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Induction of an auto-anti-IgE response in rats

III. INHIBITION OF A SPECIFIC IgE RESPONSE

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SUMMARY

An auto-anti-IgE response was induced in conventional (PVG-RT1^U and high IgE-producing (BN) rat strains by immunization with a highly purified rat IgE myeloma IR2. Earlier work established that total serum IgE levels were decreased by this procedure (Marshall & Bell, 1985) but only in the PVG-RT1^U strain. IR2-immunized rats were tested for their ability to produce a specific IgE response to ovalbumin (OVA). The primary anti-OVA IgE response was inhibited by 60–75% in both rat strains, regardless of whether the total serum levels of IgE were reduced. The secondary IgE response to OVA was also inhibited in anti-IgE-producing animals but not in rats primed with OVA before anti-IgE induction. The inhibition of the anti-OVA response was isotype specific; the IgG response to OVA was unaffected. These studies may help elucidate the regulatory role played by naturally occurring anti-IgE antibodies found particularly in atopic individuals.

INTRODUCTION

A variety of potential methods of modulating IgE responses aimed at improving the treatment of allergic disease have been investigated in the past. Amongst these are experimental systems involving the use of heterologous anti-isotype antibodies such as anti-μ (Manning & Jutila 1972a; Manning, Manning & Reed, 1976) or anti-ε (Dessain *et al.*, 1981; Bozelka *et al.*, 1982), which were shown to suppress immunoglobulin (Ig) responses *in vivo*. However, the large quantities of foreign anti-Ig that must be given and the requirement for neonatal treatment (Manning & Jutila, 1972b) clearly places a limit on the potential clinical use of this approach. Antigen-specific modulation of IgE responses by anti-idiotype (Blaser, Nakagawa & de Weck, 1980) is also limited; control is possible only of a few well-defined antigens, whereas atopic patients may give high IgE responses to a wide variety of antigens or different epitopes on the same antigen.

Previous work in this laboratory has demonstrated a novel experimental method for regulating overall serum IgE levels on a prolonged basis. By actively immunizing rats with purified rat IgE (IR2), a specific IgG class auto-anti-IgE response was induced which had a sustained effect on total serum IgE levels (Marshall & Bell, 1985), on mast cell populations (Marshall *et al.*, 1987a) and on the ability of animals to eliminate an intestinal helminth infection (Marshall, Wells & Bell, 1987b).

Auto-anti-IgE antibodies have also been reported in human subjects, particularly patients with eczema (Nawata *et al.*, 1985) and asthma (Inganas, Johansson & Bennich, 1981; Nawata *et al.*, 1984). The role of naturally occurring auto-anti-IgE in the pathogenesis of allergic disease is unknown. Interestingly our experimental model illustrates that anti-IgE antibodies could influence the disease process by affecting IgE synthesis (Marshall & Bell, 1985) or equally by acting on mast cells (Marshall *et al.*, 1987a).

The present study was undertaken to assess the ability of auto-anti-IgE induction to regulate specific IgE (and IgG) production in response to primary and secondary challenge with the antigen ovalbumin (OVA). Two rat strains were investigated a conventional (i.e. low) IgE-producing PVG-RT1^U strain and a high IgE-producing BN strain. We show that whereas auto-anti-IgE induction reduced total IgE levels only in the PVG-RT1^U strain, specific IgE production was inhibited in both low and high IgE-producing rats.

MATERIALS AND METHODS

Animals

The high IgE-producing BN strain and the PVG-RT1^U congenic strains of rat were bred and maintained in the Animal Unit of the Medical School, Manchester University. The PVG-RT1^U strain was bred in isolator conditions. At weaning offspring were removed from the isolator and maintained in a conventional environment. All animals were 8–11 weeks old at the start of experimentation, and were age and sex matched with controls.

Reagents

IR2 (IgE), IR162 (IgE) and IR1060 (IgA) myeloma proteins were purified as previously described (Marshall & Bell, 1985)

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Table 1. The effect of auto-anti-IgE induction on the specific IgE response to OVA in PVG-RT1^U rats

Stage in IR2* immunization schedule	*n*	Total serum† (IgE) (ng/ml)	Anti-IgE‡	Specific anti-OVA (IgE) U/ml† days after priming with OVA 14	21	28
Control	6	240±80	—	52±15	45±15	23±3
Day 14	6	129±42	—	31±6	27±3	18±1
Day 42	7	56±19§	352	20±4§	10±4¶	19±2

* Day after first injection of ap IR2 on which 10 μg ap OVA was injected i.p. Day 42 group received a second injection of ap IR2 on Day 14.

† Means±SE.

‡ Values represent ng sheep anti-rat IgG bound/ml serum; — denotes no significant binding above normal serum.

§ $P<0.01$: experimental versus control.

¶ $P<0.05$.

from ascites fluids provided by Professor H. Bazin. Sheep anti-rat IgE for use in the PRAST assay was raised against IR2 then absorbed against an Ig preparation from IgE-free nude rat serum and eluted from IR162 coupled to Sepharose 4B (Sigma). It showed no reactivity against nude rat serum, purified rat IgG2a and 2b or rat myeloma IgA. ^{125}I-sheep anti-rat IgG was prepared as previously described (Marshall & Bell, 1985) for use in the auto-anti-IgG assay. OVA, human gamma globulin (HGG), and alkaline phosphatase-coupled anti-rat IgG, for use in the IgG anti-OVA assay, were purchased from Sigma, Poole, Dorset, U.K.

IgE assays

Paper radioimmunosorbent (PRIST) and paper radioallergosorbent (PRAST) assays were carried out according to the method of Karlsson *et al.* (1979).

IR2 immunization

An anti-IgE response was induced by two i.p. injections of 100 μg alum-precipitated IR2 (ap IR2) 14 days apart. Animals were bled via the tail artery.

Iodination

Proteins were labelled with ^{125}I (IMS-30; Amersham, Bucks, U.K.) using iodogen (Pierce Chemical Co., Rockford, IL). Free iodine was removed on Dowex AG 1 × 8 anion exchange resin in 0·2 M borate-buffered saline, pH 8·2 (BBS).

IgG anti-IgE assay (auto-anti-IgE)

A plate radioimmunoassay was used as previously described (Marshall & Bell, 1985) with slight modifications. Briefly, activated microtitre plates (Flow Ltd) were coated with IR162 rat IgE myeloma at 100 μg/ml in BBS. The plates were washed, the remaining binding sites blocked with a 1% w/v solution of bovine serum albumin (BSA) in BBS and 50 μl of sera diluted 1/10 and 1/40 in 0·15 M phosphate-buffered saline (PBS) + 0·3% BSA and 0·02% polyoxyethylene sorbitan monolaurate (Tween 20). The results from sera diluted 1/40 were used unless binding of less than 20% above normal serum was obtained at this dilution, in which case results at a 1/10 dilution were examined.

The plates were incubated for 90 min at room temperature and washed. Rat IgG bound to the plate was detected using a ^{125}I-labelled sheep anti-rat IgG that had been extensively absorbed against IgE as described elsewhere (Marshall & Bell, 1985).

IgG anti-OVA assay

An ELISA assay for IgG anti-OVA was carried out according to the method of Voller *et al.* (1974). Rat IgG bound to OVA was detected using alkaline phosphatase-conjugated anti-rat IgG. Titres were determined as dilution of sera which gave half maximal binding.

Statistics

Statistical analysis was performed using Student's *t*-test.

RESULTS

Our previous work (Marshall & Bell, 1985) showed that a specific and persistent autoantibody response directed against IgE was induced by injecting adult rats twice, 14 days apart, i.p. with 100 μg ap IR2.

Initial experiments investigated the primary anti-OVA IgE response of animals during immunization with IR2 compared with unimmunized control animals. Groups of ♀ PVG-RT1^U and BN rats were injected with 10 μg ap OVA alone (control), 14 days after a single injection of IR2 (Day 14) or 28 days after a second (i.e. Day 42 after the first) injection of IR2. Specific IgE production against OVA was inhibited in auto-anti-IgE-producing PVG-RT1^U rats (Table 1) 14 and 21 days after OVA injection. Although a small reduction of anti-OVA IgE was observed in PVG-RT1^U rats given a single injection of IR2 (Table 1, Day 14 group), a statistically significant reduction of 61–77% was seen only in rats with circulating levels of anti-IgE antibodies (Table 1, Day 42 group). Note also that the total serum IgE was significantly depressed in the Day 42 group of PVG-RT1^U animals ($P<0.01$).

The BN rat strain has serum IgE values four or five times higher than the PVG-RT1^U strain and may give specific IgE responses 10–100 times higher. As a more rigorous test of the effect if auto-anti-IgE induction on specific IgE against OVA, BN rats were examined using the same protocol as above.

Table 2. The effect of auto-anti-IgE induction on the specific IgE response to OVA in high IgE-producing BN rats

Stage in IR2* immunization schedule	*n*	Total serum† IgE (ng/ml)	Anti-IgE‡	Specific anti-OVA (IgE) U/ml† days after priming with OVA 14	21	28
Control	6	952±63	—	4910±227	678±50	626±133
Day 14	6	1028±92	56	1404±208§	436±23¶	608±23
Day 42	6	1211±201	127	1210±397§	200±21§	300±152

* Day after first injection of ap IR2 on which 10 μg ap OVA was injected i.p. Day 42 group received a second injection of ap IR2 on Day 14.

† Means±SE.

‡ Values represent ng sheep anti-rat IgG bound/ml serum; — denotes no significant binding above normal serum.

§ $P<0.001$: experimental versus control.

¶ $P<0.01$.

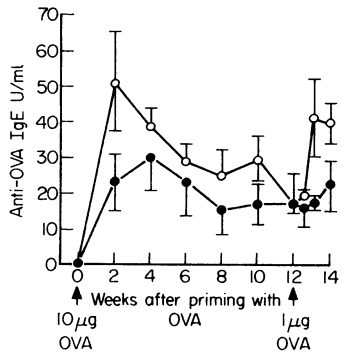

Figure 1. Specific anti-OVA IgE responses in auto-anti-IgE-producing PVG-RT1$_U$ rats following priming and challenge with OVA. Rats immunized 42 and 28 days before with 100 μg ap IR2 (●) or ap HGG (○) (control) were primed with 10 μg of ap OVA + 10^{10} *Bordetella pertussis* organisms i.p. and challenged with 1 μg ap OVA 12 weeks later. Mean values of six animals per group±SE.

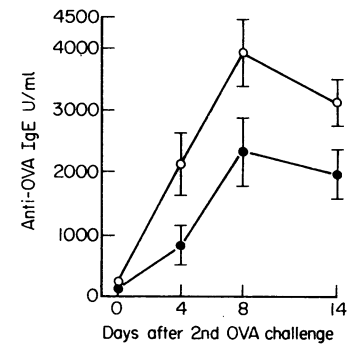

Figure 3. Auto-anti-IgE production inhibits specific IgE responses in BN rats following a secondary challenge with OVA. Animals were immunized with 100 μg of AP IR2 (●) or AP HGG (○) i.p. on Days 0 and 14, primed with 10 μg of sol OVA + 10^{10} *Bordetella pertussin* organisms on Day 28 and rechallenged with 1 μg of sol OVA i.d. on Day 84. Mean values of six to seven rats per group±SE.

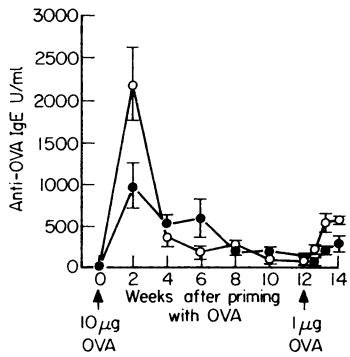

Figure 2. Specific anti-OVA IgE responses in auto-anti-IgE-producing BN rats following priming and challenge with OVA. Rats immunized 42 and 28 days before with 100 μg ap IR2 (●) or ap HGG (○) (control) were primed with 10 μg of ap OVA + 10^{10} *Bordetella pertussis* organisms i.p. and challenged with 1 μg ap OVA 12 weeks later. Mean values of six animals per group±SE.

A single or two injections of IR2 induced detectable amounts of auto-anti-IgE in BN rats (Table 2). The Day 14-specific anti-OVA IgE was reduced from 4910 U/ml in controls to 1410 U/ml and 1210 U/ml in BN rats injected once or twice, respectively, with IR2 (Table 2). This reduction of about 70% in the specific anti-OVA IgE response was seen despite the previously reported (Marshall & Bell, 1985) and here confirmed inability of auto-anti-IgE induction to reduce total IgE levels in BN rats. In a further experiment animals were immunized with IR2 or with an alternative Ig (HGG). The primary IgE anti-OVA response was inhibited in both PVG.RT1^U rats and BN rats at Day 14 ($P<0.05$ PVG.RT1$_U$, Fig. 1; $P<0.05$ BN, Fig. 2).

The suppressive effect of auto-anti-IgE induction was not limited to a specific primary IgE response. When IR2-treated PVG-RT1^U animals which gave responses on Day 14 of primary OVA immunization of 51·0±11 (control) and 21·5±18 (anti-IgE), respectively, were challenged with OVA again the secondary anti-OVA IgE response was also significantly ($P<0.05$) reduced (Fig. 1). A very similar effect was observed in BN rats (Fig. 2), although as expected both primary and secondary specific IgE responses were 10–40 times higher in this strain.

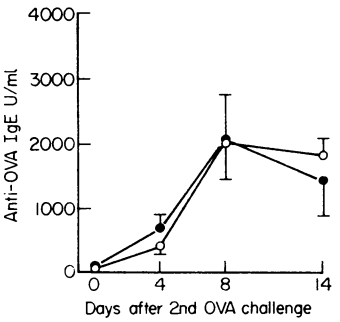

Figure 4. Subsequent auto-anti-IgE induction fails to inhibit specific anti-OVA IgE responses in OVA-immunized BN rats. Animals were primed with 10 μg of sol OVA + 10^{10} *Bordetella pertussis* organisms on Day 0, immunized with 100 μg of ap IR2 (●) or ap HGG (○) on Days 28 and 42 and challenged with 1 μg of sol OVA i.d. on Day 84. Mean values of six rats per group ± SE.

Table 3. IgG anti-OVA responses in IR2-immunized (anti-IgE) and HGG-immunized (control) rats

Strain	Day	Group*	IgG anti-OVA†
PVG-RT1$_U$	14 of 1° response	1 Anti-IgE	2·82 ± 0·27
PVG-RT1$_U$	14 of 1° response	2 Control	2·69 ± 0·20
BN	14 of 1° response	3 Anti-IgE	2·42 ± 0·21
BN	14 of 1° response	4 Control	2·56 ± 0·10
BN	8 of 2° response	5 Anti-IgE	2·74 ± 0·21
BN	8 of 2° response	6 Control	2·73 ± 0·41
BN	8 of 2° response	7 Anti-IgE	2·61 ± 0·17
BN	8 of 2° response	8 Control	2·55 ± 0·26

* Corresponding specific IgE values are found in Fig. 1, Groups 1 and 2; Fig. 2, Group 3; Fig. 3 Groups 5 and 6; Fig. 4, Groups 7 and 8.

† Values are mean log titres ± SE of six to seven rats per group, measured by ELISA, where titre is defined as dilution of antiserum that gives half maximal binding.

It was necessary to examine the effect of the anti-IgE response in more detail. Firstly, because the OVA immunization of Figs 1 and 2 was induced by an alum-precipitated antigen which may not be optimal for a secondary IgE response (Jarrett *et al.*, 1980), subsequent experiments used a soluble (sol) antigen for priming and challenge. Secondly, the inhibition of the secondary response to OVA in the sequence of injections IR2, IR2, OVA, OVA could be largely influenced by the lack of a vigorous primary rather than a true effect on a secondary anti-OVA IgE response. Therefore we wanted to determine whether auto-anti-IgE induction could alter an already established anti-OVA IgE response.

The following immunization regimes were tested in BN rats: (i) ap IR2 (×2), sol OVA (primary), sol OVA (secondary); (ii) sol OVA (primary), ap IR2 (×2), sol OVA (secondary).

When auto-anti-IgE induction preceeded the primary OVA injection, the secondary IgE response to sol OVA was decreased (Fig. 3); similar results were obtained using ap OVA challenge (Figs 1 and 2). However, when IR2 immunization followed a primary OVA injection, the secondary IgE response was not inhibited (Fig. 4). Auto-anti-IgE induction was not able to inhibit a previously established anti-OVA IgE response.

The ability of auto-anti-IgE induction to inhibit the IgE class of Ig selectively was examined by measuring the IgG class of anti-OVA in the same serum samples of all groups of animals. The results from the most relevant groups are shown in Table 3. IgG antibody directed against OVA was detected in all the OVA immunized groups but at no time was there any significant difference between the IR2-immunized and the respective control group(s) of animals in the amount of OVA-specific IgG detected in sera.

DISCUSSION

The induction of auto-anti-IgE antibodies in conventional and high IgE-producing rats compromised the ability of these animals to produce a specific IgE response. Challenge and rechallenge with OVA of IR2 immunized rats resulted in significantly lower levels of anti-OVA IgE antibody compared with HGG-immunized or unimmunized controls. However, IR2 immunization was unable to affect specific IgE in rats previously primed to OVA; auto-anti-IgE induction did not prevent the stimulation of memory cells for IgE.

It has been reported that the use of alum as an adjuvant is detrimental to the induction of a secondary IgE response (Jarrett *et al.*, 1980). In order to evaluate whether alum was influencing the results, two changes were made in the protocol. Firstly in place of unimmunized controls ap HGG-injected rats were substituted. Secondly primary and secondary anti-OVA responses were induced with sol OVA in place of ap OVA. Auto-anti-IgE induction inhibited the secondary specific anti-OVA IgE as before even though a much greater secondary anti-OVA response was elicited (Figs 1–3).

The inhibition of specific IgE was not dependent on auto-anti-IgE suppressing the entire IgE class. As previously reported (Marshall & Bell, 1985) and confirmed here, total IgE levels were inhibited only in anti-IgE-producing PVG-RT1^U rats but were not significantly or consistently reduced in the BN rat strain. The specific anti-OVA IgE, however, was suppressed in both strains.

It may be argued that the auto-anti-IgE blocked the binding of specific IgE to the anti-IgE-coated paper discs (PRAST assays) and that the apparent reduction of specific IgE was a limitation of the assay. This is unlikely since in the BN strain there was no correlation between reduced total and reduced specific IgE although both assays employed the same heterologous anti-rat-IgE reagent. Furthermore, we have previously shown (Marshall & Bell, 1985) that the auto-anti-IgE antibodies do not compete for the same binding sites on the IgE molecule as the heterologous anti-IgE employed in the PRIST and PRAST assays.

Since auto-anti-IgE was shown to be capable of causing mast cell degranulation *in vivo* (Marshall *et al.*, 1987a) it was conceivable that products of degranulation such as histamine were exerting an immunosuppressive effect that would also involve the IgE system. However, there was no evidence of a generalized suppression of antibody synthesis. IgG anti-OVA levels were not significantly altered by auto-anti-IgE induction.

The inability of auto-anti-IgE induction to contain a secondary IgE response in previously immunized rats places certain constraints on the clinical potential of this approach for

therapeutic application. Nevertheless our model will be useful to investigate the underlying mechanisms of IgE regulation and could clarify the role of naturally occurring auto-anti-IgE antibodies in atopic patients (Nawata *et al.*, 1985, 1984; Inganas *et al.*, 1981).

There are many possible mechanisms by which IR2 immunization might inhibit total IgE levels or specific IgE responses. The most obvious are those which depend on binding of anti-IgE to IgE-bearing cells. It is known that heterologous anti-Ig antibodies can be cytotoxic for lymphocytes in the presence of complement (Hartmann, Reed & Mehner, 1971) or that opsonization of lymphocytes may occur (Basten *et al.*, 1971). There is, however, little evidence that these mechanisms operate *in vivo*. In adult animals attempts to suppress antibody production by treatment with heterologous anti-Ig antibodies have proved unsuccessful (Manning, 1972, 1980). In fact, anti-μ treatment of adult rats led not to suppression but to an increased production of IgM of an aberrant type (Manning, 1980). Manning (1972) also found that when heterologous anti-α antisera treatment was delayed in young mice until IgA was detectable in the serum, there was a uniform failure of the animals to exhibit suppression of either faecal or serum IgA. Although neonatal injections of heterologous anti-IgE were effective at inhibiting specific IgE in rodents (Bozelka *et al.*, 1982; Dessain *et al.*, 1981), such treatment of adult animals was ineffective. Why, therefore should inducing an auto-anti-IgE response prove to be inhibitory.

There are important differences between autologous and heterologous anti-Ig antibodies. For instance an autoantibody might be expected to fix complement or bind to Fc receptors on cells with a greater affinity, inducing a more efficient Fc-dependent suppression. Cells other than B cells could also be involved; T cells (Fritsche & Spiegelberg, 1978), eosinophils (Capron *et al.*, 1986) and mast cells (Froese, 1980) all have receptors for IgE.

Using a related system in which an autologous response was induced against the Ig variable region (instead of the constant region) the resulting anti-idiotypes suppressed specific IgE (as well as other isotypes) (Blaser *et al.*, 1980). The induction of T cells which suppress specific IgE was also reported by Chen & Katz (1983) who treated neonatal mice with a tolerogenic preparation of isologous IgE. We have no information as to whether T cells are involved in our auto-anti-IgE system. Attempts to analyse this model by adoptive transfer were complicated by the fact that lymphocyte-injected, irradiated recipients produced high levels of IgE non-specifically (J. S. Marshall and E. B. Bell unpublished observations) and that auto-anti-IgE-producing cells from the thoracic duct or mesenteric lymph nodes transferred anti-IgE synthesis only to irradiated but not unirradiated rats.

On the basis of these results, auto-anti-IgE antibodies in atopic individuals might be expected to down-regulate the development of IgE responses to primary antigen challenge whilst not necessarily reducing the overall serum IgE levels—a hypothesis that remains to be tested. This would be in keeping with the observation that some groups of patients with high IgE levels also have high levels of auto-anti-IgE. The recent development of mouse monoclonal auto-anti-IgE antibodies could be useful in elucidating the precise mechanisms by which anti-IgE affect IgE production in adult animals (Haba & Nisonoff, 1987a, b) while the rat model of auto-anti-IgE induction should provide further information on the many potential effects of circulating polyclonal auto-anti-IgE antibodies.

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