mice and Balb/c mice. Antigen specific IgE was quantitated in CFW mice by the passive cutaneous anaphylaxis (PCA)assay. Alum, complete Freunds adjuvant and saline extract of Bordetella pertussis were used as adjuvants. Nude mice failed to produce specific IgE following both primary and secondary immunization with hen egg albumin (EA), crude ascaris extract and crude ascaris extract coupled to dinitrophenyl groups. In contrast, NLM and Balb/c mice made strong IgE responses.

Nude mice implanted with thymus glands from neonatal Balb/c or NLM donors made IgE responses equal to the responses of NLM mice. These data indicate that there is an absolute requirement for thymus-derived cells in the formation by mice of specific IgE.

Nude mice were passively sensitized intradermally with an IgE-positive serum; when challenged intravenously with specific antigen, these nude mice demonstrated strong PCA reactions. It is clear, therefore, that although nude mice cannot make specific IgE, they do have the mechanism necessary to elicit PCA reactions.

Attempts were made to show a negative regulatory effect of the thymus on IgE production by using splenectomized, 60Co-irradiated or antilymphocyte serum-treated mice. No enhancement of IgE production was observed in these experiments. In contrast to results obtained by others using rats, infection of immunized mice with the nematode Nippostrongylus brasiliensis did not potentiate specific IgE production.

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ABSTRACT

The regulatory effects of the thymus gland on the production of reaginic immunoglobulin E (IgE) were studied using congenitally athymic (nude) mice, phenotypically normal littermates (NLM) of nude mice and Balb/c mice. Antigen specific IgE was quantitated in CFW mice by the passive cutaneous anaphylaxis (PCA)assay. Alum, complete Freunds adjuvant and saline extract of _Bordetella pertussis_ were used as adjuvants. Nude mice failed to produce specific IgE following both primary and secondary immunization with hen egg albumin (EA), crude ascaris extract and crude ascaris extract coupled to dinitrophenyl groups. In contrast, NLM and Balb/c mice made strong IgE responses. Nude mice implanted with thymus glands from neonatal Balb/c or NLM donors made IgE responses equal to the responses of NLM mice. These data indicate that there is an absolute requirement for thymus-derived cells in the formation by mice of specific IgE.

Nude mice were passively sensitized intradermally with an IgE-positive serum; when challenged intravenously with specific antigen, these nude mice demonstrated strong PCA reactions. It is clear, therefore, that although nude mice cannot make specific IgE, they do have the mechanism necessary to elicit PCA reactions.

Attempts were made to show a negative regulatory effect of the thymus on IgE production by using splenectomized, ^{60}Co-irradiated or antilymphocyte serum-treated mice. No enhancement of IgE production was observed in these experiments. In contrast to results obtained by others using rats, infection of immunized mice with the nematode _Nippostrongylus brasiliensis_ did not potentiate specific IgE production.

INTRODUCTION

Properties of Homocytotropic Antibodies. In humans, reaginic antibodies, which are responsible for some allergic reactions, are in the immunoglobulin class IgE. IgE is a glycoprotein with a molecular weight of 200,000 and is synthesized by plasma cells predominantly in the respiratory and gastrointestinal tracts. It is capable of agglutinating red blood cells coated with antigen but possess no complement-fixing activity (1). It differs from other classes of immunoglobulin in that it is non-precipitating. It does not have the ability to cross the placenta and is not found in human colostrum (2). Zone electrophoresis has shown that IgE moves in the "fast γ" region while zone centrifugation in buffered sucrose gradient has shown that it sediments in the 7S region (2). Human IgE can sensitize the skin of humans, but not the skin of most other species, for immediate-type hypersensitivity reactions.

A similar type of antibody, possessing properties of the human reaginic antibody, IgE, has been reported in mammals other than man by several investigators. Thus, reaginic IgE-like, homocytrotropic antibodies (HTA) have been reported in several animal species including the guinea pig (3), rat (4), and mouse (5). Like the human reagin, these antibodies are heat-labile; heating the undiluted serum at 56°C for 30 minutes destroys their skin-sensitizing ability. Many species, including the mouse, also produce another homocytotropic antibody

which belongs to the IgG_1 subclass (5). IgG_1 does not persist cytophilically in the skin for long periods of time (hours, as compared to weeks for IgE) but does persist in the serum longer than the reaginic-type antibody. IgG_1 is heat-stable, so heating for 30 minutes at 56^oC does not destroy its activity (5). It is, therefore, possible to distinguish between the activities of IgG_1, and IgE HTA in the passive cutaneous anaphylaxis (PCA) assay by heating serums or varying the time of antigen challenge after skin-sensitization. IgG_1-mediated reactions are typically developed 2 hr after passive sensitization, whereas IgE-mediated reactions are developed 72 hr after passive sensitization.

It is thought that IgE attaches to mast cells and causes the release of chemical mediators of the PCA reaction (1). Mota (6) also reported that rat mast cells can be sensitized passively with serum containing what he called "mast cell sensitizing antibody," which is comparable to human IgE. He found that sensitized mast cells release histamine and 5-hydroxytryptamine after the addition of specific antigen. It is thought that serotinin (5-hydroxytryptamine) is the main chemical mediator of anaphylaxis in the rat and the mouse (6).

In mice, IgE production depends on the antigen-adjuvant system as well as on the strain of animal used. Revoltella and Ovary (7) reported that three of six strains of mice immunized with DNP-haemocyanin and $AL(OH)_3$ were able to produce IgE. SWR/J, C57BL/6J and

A/HeJ made IgE, whereas CBA/J, DBA/J and SW made high levels of IgG_1 but no IgE (7). Clausen et al (8) found that the inbred strains of mice NBL/n, C57Bl/6J and C57BL/10J were far superior to the strains AKR/n, STR/n and RML in production of IgE when challenged with hen egg albumin (EA) and saline extracts from Bordetella pertussis (PE).

Several different antigens and adjuvants have been used to elicit IgE production. Using EA as antigen, Clausen, et al., were successful in stimulating IgE with SE as adjuvant, whereas incomplete Freund's adjuvant was superior in stimulating 2 hr PCA antibody, IgG_1 (9). Tumor antigens and alloantigens have also been used to induce IgE production in mice. Thus C57BL and Balb/c mice immunized with 1×10^6 viable tumor cells from the B16 melanoma established in C57Bl mice, responded with production of IgE antibody (10). Kind and Macedo-Sobrinho were able to get IgE production in mice in response to rabbit anti-mouse thymocyte serum (11).

Small doses of antigen such as pollen, which cause human allergies, seem to be best for production of IgE (12). In guinea pigs, Perini and Mota (13) demonstrated that low doses of EA together with low doses of lipopolysaccharide (LPS) were effective in stimulation of IgE production, and high doses of antigen and adjuvant resulted in high production of IgG_1.

It has also been reported that a state of tolerance can be induced

to the IgE antibody class. Katz and co-workers (14) showed that two pretreatments of mice with 500 µg of the copolymer DNP-D-glutamic acid and D-lysine significantly reduced the animals' ability to respond to DNP-crude ascaris extracts (DNP-CAE) with production of IgE antibody. This tolerance could be transferred to syngeneic, unprimed, irradiated recipients with spleen cells from mice primed with DNP-CAE and alum and given two 500 µg injections of DNP-D-glutamic acid and D-lysine 25 days later. Saverbronn Maia, et al, showed that three immunizations of 100 ug EA significantly reduced IgE production of DBA/1J mice when immunized subsequently with 0.1 µg EA.

Thymus Dependency of IgE. Thymus-derived cells (T cells) are necessary for production of IgM or IgG to the so-called "Thymus-dependent" antigens. Although most antigens, such as heterologous proteins and heterologous erythrocytes, are thymus dependent, there are some "thymus-independent" antigens, such as endotoxin, pneumococcal polysaccharide, and Vi antigen.

Until recently, only speculations have been made on the T-cell collaboration with B cells in relationship to the IgE response. For example, Okumura and Tada (16) demonstrated that neonatal thymectomy of rats caused a reduction in the production of IgE in response to dinitrophenol-crude Ascaris extract (DNP-CAE).

The most meaningful data supporting T-cell dependency of the IgE

response is seen in the work of Michael and Bernstein (17). They found that congenitally athymic (nude) mice failed to respond with IgE production to EA, bovine serum albumin, and human gamma globulin, whereas their normal thymus-bearing littermates did respond to these antigens. However, nude mice that had received thymus cells from normal Balb/c mice were able to make an IgE response to these antigens.

Some unpublished work of Reed and Munoz further indicates that IgE responses may be thymus dependent. They found that normal littermates to nudes could make an IgE response to EA and SE, bovine gamma-globulin and SE and Keyhole Limpet hemocyanin, whereas nude mice did not respond to these antigens. They also measured the IgE response to two antigens known to be thymus independent for an IgM response, namely, SIII and endotoxin. Neither nudes nor their normal littermates made any IgE response to these antigens.

Thymus Regulation of the IgE Response. IgE production may be thymus dependent but may also be regulated by the thymus. Tada and coworkers (16), for example, demonstrated that while neonatal thymectomy of rats decreases the IgE response, adult thymectomy increases this response. They showed a similar enhancement of IgE production with 400 rad X-irradiation (18), splenectomy (16), some immunosuppressive drugs (19) and antithymocyte treatment (20). One explanation for such an enhanced effect is the "turning off" of suppressor T-cells or the

destruction of T-cells, allowing B-cells to produce IgE in an unregulated manner.

Tada and Okumura (21) demonstrated that HTA formation can be suppressed by passive administration of homologous antibody against the same antigen. This suppression appeared selective in that the HTA response was affected, whereas the IgM response was not. They suggested that blocking antibody in the passively administered serum acts by suppressing the synthesis of reagin rather than by preventing the combination of allergen and reagin (21). Their reasons for this conclusion were: the fact that the amounts of passive antibody used were much less than those needed to cover all of the antigenic determinants; and also the fact that IgE was suppressed while hemagglutinating antibodies were not.

Okumura and Tada (22) have used a hapten-carrier molecule to induce reagin formation. By transferring thymocytes from rats hyperimmunized with crude Ascaris extract (CAE) or DNP-CAE into recipients that were producing high titers of HTA, they achieved a drastic reduction of HTA within two days of transfer. Because this effect only took place when the carrier was the same in donor and recipient, they concluded that carrier-specific, thymus-derived lymphocytes can negatively regulate the HTA formation which is known to be hapten-specific (23). They further investigated this negative control to find what they

called an "antigen-specific T-cell factor" which they felt was responsible for the suppression of IgE production. They treated the thymocyte extracts from hyperimmunized donors with different nucleases and proteinases and found that the T-cell factor was not digestible by RNase but is destroyed with trypsin and pronase, therefore, they concluded, the T-cell factor was of protein nature. Its activity sedimented slower than IgG hemagglutin in sucrose density gradient ultracentrifugation and was electrophoretically located in regions corresponding to alpha and beta globulins. They suggest, therefore, that the T-cell factor may be an unique receptor of the T-cell and not an unusual immunoglobulin (24).

In working with rats, White and Holm (25) demonstrated that administration of equine antilymphocyte serum before immunization with KLH yielded an enhancement of early systhesis of reaginic antibody but giving equine antilymphocyte serum after administration of KLH resulted in late synthesis enhancement (25). Antilymphocyte serum pretreatment was suppressive for KLH agglutin in production.

Kind and Macedo-Sobrinho (11) have reported a similar regulatory control of the thymus on IgE formation in mice. They found that X-irradiation (450 rad) resulted in sustained level of IgE production in mice treated with rabbit anti-mouse thymocyte serum. Splenectomy and thymectomy did not result in increased IgE production.

Effects of Nippostrongylus brasiliensis on IgE Production. Several investigators have reported a potentiated reagin response following nematode infection. Thus, Block, et al (26) demonstrated that rats infected with Nippostrongylus brasiliensis larvae secondary to immunization with EA or KLH plus alum exhibited increased reagin production of short duration. The potentiated response was only found in IgE production and not in the other classes of antibody. Jarrett, et al (27), in the rat, showed that both antigen and adjuvant must preceed infection with N. brasiliensis for a potentiated response, which could rise as much as 100-fold. They showed that no potentiated response occurred if PE was substituted for the parasite, leading them to conclude that the N. brasiliensis did not act as an adjuvant.

The Thymus as the Origin of the Mast Cell. It has been hypothesized by Csaba et al (28) and also by Ginsberg (29) that the thymus is the origin of the mast cell. Pritchard, Viklicky and Sima (30), however, studied the connective tissue of the skin and the lymph nodes of nude mice and found what they proposed to be mast cells. Because the nude mouse is congenitally athymic, they concluded that the origin of the mast cell could not possibly be the thymus. If nude skin was able to elicit a PCA reaction, there would be good evidence in support of Pritchard's conclusion.

The following experiments were designed to evaluate the role of the thymus in IgE production.

MATERIALS AND METHODS

Animals. Balb/cJ mice were obtained from the Jackson Laboratory, Bar Harbor, Maine. Congenitally athymic (nude) mice (nu/nu) (31) and their phenotypically normal littermates (NLM; +/nu or +/+) were obtained from the Montana State University Colony in which the mutant gene, nu, is being placed onto a Balb/c background by backcross-intercross mating scheme (32).

Balb/cJ, nude and NLM mice, 1-3 months of age, were immunized by various schemes designed to induce the production of HTA. No attempt was made to distinguish between the HTA response of males and females.

CFW female mice, 1-3 months of age, obtained from Carworth Farms, Portage, Mich., were used as serum recipients in PCA assays.

All animals were maintained in a clean environment and were given acidified-chlorinated water and autoclaved Purina 5010 feed.

Antigens. Hen egg albumin (EA), 5-times crystallized, was obtained from Miles Laboratories Inc., Kankakee, Ill. The crystallized EA was stored at -20°C. For immunizations, EA was dissolved at the appropriate concentration in saline or phosphate buffered saline (PBS). Fresh EA solution was prepared for each experiment.

Crude Ascaris Extract. Dr. Herbert J. Morse, Ill, Laboratory of Microbial Immunity, National Institutes of Health, Bethesda, Maryland,

made and provided the crude ascaris extract (CAE). CAE was prepared by homogenizing saline-washed Ascaris organisms, dialyzing the homogenate against several changes of PH 8.2 borate buffered saline and clarifying dialysate by centrifugation (10,000 x G - 30 minutes). CAE was stored in small aloquates at -20°C. For use, CAE was thawed and diluted to the desired concentration in saline.

DNP-Ascaris. Dinitrophenylated crude Ascaris extract (DNP-CAE) was also made and provided by Dr. H. Morse. The DNP group was combined with ascaris extract in the ratio of 48 DNP/10^5 daltons.

Vi-antigen (Vi) was purified by Dr. F. Jarvis, Idaho State University, Pocatello, Idaho, by a continuous-flow paper curtain electrophoresis procedure (33). This antigen, a polymer of N-acetyl galactosaminuronic acid (33), was provided by Dr. J. T. Ulrich of Idaho State University. The lyophilized Vi was stored at 4°C. for use in experiments, Vi was dissolved in saline.

Adjuvants. Alum was prepared as follows: 75 grams of aluminum ammonium sulfate (Humco Laboratory, Texarkana, Texas) was added to 180 ml of distilled water and then 75 ml of 1 N NaOH was slowly added with continuous mixing. The resulting precipitate was washed 8 times with sterile distilled water and was finally resuspended in sterile distilled water at a concentration of 60 mg/ml. This adjuvant was mixed by pipetting with the different antigens used prior to immunizations.

Pertussis Extract. A saline extract of Bordetella pertussis (PE) was made (9) and provided by Dr. John Munoz, Rocky Mountain Laboratory, Hamilton, Montana. The lyophilized extract was stored at 4°C and was dissolved in saline for use as an adjuvant. At times it was mixed with antigen before immunization.

Lipopolysaccharide (LPS) was extracted from Escherichia coli 0113 by the hot phenol-water method (34) and was provided by Dr. J. A. Rudbach, University of Montana, Missoula, Mt. LPS was dissolved in PBS at a concentration of 1 mg/ml and stored at -20°C until diluted in PBS for use in experiments.

Complete Freund's Adjuvant (CFA) was purchased from Difco Laboratories, Detroit, Michigan.

Antilymphocyte Serum. Rabbit anti-mouse lymphocyte serum (RAMLS), lot 13096, was purchased from Microbiological Associates, Bethesda, Maryland. Other lots were not used in this experiment.

Nematodes. In some experiments designed to potentiate HTA production, mice were injected subcutaneously with 600 L_3 larvae of a mouse-adapted strain of the nematode Nippostrongylus brasiliensis (35).

Irradiation. Mice and rats were given 400-450 rad whole-body irradiation using a Picker 60 Cobalt therapy unit. To insure uniform

dose during irradiation, the mice were held in a plexiglass cage placed on top of a rotating turntable. The dose rate was approximately 75 rad/min.

Splenectomy. Spleens were removed surgically from Balb/c mice at approximately six weeks of age. The mice were anesthetized with the proper dose of Nebutol (36). The spleen was ligated and cauterized before removal. The peritoneum and skin were sutured separately. Care was taken not to tear the spleen and not to leave any spleen remnants in the mice.

Thymus Grafts. One month old nude mice were anesthetized with Nebutol (36) prior to implantation of two to four thymus glands from donors less than three days of age. Implants were made under the fascia in the axillary region of the nude. Either Balb/c or NLM were used as thymus donors. Only thymus-grafted nudes that outwardly appeared healthy were used in experiments.

Thymus Cell Injections. Thymocyte preparations were made by screening young adult Balb/c thymuses through stainless steel 80-mesh screens into PBS containing 1% fetal calf serum (PBS-FCS). The thymocytes were then washed once with PBS-FCS. Viability was determined by a Trypan Blue Exclusion test, and 1.4×10^8 viable cells contained in 0.7 ml were injected slowly into the tail vein of recipient nude mice.

Immunization Procedures. To obtain HTA, mice were given varying doses of antigen and adjuvant on day 0. Antigen and adjuvant were always mixed prior to immunization. CFA was mixed with antigen with a double syringe while all other adjuvants used were mixed with a pipette. The injections were usually given intraperitoneally, although there were some experiments in which the route of injection was intravenous.

The animals were bled on days 10 and 20 after initial immunization of antigen alone because I found that adjuvant has a deleterious effect on antibody production if given at this time. The animals were then bled on day 30, 10 days after the booster injection. Serum was collected from individual animals and stored separately at -30°C because I and others (37) have found that reaginic antibody denatures at 0°C.

Passive Cutaneous Anaphylaxis. A pool was made from the serum of individual animals within a given experimental group. Two-fold serial dilutions of the serum pool were made in saline. 0.05 ml of a given serum dilution was injected intradermally on the back of a recipient CFW mouse. The hair was clipped from the back of recipient mice 24 hours prior to the intradermal injection. Recipient mice were injected intravenously with 0.2 ml of antigen solution (1% solution of EA, 0.2 mg DNP-CAE or 0.2 mg CAE) in saline containing 0.5% Evans Blue dye. Thirty minutes after intravenous challenge with antigen and Evans

Blue the animals were killed by cervical dislocation and the skin on the back was incised and inverted. A positive reaction was noted by a blue circular area of the skin at the injection site. Vertical and horizontal measurements were recorded. An area less than 4mm x 4mm was recorded as a negative reaction, whereas an area greater or equal to 4mm x 4mm was recorded as a positive reaction. Reactions were given a subjective rating of 1+ to 4+ determined by the depth of color, a 4+ reaction being the deepest blue. The endpoint was defined as the last dilution giving a positive reaction.

It should be noted that two different HTAs are measured by the PCA assay. If the recipient animals are allowed to wait two hours before challenge with antigen and Evans Blue dye, then IgG_1 is detected. IgG_1 is heat stable, has a short duration in the skin and a long duration in the serum. In contrast, if antigen and Evans Blue dye are injected 72 hours after skin sensitization, the PCA reaction detects IgE, which is heat labile, has a longer duration in skin but a shorter duration in the serum.

RESULTS

IgE Production in the Mouse. Heat sensitivity is a good property to use to distinguish between heat-stable IgG_1 and heat-labile IgE. Thus, it was determined from heating undiluted serum that the 2-hour PCA reaction remained while the 72-hour PCA diminished. This same result was found in sera from both antigens used for immunization and from serum collected after a primary immunization on day 10 and a secondary immunization collected on day 30 (Table I). It should be noted that the 72-hour response remained if the serum was not heated. These results suggest that the antibody observed at 72-hours is IgE. Because of these results, I routinely used the 72-hour PCA response as a measure of IgE.

Preliminary Studies on the Ability of Different Antigens to Elicit an IgE Response in Mice. Several different antigen-adjuvant combinations were tested for their ability to elicit IgE antibody production in Balb/c mice (Table II). Mice immunized with 10 μg CAE plus 50 μg PE failed to produce IgE (line A), even when boosted with 10 μg CAE on day 20. Negative results were also obtained with 125 μg EA plus 50 μg PE (line B) and 10 μg CAE plus 1 μg LPS (line C); again, booster injections were not effective. CAE, however, was a good antigen to elicit IgE when used with either CFA or alum adjuvants. 100 μg CAE plus 20 mg alum (line D) gave 72 hr PCA reactions on day 10 after a primary immunization and day 30 after a secondary immunization. A slightly higher

TABLE I

Effects of heating a homocytotropic antibody positive serum from Balb/c mice, 30 minutes at 56°C as demonstrated by passive cutaneous anaphylaxis reactions

Immunization Day 0	Immunization Day 20	Heated	No. of Serum in Pool	Day Serum Collected	Sensitization Period	PCA[h] Titer of Serum Pool
10 µg EA[a], Al[b]	10 µg EA	+	5	30	2 hr	256[c]
same[f]	same	+	5	30	72 hr	neg[g]
same	same	-	7	30	72 hr	128
10 µg CAE[d], CFA[e]	none	+	5	20	2 hr	64
same	same	+	5	20	72 hr	neg
same	same	-	7	20	72 hr	128

[a]EA = hen egg albumin

[b]Al = 10 mg alum

[c]256 = titer reciprocal of serum pool

[d]CAE = crude ascaris extract

[e]CFA = complete Freunds adjuvant in equal volume as antigen

[f]same = same serum as above

[g]neg = not detected at 1/8 dilution

[h]PCA = passive cutaneous anaphylaxis

titer on day 10 was obtained if the dose was reduced to 10 μg CAE plus 10 mg alum (line E). When CFA was used (line F), the mice produced IgE on days 10 and 20, and also after a booster injection. Very good primary titers were obtained with 10 ug DNP-CAE plus 10 mg alum (line G), and also after a booster injection of DNP-CAE.

Egg albumin with alum as adjuvant was by far the easiest compound to use and also elicited high titers of IgE on days 10, 20 and 30. The optimal dose was 10 μg EA plus 10 mg alum (line H). CFA was also effective with 125 μg EA in production of IgE on days 20 and 30 although the titers are much lower than if alum was used as adjuvant.

After all these tests were completed, it was decided that 10 μg EA plus 10 mg alum (line L) was the best antigen-adjuvant combination with which to observe the IgE response.

Thymus Dependency of the IgE Response. An attempt was made to determine whether or not IgE exhibits the usual thymus-derived cell (T-cell)-bursa-equivalent (B cell) collaboration required for production of other immunoglobulin classes. To test for such a requirement, attempts were made to induce IgE production in the nude mouse (Table III). Because this animal lacks T cells from birth, it provides an excellent system in which to observe thymus dependency. Both nude and NLM mice were immunized with 10 μg EA plus 10 mg alum, and were boosted with 10 μg EA on day 20. The NLM responded on days 10, 20 and 30 with

TABLE II

Effectiveness of different antigen-adjuvant combinations injected intraperitoneally in eliciting IgE production in Balb/c mice

Line	Antigen-Adjuvant Combinations Day 0	Immunization Day 20	No. of Serum in Pool	72 hr PCA[a] Titer of Serum Pool		
				Day 10	Day 20	Day 30
A	10 µg CAE[b], 50 µg PE[c]	10 µg CAE	4	neg[d]	neg	neg
B	125 µg EA[e], 50 µg PE	125 µg EA	5	neg	neg	neg
C	10 µg CAE, 1 µg LPS[f]	10 µg CAE	4	neg	neg	neg
D	100 µg CAE, 20 mg Al[g]	100 µg CAE	4	8[h]	neg	128
E	10 µg CAE, 10 mg Al	10 µg CAE	4	16	neg	128
F	10 µg CAE, CFA[i]	10 µg CAE	4	64	128	32
G	10 µg DNP-CAE[j], 10 mg Al	10 µg DNP-CAE	4	64	32	256
H	10 µg EA, 10 mg Al	10 µg EA	4	64	16	128
I	125 µg EA, CFA	10 µg EA	4	neg	16	8

[a]PCA = passive cutaneous anaphylaxis

[b]CAE = crude ascaris extract

[c]PE = saline extract of Bordetella pertussis

[d]neg = not detected at 1/2 dilution

[e]EA = hen egg albumin

[f]LPS = lipopolysaccharide

[g]Al = alum

[h]8 = titer reciprocal of serum pool

[i]CFA = complete Freunds adjuvant

[j]DNP-CAE = dinitrophenylated crude ascaris extract

TABLE III

Thymus Dependency of the IgE Response

Line	Immunization Day 10	Immunization Day 20	Group	Time of Graft	PCA[a] Titer of Serum Pool Day 10	Day 20	Day 30
A	10 µg EA[b], Al[c]	10 µg EA	NLM[d]	NA[e]	32[i]	32	32
B	same[f]	same	Nu[g]	NA	neg[j]	neg	neg
C	same	same	Nu-TC[h]	2 months	neg	neg	16
D	10 µg EA, Al	10 µg EA	NLM	NA	64	8	32
E	same	same	Nu	NA	neg	neg	neg
F	10 µg EA, Al	10 µg EA	Balb/c	NA	64	32	32
G	same	same	Nu-TG[k]	7 months	32	128	not done
H	10 µg EA, Al	10 µg EA	Balb/c	NA	64	128	128
I	same	same	Nu-TC	2 months	neg	neg	16
J	10 µg CAE, Al	10 µg CAE	NLM	NA	128	32	16
K	same	same	Nu	NA	neg	neg	neg
L	same	same	Nu-TG	4-6 months	neg	neg	2
M	10 µg EA, Al	10 µg EA	Balb/c	NA	16	8	not done
N	same	same	Nu-TC	7 months	neg	neg	not done
O	same	same	Nu-TG	6-12 months	16	4	not done

[a]PCA = passive cutaneous anaphylaxis

[b]EA = hen egg albumin

[c]Al = 10 mg alum

[d]NLM = normal littermate to nude

[e]NA = not apply

[f]same = same serum as above

[g]Nu = nude, athymic mouse

[h]Nu-TC = nude with thymus cells

[i]32 = titer reciprocal of serum pool

[j]neg = not detected at 1/2 dilution

[k]Nu-TG = nude with thymus graft

production of IgE. Thus, NLM responded after a primary immunization as seen on days 10 and 20 and also after a secondary immunization as seen on day 30 (line A). The nude mice, however, made no such responses (line B).

Repetitions of this experiment yielded similar resulta (Table III, lines D and F).

Attempts were also made to induce IgE production in nude mice with another antigen, CAE (Table III). The immunization dose was 10 µg CAE plus 10 mg alum. Again, NLM responded on days 10, 20 and 30 with production of IgE antibody, and again, nude mice failed to respond to the antigen even after secondary immunization.

IgE Production in Nude Mice Reconstituted with Thymus Cells. Because nude mice failed to produce IgE in response to EA and alum, their response was checked following reconstitution with 1.4×10^8 thymus cells from normal Balb/c mice. Two months after the thymus cell injections were made, the mice were immunized with 10 µg EA plus 10 mg alum (Table III, line C). Thymus cell-injected nude mice did not respond until after the secondary immunization of 10 µg EA. The titer of the response was not as high as the Balb/c control although there was a response (16) in contrast to the total lack of a response in normal nude mice. This experiment was repeated with similar results (line I).

IgE Production in Nude Mice Reconstituted with Thymus Glands. Nude mice were also implanted subcutaneously with 2 to 4 thymus glands to make them immunologically competent. Seven months later, these mice and Balb/c controls were immunized with 10 μg EA plus 10 mg alum on day 0 and 10 μg EA alone on day 20. The Balb/c mice responded with production of IgE on days 10, 20 and 30 (Table III, line F). The nude mice which had received the thymus glands also responded well on days 10 and 20 (line G). No test was done on day 30. In a repeated experiment nude mice that received thymus glands 6 to 12 months before immunization with 10 μg EA plus 10 mg alum made an IgE response similar to the response of the Balb/c controls. Similar response was observed on days 10 and 20 (Table III, lines M and O).

Thymus-implanted nude mice were also immunized with 10 μg CAE plus 10 mg alum 4 to 6 months after grafting. In this case, they made no response on days 10 and 20 but did make a slight response after secondary immunization. The Balb/c controls responded on all three days (Table III, lines J and L).

IgE Response of Nude Mice to a Thymus Independent Antigen. Nude mice and NLM were immunized with 1 μg Vi mixed with 10 mg alum. Vi is known to be a thymus independent antigen for the IgM response. Attempts were made to induce IgE production in nude mice with this antigen. Unfortunately, Vi does not stimulate IgE production in NLM. It did

not elicit IgE production in nude mice (Table IV).

The Thymus as the Origin of the Cells Responsible for PCA Reactions. Mota (6) has suggested that the cell responsible for the anaphylatic reaction in rats and mice is the mast cell; the combination of antigen and antibody stimulates the mast cell to release the pharmacological mediators of the PCA reaction, histamine and serotonin (6). Therefore, if nude mice were able to produce a PCA reaction the origin of the mast cell could not be the thymus as others have suggested (28, 29).

To test this hypothesis, nude mice and CFW mice were sensitized with serum known to be positive for a PCA reaction. As shown in Table V, nude mice in all cases gave a PCA reaction at least as good as the CFW controls for both 72 hr and 2 hr PCA reactions.

Effects of Immunosuppressive Agents on the IgE Response in Mice. Tada demonstrated that rats showed an enhanced production of IgE when treated with 400 rad X-irradiation (18), splenectomy (16), some immunosuppressive drugs (19) and antithymocyte treatment (20). Attempts were made to show this same enhancement effect in mice. Balb/c mice were injected with 0.3 ml ALS 4 days after immunization with 10 μg EA and 10 mg alum. A decreased titer was observed on days 10, 20, 30 and 40 (Table VI, lines A and B) in animals that were treated with ALS. Similar results were obtained when this was repeated changing only the time of administration of ALS. When ALS was administered at the time of

TABLE IV

IgE response of mice to a thymus independent antigen, Vi

Immunization Day 10	Group	PCA[a] Titer of Serum Pool Day 10	Day 20
1 μg Vi, Al[b]	NLM[c]	negative[d]	negative
same as above[e]	Nu[f]	negative	negative

[a]PCA = passive cutaneous anaphylaxis
[b]Al = 10 mg alum
[c]NLM = normal littermate of nude
[d]negative = not detected at a 1/2 dilution
[e]same as above = denotes the same serum as above
[f]Nu = congenitally athymic nude mouse

TABLE V

Passive cutaneous anaphylaxis response of nude[a] and CFW mice to homocytotropic antibody positive serum

Recipient Mouse	No. of Serum in Pool	Period of Sensitization	PCA[d] Titer of Serum Pool Day 10	Day 30[e]
Nude*	5	2 hours	ND[b]	64[c]
CFW*	5	2 hours	ND	64
Nude*	5	72 hours	16	64
CFW*	5	72 hours	16	32
Nude**	4	72 hours	64	64
CFW**	4	72 hours	64	64

[a]nude = congenitally athymic nude mice

[b]ND = not done

[c]64 = titer reciprocal of serum pool

[d]PCA = passive cutaneous anaphylaxis

* = denotes serum pool #1

** = denotes serum pool #2

TABLE VI

Thymus regulation of the IgE response

Line	Immunization Day 10	Immunization Day 20	Group	No. Serum in Pool	PCA[a] Titer of Serum Pool Day 10	20	30*	40
A	10 μg EA[b], Al[c]	10 μg EA	Balb/c	7	64[d]	128	128	32
B	same[e]	same	ALS[f]	7	4	16	32	32
C	same	same	400 R[g]	7	neg[h]	32	16	4
D	same	same	Spx[i]	7	64	32	64	8
E	10 μg CAE[j], Al	10 μg CAE	NLM[k]	4	128	32	16	
F	same	same	ALS	4	neg	4	neg	
G	10 μg CAE, Al	10 μg CAE	NLM	5	2	neg	neg	
H	same	same	ALS	5	8	4	2	
I	10 μg EA, Al	10 μg EA	NLM	4	64	8	32	
J	same	same	ALS	4	neg	neg	4	

[a]PCA = passive cutaneous anaphylaxis

[b]EA = hen egg albumin

[c]Al = 10 mg alum

[d]64 = titer reciprocal of serum pool

[e]same = same serum as above

[f]ALS = rabbit anti-mouse lymphocyte serum

[g]400 R = 400 rad X-irradiation

[h]neg = not detected at 1/2 dilution

[i]Spx = splenectomy

[j]CAE = crude ascaris extract

[k]NLM = normal littermate to nude

* = denotes serum after second immunization

of immunization with 10 μg EA and 10 mg alum, the IgE response was abolished on days 10 and 20 and barely detectable on day 30 (lines I and J). When ALS was administered at the same time as immunization with 10 μg CAE plus 10 mg alum, a slightly higher titer was observed for one experiment (Table VI, lines G and H) whereas a much lower titer was observed in another experiment (Table VI, line E and F). 400 rad X-irradiation (line C) and splenectomy (line D) also decreased the IgE response. From these data, it can be seen that immunosuppressive agents depress the IgE response instead of enhancing it.

Effect of Infection with Nippostronglus brasiliensis on the IgE Response. Other investigators (26, 27) have demonstrated in rats a potentiated IgE response to several different antigens following nematode infection. An attempt was made to find this potentiated response in mice. Mice were inoculated subcutaneously with 600 larvae of the parasite Nippostrongylus brasiliensis ten days after initial immunization of 10 μg EA plus 10 mg alum. Balb/c mice without injected larvae served as controls. Sera were tested by the PCA method on days 10, 20, 30 and 40 (Table VII). The mice received a secondary immunization on day 20 following collection of serum. Mice that received the 600 larvae showed a slightly higher titer on day 30 (256 vs 128). Because there was only one dilution separating the worm infected from the controls, these data are not very meaningful. One dilution variation can be seen throughout this experiment. It should be noted, how-

TABLE VII

Effects on IgE production of infection with Nippostrongylus brasiliensis ten days after initial immunization with 10 µg egg albumin and 10 mg alum

Group	No. of Serum in Pool	72-hr PCA[a] Titer of Serum Pool			
		Day 10	Day 20	Day 30*	Day 40
Balb/c	7	64[b]	128	128	64
Balb/c with worm infection[c]	7	64	64	256	32

[a]PCA = passive cutaneous anaphylaxis

[b]64 = titer reciprocal of serum pool

[c]worm infection = 600 larvae of the worm Nippostrongylus brasiliensis were injected subcutaneous

* = mice received a second immunization on day 20 of 10 µg egg albumin

ever, that the PCA reactions were of a deeper blue in the worm infected mice.

DISCUSSION

Because the mouse produces two different homocytotropic antibodies, it was necessary to make certain that the antibody with which I was working was IgE and not IgG_1. IgG_1 is present in the skin of sensitized animals two hours after sensitization and is heat resistant for 30 minutes at 56°C. IgE, on the other hand, is present in the skin for longer periods of time, beyond 72 hours after sensitization, and is heat labile at 56°C for 30 minutes. To make sure the IgG_1 was not persisting in the skin for 72 hours and thus giving false positive reactions, the undiluted serum was heated at 56°C for 30 minutes, and recipient animals were challenged with antigen and Evans Blue dye 72 hours after sensitization. No reaction was observed at this time, which indicated that at 72 hours only IgE was present. IgE was only detected if the serum was not heated. From these observations, I knew the antibody being observed was IgE.

Several different antigens were used to elicit an IgE response. Although others have been able to obtain a response using PE as an adjuvant (25, 8, 9), I was not. Oftentimes with this adjuvant, there was bluing of the entire skin, which probably was an indication of an IgG_1 response. The best immunizing agent tried was 10 ug EA mixed with 10 mg alum. CAE was not a reliable antigen. Sometimes CAE would elicit a good response, but at other times it would not. DNP-CAE was an excellent immunogen. It was also mixed with alum prior to immunization.

Other investigators have implied that the IgE response is thymus dependent (17, 21). The data I have collected provides good verification that the IgE response is thymus dependent. Tada et al (21) found that neonatal thymectomy results in a decreased IgE response. It may not completely eliminate the IgE response because with neonatal thymectomy there is a possibility of leaving some thymus cells in the animal, either due to failure to remove all of the gland or to seeding of T-cells to other organs before birth. For these reasons, the nude mouse offers an ideal system in which to observe thymus dependency because this animal lacks thymus cells from birth. Nude mice immunized with 10 μg EA and 10 mg alum failed to produce any detectable IgE, whereas, NLM produced IgE in high quantities. Nude mice also failed to produce any IgE when immunized with 10 μg CAE mixed with 10 mg alum. The failure of the nude mouse to produce IgE provides a good indication of the thymus dependency of the IgE response. To say that the reason the nude mouse does not produce IgE is that it has no thymus, it is necessary to show that lack of a thymus is the only reason the nude mouse failed to produce IgE. To establish this, thymus glands were implanted in nude mice before they were immunized with 10 μg EA and 10 mg alum. If the nudes were first implanted with a thymus from a NLM or Balb/c mouse, they were able to produce IgE; without the thymus grafts from normal animals, they could not produce any IgE. Thymus cell injections also restored the response to some nudes. Perhaps if the

mice were to have more time between thymus cell injection and immunization, they would all have responded with production of IgE. From these data, one may conclude that the IgE response is thymus dependent.

I attempted to induce IgE production in nude mice with Vi, an antigen known to be thymus independent for the IgM response. Neither nude mice nor their NLM responded with production of IgE to this antigen. It can be concluded that Vi does not induce detectable levels of IgE in nude or normal mice.

Although nude mice were unable to produce IgE, the possibility that they also lack cells capable of eliciting a PCA reaction was checked. Mota (6) has called these cells "mast cells." They are the cells which combine with IgE antibody and, in the presence of the specific antigen, release the pharmacological mediators of the PCA reaction, which is primarily serotonin in the mouse. Both nude mice and CFW controls were sensitized with serum collected from animals known to be positive for a PCA reaction. Because the nude mice reacted as well as the CFW controls, it was concluded that they do have the cells necessary for PCA reactions. Inasmuch as nude mice lack a functional thymus, it was also concluded that the origin of such a cell is not the thymus and it is not a thymus-dependent cell type.

Several investigators have shown that agents which are immunosuppressive for other types of antibody have an enhancing effect on IgE

production. Tada and co-workers found this to be true in rats (16, 18, 19, 20). White and Holm (25) found enhancement of IgE in rats given ALS. Kind and Macedo-Sobrinho (11) showed similar results when mice were treated with 450 rad X-irradiation. In my experiment, Balb/c mice were given ALS or X-irradiated. Neither of these showed an increase in production of IgE. Splenectomy of Balb/c mice also showed no enhancing effect. In most cases, immunosuppressive agents caused a decrease in IgE production. The immunosuppressive agents I used did not demonstrate that the thymus has a negative regulatory effect on IgE production. It should be noted that these experiments were done with Balb/c mice, and another strain of mice may show different results. Balb/c mice were selected for these studies because they make good IgE responses; perhaps they make good responses because they lack suppressor T-cells. If this is the case, one would not expect immunosuppressive treatments to enhance IgE production in this strain. Such treatment might, however, enhance IgE production in strains which normally make poor IgE responses.

A potentiated IgE response has been observed in rats following nematode infection (26, 27). In my study, Balb/c mice were inoculated subcutaneously with 600 larvae of the nematode Nippostrongylus brasiliensis ten days after immunization with 10 μg EA and 10 mg alum. No meaningful potentiated response was observed in the mice that received the worm infection. The PCA reactions, however, were a much

deeper blue than in the control animals. The titers between the worm infected and the controls were not meaningful. More experimentation on this subject is needed.

SUMMARY

The regulatory effects of the thymus gland on the production of reaginic immunoglobulin E (IgE) were studied using congenitally athymic (nude) mice, phenotypically normal littermates (NLM) of nude mice and Balb/c mice. Antigen specific IgE was quantitated in CFW mice by the passive cutaneous anaphylaxis (PCA) assay. Alum, complete Freunds adjuvant and saline extract of Bordetella pertussis were used as adjuvants. Nude mice failed to produce specific IgE following both primary and secondary immunization with hen egg albumin (EA), crude ascaris extract and crude ascaris extract coupled to dinitrophenyl groups. In contrast, NLM and Balb/c mice made strong IgE responses. Nude mice implanted with thymus glands from neonatal Balb/c or NLM donors made IgE responses equal to the responses of NLM mice. These data indicate that there is an absolute requirement for thymus-derived cells in the formation by mice of specific IgE.

Nude mice were passively sensitized intradermally with an IgE-positive serum; when challenged intravenously with specific antigen, these nude mice demonstrated strong PCA reactions. It is clear, therefore, that although nude mice cannot make specific IgE, they do have the mechanism necessary to elicit PCA reactions.

Attempts were made to show a negative regulatory effect of the thymus on IgE production by using splenectomized, ^{60}Co-irradiated or antilymphocyte serum-treated mice. No enhancement of IgE production

was observed in these experiments. In contrast to results obtained by others using rats, infection of immunized mice with the nematode *Nippostrongylus brasiliensis* did not potentiate specific IgE production.

BIBLIOGRAPHY

1. Ishizaka, K.,and T. Ishizaka. 1970. The significance of immunoglobulin E in reaginic hypersensitivity. Ann. Allergy 28: 189-202.

2. Stanworth, D. R. 1971. Immunoglobulin E (reagin) and allergy. Nature. 1971. 233:310-316.

3. Mota, I., and A. Perini. 1970. Susceptible homocytotropic antibody in the guinea pig. Life Sci. 9:923-930.

4. Mota, I. 1961. Mast cell lytic antibody. Nature. 192:4808-4814.

5. Mota, I., D. Wong and E. H. Sadun. 1968. Mouse Homocytotropic antibodies. I. Specific differentiation between mouse $7s_1$ and reagin-like antibodies. Life Sci Pt. 2. 7:1289-1293.

6. Mota, I. 1964. The mechanism of anaphylaxis. I. Production and biological properties of 'mast cell sensitizing' antibody. Immunology 7:681-699.

7. Reveltella, R.,and Z. Ovary. 1969. Reaginic antibody in different mouse strains. Immunology 17:45-54.

8. Clausen, C. R., J. Munoz and R. K. Bergman. 1970. A reaginic type of antibody stimulated by extracts of Bordetella pertussis in inbred strains of mice. J. Immunol. 104:312-319.

9. Clausen, C. R., J. Munoz and R. K. Bergman. 1969. Reaginic-type antibody in mice stimulated by extracts of Bordetella pertussis. J. Immunol. 103:768-777.

10. Bartholomaeus, W. N.,and D. Keast. 1972. Reaginic antibody to tumour and alloantigens in mice. Nature-New Bio. 1239:206-207.

11. Kind, L. S.,and B. Macedo-Sobrinho. 1973. Reaginic antibody formation in mice injected with rabbit anti-mouse thymocyte serum. Int. Arch. Allergy Appl. Immunol. 45:780-788.

12. Stanworth, D. R. 1973. Immediate hypersensitivity, the molecular basis of the allergic response. p. 170. North-Holland Publishing Company, Amsterdam and London.

13. Perini, A., and I. Mota. 1973. The production of IgE and IgG antibodies in guinea-pigs immunized with antigen and bacterial lipopolysaccharides. Immunology 25:297-305.

14. Katz, D. H., T. Hamzoka and B. Benacerraf. 1973. Induction of immunological tolerance in bone marrow-derived lypmhocytes of the IgE antibody class. Proc. Nat. Acad. Sci. 70:2776-2780.

15. Sauerbronn Maia, L. C., N. M. Vas and E. M. Vas. 1974. Effects of soluble antigen on IgE responses in the mouse. Int. Arch. Allergy. 46:339-344.

16. Okumura, K.,and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. J. Immunol. 106:1019-1025.

17. Michael, J. G.,and I. L. Bernstein. 1973. Thymus dependence of reaginic antibody formation in mice. J. Immunol. III:1600-1601.

18. Tada, T., M. Taniguchi and K. Okumura. 1971. Regulation of homocytotrepic antibody formation in the rat. II. Effect of X-irradiation. J. Immunol. 106:1012-1018.

19. Taniguchi, M., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. IV. Effect of various immunosuppressive drugs. J. Immunol. 107:579-585.

20. Okumura, K., and T. Tada. 1974. Effect of antithymocyte serum on reaginic antibody formation in the rat. Immunology 26:257-268.

21. Tada, T., and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. I. Feed-back regulation by passively administered antibody. J. Immunol. 106:1002-1011.

22. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. VI. Inhibitory effect of thymocytes on the homocytotropic antibody response. J. Immunol. 107:1682-1689.

23. Tada, T., and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. V. Cell cooperation in the antihapten homocytotropic antibody response. J. Immunol. 107:1137-1145.

24. Okumura, K., and T. Tada. 1974. Regulation of homocytotropic antibody formation in the rat. IX. Further characterization of the antigen-specific inhibitory T cell factor in hapten-specific homocytotropic antibody response. J. Immunol. 112:783-791.

25. White, G. J., and M. S. Holm. 1973. Induction of reagin synthesis in the rat with associated and dissociated hemocyanin: Effect of antilymphocyte serum. J. Immunol. 110:327-334.

26. Bloch, K. J., J. L. Ohman, J. Waltinand, R. W. Cygan. 1973. Potentiated reagin response: initiation with minute doses of antigen and alum followed by infection with Nippostrongylus brasiliensis. J. Immunol. 110:197-204.

27. Jarrett, E. E., D. Henderson, P. Riley and R. G. White. 1972. The effect of various adjuvant regimens and of nematode infection on the reaginic antibody response to egg albumin in the rat. Int. Arch. Allergy. 42:775-781.

28. Csaba, G., L. Surjan, J. Fischer, J. Kiss and I. Toro. 1969. On the mechanism of mast cell formation. Acta biol. Acad. Sci. hung. 20:57-63.

29. Ginsberg, H. 1963. The in vitro differentiation and culture of normal mast cells from mouse thymus. Ann. N. Y. Acad. Sci. 103:20-25.

30. Viklicky, V., P. Sima and H. Pritchard. 1973. On the origin of mast cells in adult life. Folia. Biol. Prague. 19:247-251.

31. Rygaard, J. 1973. Thymus and self: Immunobiology of the mouse mutant nude. F. A. D. L., Copenhagen, Denmark.

32. Billingham, R. E., and W. K. Silvers. 1971. The immunobiology of transplantation. Prentice-Hall, Inc., Englewood Cliffs, N. J.

33. Landy, M., R. J. Trapani, M. E. Webster and F. G. Jarvis. 1963. Immunological properties of Vi antigen isolated by chemical fractionation and by electrophoresis. Tex. Rep. Biol. Med. 21:214-229.

34. Westphal, O., O. Luderitz and F. Bister. 1952. Uber dee extraktion von bacterien mit Phenol/Wasser. Z. naturforsch 7:148-155.

35. Westphal, O., and A. C. Todd. 1966. Adaptation of Nippostrongylus brasiliensis to the mouse. J. Parasito. 52:233-236.

36. Pilgrim, I., and K. DeOme. 1955. Intraperitoneal pentobarbitol anesthesia in mice. Exp. Med. Surg. 13:401-403.

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