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IgE suppression by maternal IgG

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Summary. Rats born of egg albumin immunized mothers have a diminished capacity to produce IgE antibody to egg albumin persisting for at least 13–14 weeks after birth. At the same time the primary IgG response to the antigen is usually enhanced. Previous studies indicated that these effects were mediated by factors transferred in maternal milk.

The phenomenon can be duplicated by the administration of small quantities of immune serum to rats during the first 3 weeks of life. The active component of immune serum is shown to be specific antibody.

Suckling rats acquire egg-albumin-specific IgG from the immune mother via the milk. Their serum level approaches that of the mother by 20 days but declines rapidly after weaning to become undetectable by 6–8 weeks. As maternal influence on the immune responsiveness of the offspring persists for several weeks beyond this time, it is unlikely that the mechanism involves a simple blocking by circulating antibody of the access of antigen to cellular receptors. Alternative mechanisms are briefly discussed.

Attention is drawn to the possibility that the suppression of IgE antibody responsiveness by maternal IgG may represent a physiological regulatory process with a role in inhibiting the development of infantile allergies.

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INTRODUCTION

The immunological response of young mammals is influenced in a number of ways by maternal immunity. The most documented effects are those arising from the transfer of maternal antibody (Brambell 1970; Solomon 1971; Weiler, 1981) but evidence for sensitization by transferred maternal lymphocytes or their soluble products is also substantial (Cramer, Kunz & Gill, 1974; Beer, Billingham, Head & Parmley, 1977).

In investigating the influence of the mother on IgE antibody responsiveness of young rats we observed that maternal immunization could produce distinct effects on antibody in different immunoglobulin classes: IgE responses to egg albumin (EA) were markedly diminished in rats whose mothers had been immunized with the antigen while the primary IgG response was augmented (Jarrett & Hall, 1979). Foster nursing experiments showed that this altered immune responsiveness was mediated by factors transferred in maternal milk. Circumstantial evidence indicated that transferred antigen was not the cause. The object of the experiments described here was to examine the possibility that antibody in the milk was responsible for these effects.

MATERIALS AND METHODS

Rats

The breeding rats were random-bred Hooded Lister (Animal Suppliers, Welwyn, Herts and latterly Olac Ltd, Bicester).

Immunization and blood sampling
Immunization, by i.p. injection of EA (Grade V, Sigma Chemical Co., St. Louis, Mo.) with 10^{10} heat-killed *Bordetella pertussis* (Bp) organisms (Department of Microbiology, University of Glasgow), was performed for three purposes. (i) To immunize prospective mothers, female rats aged around 3 months received 1 mg of EA and Bp one month before mating. (ii) To prepare immune serum, rats were immunized with 1 mg of EA and Bp and were exsanguinated either 1 month later or 12 days after a challenge at 1 month with 1 μg of EA. For purposes unrelated to the experiments described here IgE was removed by passing immune serum through an anti-rat Fcε immunosorbent column. Serum was stored at $-20°$. (iii) To test for the effect on the immune response of maternal immunization or of neonatal injections of immune serum or fractions thereof, rats were immunized, unless otherwise stated, with 10 μg of EA and Bp and challenged 1 month later with 1 μg of EA. Previous experience has shown that the primary IgE response of Hooded Lister rats immunized in this way develops within 10–12 days and thereafter maintains a plateau lasting for at least 60 days. The booster IgE response is maximal 4 days after challenge and then declines. The total antigen-binding capacity (ABC) continues to rise to reach a maximum level around 16 days after primary immunization and 12 days after challenge (Jarrett, 1978, Jarrett & Hall, 1981). As a standard procedure to monitor these responses, rats were bled (from the tail or finally by cardiac puncture) on day 0 and between days 18 and 21 after primary immunization and on day 4 and also often on day 12 after challenge.

Measurement of antibodies
During the course of the experiments described here, we changed from a passive cutaneous anaphylaxis test (PCA; Ovary 1964) to a paper radioallergosorbent test (PRAST, Karlsson, Ellerson, Haig, Jarrett & Bennich, 1979) for the measurement of rat IgE antibody. The techniques were shown to give comparable results. The PCA tests were performed by the i.d. innoculation of 0·1 ml of dilutions of test serum in saline into the shaved back of duplicate recipient rats. After 48 hr, the animals were injected i.v. with 2·5 mg of EA and 0·5 ml of 1% Evan's blue. The PCA titre was the highest dilution giving a clearly visible skin reaction. The PRAST test was performed as previously described. Briefly, EA was conjugated to cyanogen bromide-activated filter paper discs (Whatman No. 1, Whatman Lab. Sales Ltd, Maidstone) which were incubated first with test serum and subsequently with ^{125}I-labelled purified rabbit anti-rat Fcε. The uptake of labelled antibody was compared with the uptake in a reference system using an antigen-positive serum with a known PRAST score, calculated as described by Karlsson *et al.* (1979) and expressed as units/ml. The mean PRAST units and standard error of the means (SE) shown in the Figures were calculated from the $\log_{10}$ values. Samples without detectable antibody were assigned a value of 1.

Total antigen-binding capacity (ABC) was measured by the Farr technique (Farr, 1958) as modified by Mitchison (1964). Iodination of EA was carried out with the ^{125}I-labelled Bolton and Hunter reagent (Bolton & Hunter, 1973; Radiochemical Centre, Amersham) using 5 mg of EA and 1 of mCi (=37 MBq) of the labelled reagent to give a specific activity of 6–12 × 10^4 c.p.m./μg. Labelled EA was added to dilutions of test and normal control serum to give a final concentration of 1 μg EA/ml. After incubation overnight, precipitates at 50% ammonium sulphate saturation were counted together with tubes containing the total amount of ^{125}I-label added. ABC was calculated as described by Mitchison (1971) and expressed as micrograms of antigen bound per millilitre of undiluted serum. The mean ABC and SE were calculated as described for the PRAST values. The lower limit of sensitivity of the test was an ABC of 0·50 μg/ml. Comparison of antibody levels in assays for ABC and an IgG PRAST (Karlsson *et al.*, 1979) has indicated that the greater part of the binding capacity for antigen in the serum of rats immunized as above is attributable to IgG antibody.

Estimation of immunoglobulins
Estimation of the level of immunoglobulins in serum fractions was by the single radial immunodiffusion method of Mancini, Carbonara & Heremans (1965) using antisera monospecific to each Ig class (antisera to IgA and IgM were a kind gift from H. Bazin, antiserum to IgG was from Miles Laboratories Ltd, Slough). Normal rat serum with known Ig concentrations (titrated against purified Igs of each class) was used as standard.

Preparation of immunosorbent-purified EA-antibodies
EA was bound, according to the manufacturers instructions, to CNBr-activated Sepharose 4B (Pharmacia U.K. Ltd, London). Immune serum was dialysed against Tris (0·1 M pH 8·0) and passed through a

column of immunosorbent. After extensive washing of the column, EA-antibodies were eluted with glycine HCl (0·1 M pH 3·0) and dialysed against Tris as above. The efficacy of immunosorption was determined by assay of the starting and antibody-depleted serum and of the eluted antibodies for ABC.

Statistical methods

The Mann-Whitney U test was used to compare antibody levels of one group with another as assumptions necessary for use of the parametric test could not be validated. Tables of critical values of U for determination of probability were from Siegal (1956).

RESULTS

IgE suppression transferred to neonates by immune serum

In order to find if an IgE suppressive factor, similar to that normally transferred in maternal milk, was present in maternal serum, immunized mothers were exsanguinated on the day that their litters were weaned and their serum was administered to normal rat pups. Each rat in three litters received 0·1 ml of serum i.p. three times per week for 3 weeks after birth. Animals in control litters (two–three litters per group) were untreated or received normal rat serum or saline. The young rats, weaned at 21 days, were immunized with EA at 6–7 weeks of age and were challenged 1 month later.

The results in Fig. 1 showed that both primary and secondary IgE responses of rats which had been treated with immune serum were significantly lower than those of untreated rats ($P<0{\cdot}05$ and $<0{\cdot}002$, respectively) and the ABC primary response significantly higher ($P<0{\cdot}002$). By contrast the responses of rats which had been treated with either normal serum or saline were similar to those of untreated rats. There was no significant difference between the responses of male and female rats.

Transfer with serum fractions

At first it was not assumed that the active constituent of immune serum was necessarily antibody; the possibility that factors derived from antigen-specific suppressor T cells might be involved was also considered. To estimate the molecular size of the IgE suppressive factor, immune serum was fractionated, first by precipitation at 50% saturated ammonium sulphate and then by gel filtration of the immunoglobulin

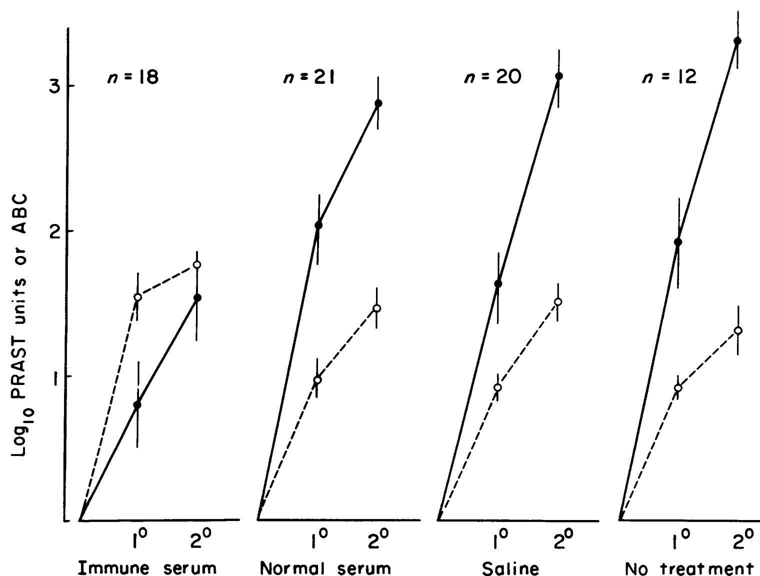

Figure 1. The effect of administration of immune serum during the suckling period on the IgE and total antibody responsiveness of young rats. The rats were immunized at 6–7 weeks of age and were challenged 1 month later. The points shown are the mean antibody levels 2 days before immunization, 19 days after immunization (1°) and 4 days after challenge (2°). Log_{10} PRAST units (●—●) or ABC (○—○).

fraction to yield preparations A–D as depicted in Fig. 2. Aliquots of 0·1 ml of either the immunoglobulin fraction, the $(NH_4)_2SO_4$ supernatant or of fractions A, B or C, all reconstituted to original serum volume, were administered to rat pups as in the previous experiment. The ABC and immunoglobulin content of the administered preparations is detailed in Table 1.

Only the rats treated with the crude immunoglobulin fraction had a significantly reduced primary IgE response. However, after challenge the IgE booster response of the rats treated with fraction C was also markedly suppressed (Table 1). Animals treated with fractions A and B showed a lesser reduction in the IgE booster response while the response of the animals treated with the $(NH_4)_2SO_4$ supernatant was similar to that of the untreated control rats. The results of this experiment suggested that the major immunosuppressive component of immune serum was contained in the IgG fraction which also had the greatest ABC.

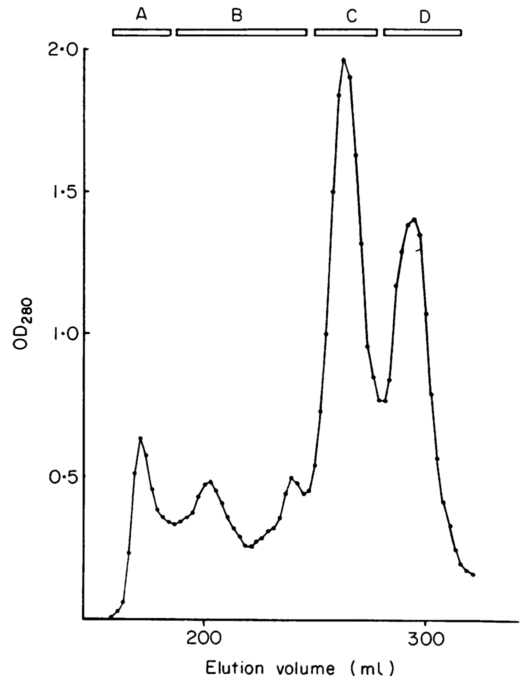

Figure 2. Gel filtration fractions of immune rat immunoglobulin administered to newborn rats. The crude immunoglobulin fraction of serum was separated in aliquots of 2 ml on Sephacryl S-300 (Pharmacia Ltd); gel column, 95 × 2·6 cm; Tris/HCl buffer (0·1 M pH 8·0 containing 0·5 M NaCl); flow rate, 10·3 ml/hr.

Transfer with immunosorbent purified antibody

To confirm that specific antibody caused suppression of IgE responsiveness, three groups of rat pups (two–three litters/group) were treated by injection of either 0·1 ml immune serum (ABC, 72·3), 0·1 ml EA-antibody-depleted serum (ABC, 0) or 0·1 ml immunosorbent-purified EA antibody (ABC, 33·6) three times a week for the first 3 weeks. Some animals in each litter were left untreated and these were used to form the control group. The rats were immunized at 6–7 weeks and challenged at 11–12 weeks. At the time of immunization low levels of antibody could still be detected in the rats which had received immune serum (ABC range 0·7 to 1·65).

The EA-IgE and the total antibody response is shown in Fig. 3. The primary EA-IgE response was significantly suppressed only in the immune serum group ($P < 0{\cdot}002$). However the booster IgE response was markedly suppressed in both immune serum and purified EA-antibody groups ($P < 0{\cdot}002$). The mean primary ABC response of these same groups was also significantly higher than that of the control group ($P < 0{\cdot}02$).

Passage of EA-antibody from immune mothers to offspring

The above experiments showed that the administration of specific antibody to rat pups could cause the type of immunological effects which we had observed in the offspring of immune mothers. Could the two phenomena be equated? To determine the amount and persistence of EA-antibody acquired from immune mothers, rat pups were exsanguinated on the occasions shown in Fig. 4 and the ABC of their serum compared with that of their mothers. The results showed that the young rats acquired most of their antibody post-natally and that the serum level increased steadily over the suckling period to reach that of the mother by the time of weaning at 3 weeks after birth. Thereafter the ABC fell rapidly to reach low levels by 5 weeks. This experiment established a broad comparability between the antibody status of rats born of immune mothers and those injected with immune serum as described above.

Duration of suppression in progeny

In previous experiments, to test for maternal effects on the immune response, we had made a practice of immunizing rats at between 5 and 8 weeks of age.

Table 1. Details of immune serum fractions administered during the suckling period and their effect on the subsequent IgE antibody response

	Immunoglobulin ($(NH_4)_2SO_4$ precipitate)	$(NH_4)_2SO_4$ supernatant	Immunoglobulin fractions† A	B	C	No treatment
ABC	92·2	0·07	1·16	3·42	55·6	—
IgG (mg/ml)	10·24	0·06	0·14	0·25	7·52	—
IgA (mg/ml)	0·45	0·02	0·02	0·27	0·02	—
IgM (mg/ml)	0·36	0·00	0·20	0·02	0·00	—
log_2 PCA titre	2·25	8·7	6·11	5·13	2·90	8·08
(Mean ± SE)	±0·49	±0·15	±0·68	±0·88	±0·55	±0·56
n*	8	11	8	8	10	14
P	0·002	0·1	0·05	0·02	0·002	—

* There were two–three litters/group: each litter was reduced to eight animal and four were injected i.p. with the above preparations (0·1 ml 3x/week for 3 weeks). Surviving treated rats were immunized at 6–7 weeks and challenged at 11–12 weeks together with a control group drawn from the untreated animals. The PCA titres shown above occurred 4 days after challenge.

† See Fig. 2.

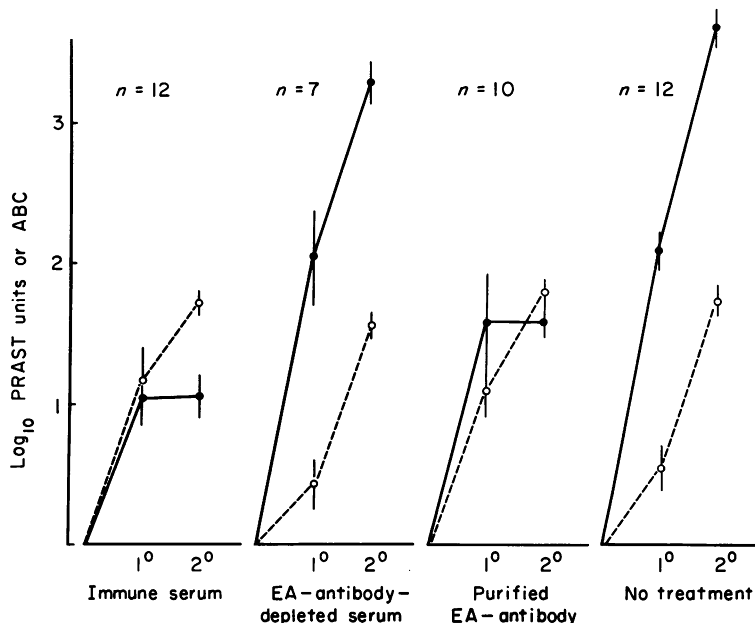

Figure 3. The effect of administration to neonatal rats of immunosorbent purified EA antibody. The rats were immunized at 6–7 weeks and challenged 1 month later. The points shown are the mean antibody levels on the day of immunization, 21 days after immunization (1°) and 4 days after challenge (2°).

Maternal antibody was still present at a low level in rats aged 5–6 weeks but was usually no longer detectable by 7–8 weeks. To find how long the maternal influence persisted after the disappearance of detectable antibody, the progeny of immune (experimental) and normal (control) mothers were immunized at either 6–7, 9–10, 13–14 or 17–18 weeks of age. In each case the rats were challenged 1 month later.

The results are shown in Fig. 5. In comparison with the control group, both primary and secondary IgE responses were significantly suppressed in the experi-

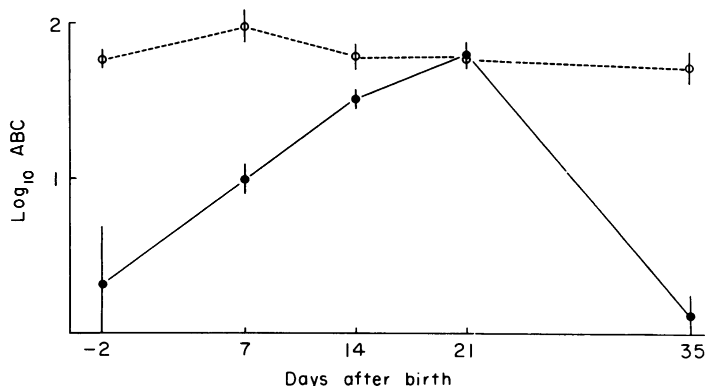

Figure 4. The rise and fall of serum EA antibody acquired passively by young rats from immunized mothers. Each of the points for maternal antibody levels (O- - -O) represent the mean of three mothers. The day −2 and day 7 points from the offspring (●——●) represent the means of pools of serum from the rats of three litters. The remaining points depict the mean of individual values from the rats of three litters.

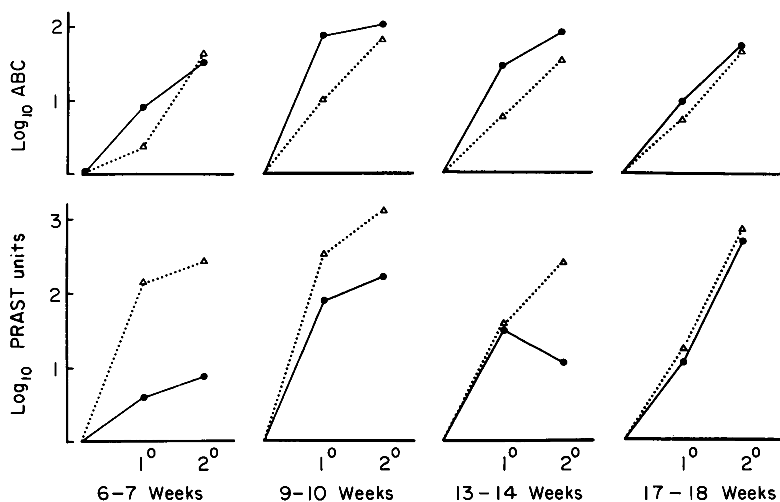

Figure 5. Duration of the altered immune responsiveness of rats born of immune mothers. The experiment involved the offspring of seven immunized (●——●) and six control untreated (Δ· · ·Δ) mothers which were born within 1 week. The litters were pooled into two groups at weaning and rats were withdrawn from the pools at random to be immunized at the above ages. They were challenged 1 month later. The mean antibody values depicted occurred on days 0 and 17 (1°) after immunization and day 4 (PRAST) or day 12 (ABC) after challenge (2°). n = Eleven–nineteen animals/group.

mental rats which received their first immunization at 6–7 or 9–10 weeks; in rats first immunized at 13–14 weeks the booster IgE response alone was suppressed; finally in the rats immunized at 17–18 weeks the IgE responses of experimental and control groups were similar. The primary total antibody response of the experimental rats was significantly elevated in the first three age groups. Residual maternal antibody could be detected only in the 6–7 week old rats (ABC range 0 to 1·3).

Effect of immunizing the offspring with a large dose of antigen

The previous experiment showed that the maternal influence on the immune response extended for several

Table 2. Effect of increasing the dose of antigen on the antibody response of the progeny of immune mothers

	Rats born to immune mothers*		Rats born to normal mothers*	
	Immunizing dose		Immunizing dose	
	10 μg	1 mg	10 μg	1 mg
Mean PRAST (u./ml)				
Primary response	0	0	79	37
Secondary response	0	0	2733	415
Mean ABC				
Primary response	42	51	21	97
Secondary response	113	87	222	268

* The experiment involved two litters of immune mothers and two of normal control mothers. At 6–7 weeks half the rats in each litter were immunized with 10 μg of EA and Bp and the other half with 1 mg of EA and Bp. All the rats were challenged with 1 μg of EA 1 month later. The geometric mean antibody levels shown above occurred 20 days after immunization and 4 days after challenge.
n = eight–nine animals/group.

weeks beyond the time that maternal antibody was detectable in the offspring. This result suggested that the mechanism involved might be other than a simple blocking, by maternal antibody, of the binding of antigen to specific receptors.

In previous experiments of this series, we had immunized the young rats with 1–10 μg EA, this being an established dose range to induce a maximal IgE response in normal rats (Jarrett, 1978). If the suppressed IgE response in the offspring of immunized mothers were the result of a complete masking of antigenic determinants by residual maternal antibody then immunization with a larger dose of antigen, by overcoming this effect, might be expected to elicit a normal response. To test this possibility rats born of immune or untreated mothers were immunized with either 10 μg or 1 mg of EA at 6–7 weeks. The results (Table 2) are particularly striking since there was a total suppression of both primary and secondary IgE responses to either dose of antigen in the offspring of immunized mothers while a substantial response occurred in the control rats. [The IgE booster response of the control rats initially immunized with 1 mg of EA was significantly lower ($P < 0{\cdot}002$) than that of the rats immunized with 10 μg of EA—a phenomenon reflecting the activation of a regulatory mechanism by the larger dose of antigen (Jarrett & Stewart, 1974; Jarrett & Hall, 1981)]. In this experiment the primary total antibody response of the experimental and control rats did not differ significantly.

DISCUSSION

The experiments described here show that a relatively long lasting suppression of specific IgE antibody responsiveness can be obtained by the administration of small amounts of specific IgG to rats during the first weeks of life. Usually but not invariably this also results in an enhanced primary IgG response. The phenomenon is in every way the counterpart of that observed in the offspring of immunized mothers suggesting that the mechanisms underlying the two are the same.

The young rat acquires the greater part of its complement of maternal antibody during the first 20 days of life by way of the maternal milk (Brambell, 1970; Halliday, 1955). The antibody content of the milk reflects the immune status of the mother (Newby, Stokes & Bourne, 1982) and while other immunoglobulin classes are represented, only IgG antibody is absorbed in an immunologically intact form by the suckling rat (Brown, 1978). The offspring of mothers immunized with EA acquire, by 20 days, a level of EA-specific antibody equal to that of their mother. However after weaning antibody falls to a low level by

5–6 weeks and becomes undetectable by 7–8 weeks after birth. The rapidity of the decline is consistent with estimations of the half-life of circulating IgG in the rat, ranging from 5·2 days for antibody absorbed from the milk (Bangham & Terry, 1957) to as little as 63 hr for polyclonal IgG in the adult rat (Peppard & Orlans, 1980).

The pattern of IgE suppression observed in the young rat appears to be a function of the amount of antibody remaining at the time of immunization. Typically both primary and secondary IgE responses are suppressed in rats first immunized at 5–6 weeks at which time maternal antibody can just still be detected, or in normal rats of the same age injected during the suckling period with immune serum of high ABC. On the other hand, frequently the booster IgE response alone is suppressed in the older offspring of immune mothers or in rats injected with fractions of immune serum having a lesser ABC.

As the altered immune responsiveness persists for several weeks after maternally-derived antibody has disappeared from the circulation, a simple explanation for the failure of the IgE response based on a complete masking of antigenic determinants by antibody is not possible. This conclusion is reinforced by the finding that a substantial increase in the immunizing dose of antigen fails to improve the IgE response. Masking of antigen could not in any case explain the augmented IgG response.

Others have observed that even minute traces of maternal antibody can depress certain immune responses such as the plaque forming response to sheep or mouse red cells in rats (Solomon, Riddell & White, 1972) or the response to measles vaccine in human babies (Albrecht, Ennis, Saltzman & Krugman, 1977). Similarly in adult animals certain immune responses can be modulated by the administration of very small amounts of antibody (reviewed by Sinclair, 1979) indicating that masking of antigenic determinants is not always the most important of the mechanisms now known to mediate the regulatory effects of antibody (Sinclair, 1979; Taylor 1982).

The question then to be asked is what other mechanisms may underly the phenomenon described here. Other possibilities which we are currently investigating are that the immune response is modulated (i) by complexes of antigen formed with very small or undetectable amounts of maternal antibody; (ii) by the binding of antigen to antibody bound to cell surface receptors; or (iii) by an alteration in the immune repertoire of the animal through the production of an anti-idiotypic response to maternal antibody. The latter mechanism is known to modulate the antibody response of mice to certain chemically defined antigens (Weiler, 1981) but is rather difficult to place in the context of the response to a multideterminant antigen showing separate effects on different immunoglobulin classes.

We are not yet sure if the enhanced IgG and the suppressed IgE responses are directly related. The effect on the IgG response is less dramatic and less consistent and our observation that IgE responses are suppressed in the occasional absence of an augmented IgG response argues against a cause and effect relationship between the two.

Adult rats and mice of most strains have a highly developed capacity to suppress the production of experimentally induced IgE responses by a multiplicity of immunoregulatory mechanisms (Jarrett, 1977; Katz, 1980). In adult Hooded Lister rats in particular, booster IgE responses are progressively diminished by the operation of an IgE-selective suppressive mechanism which is activated by exposure to minute quantities of antigen (Jarrett & Hall, 1981). The present results paint a picture of an even more comprehensive regulation of IgE responsiveness as the maternal influence merges with the endogenously developing immunoregulatory mechanisms in the maturing animal.

Finally, because of the possible practical implications for the management of IgE-mediated disease it is now a matter of importance to find if IgE suppression by maternal IgG also occurs in other species and whether this may represent a physiological regulatory process. Evidence suggesting that it may occur in human babies is contained in the work of Dannaeus and colleagues (Dannaeus, Johansson & Foucard, 1978; Dannaeus & Inganas, 1981) who found that IgG antibodies to milk and egg proteins in the serum of newborn babies reflected the corresponding maternal concentration and that high levels in cord sera appeared to protect against the onset of atopic symptoms during the first 2 years of life. The rate of catabolism of passively acquired maternal antibodies is very much slower in humans than it is in the rat (Brambell, 1970; Albrecht *et al.*, 1977) and therefore any IgE suppressive effects of human maternal antibody might be expected to last correspondingly longer.

It is an attractive idea that maternal IgG may have a role in protecting the normal infant from allergic disease while its own capacity to regulate IgE produc-

tion is developing. A separate question is whether a failure of this process may play a part in the development of allergy in atopic infants. In fact, atopic mothers appear to transfer larger amounts of (milk and egg specific) IgG to their offspring than do normal mothers (Dannaeus *et al.*,1978). To explain a failure of IgG protective effect one would therefore have to postulate either a defectiveness in the quality of the transferred antibody or an inability of the infant to capitalize on this form of regulatory mechanism. A more likely explanation is that the suppressive effect of IgG can simply be overwhelmed by an innately high IgE reactivity in the atopic infant. These problems should be addressed by further clinical studies conducted in the light of the experimental findings.

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