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Rat IgE Production

I. EFFECT OF DOSE OF ANTIGEN ON PRIMARY AND SECONDARY REAGINIC ANTIBODY RESPONSES

ELLEN E. E. JARRETT AND DIANA C. STEWART

Wellcome Laboratories for Experimental Parasitology, and Department of Bacteriology & Immunology, University of Glasgow, Glasgow

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Summary. The experiments described here form part of a series carried out to determine the conditions of antigen presentation which dispose to the production of IgE antibody in the rat. We have found that Hooded Lister rats in comparison with rats of some other strains have an exceptional ability to produce reaginic antibodies: responses can be consistently induced with very small doses of antigen and are boosted to high levels with a second dose of antigen.

The effect of the dose of antigen on these responses is as follows.

(1) *Primary reagin responses*

These could be induced with doses ranging from 1 mg to 1 μg of egg albumin (EA) injected intraperitoneally with *B. pertussis* adjuvant. The level of the primary response was unaffected by the amount of antigen given. Circulating reagins were not produced by rats given 0·1 μg of EA but could be evoked in them by a subsequent injection of antigen given without adjuvant.

(2) *Secondary reagin responses*

These could be evoked with doses ranging from 100 μg–0·001 μg of EA injected intraperitoneally without adjuvant. Injection of larger second doses resulted in fatal anaphylactic shock. The level of the secondary response was found to be determined by the amount of antigen given on the first occasion and was not influenced by the size of the second dose of antigen. Only rats injected with a small dose of antigen (e.g. 1 μg) on the first occasion, produced significant secondary responses. In contrast, large primary doses of antigen (e.g. 1 mg) were inhibitory to the production of secondary reagin responses.

The primary IgE response was long-lived, the secondary response on the other hand declined rapidly to pre-challenge levels. Tertiary booster responses could not be obtained.

The pattern of the reagin response to KLH was similar but the minimum antigen requirement was increased by a factor of 10.

The possible explanations of (a) the inhibiting effect of high primary doses of

Correspondence: Dr Ellen E. E. Jarrett, Wellcome Laboratories for Experimental Parasitology, Bearsden Road, Bearsden, Glasgow G61 1QH.

antigen on the secondary response, (b) the rapid decline of the secondary response and (c) the absence of tertiary responses are discussed. We feel that feedback inhibition by IgG antibody, or the action of suppressor T cells or more probably a combination of the two mechanisms could explain these features of the IgE response.

The results indicate that rats of suitable genetic predisposition will prove a useful model for the evaluation of factors concerned in the biosynthesis of IgE and the induction of hypersensitivity states.

INTRODUCTION

Rats are not generally considered to be good producers of reaginic antibodies, as it has been reported that only low levels are produced following immunization with conventional antigens and that the response is short-lived and cannot be significantly boosted by further doses of antigen (Binaghi and Benacerraf, 1964; Mota, 1964). In contrast, high levels of reaginic (IgE) antibody are produced by most strains of rats following infection with a variety of helminth parasites (reviewed by Ogilvie and Jones, 1969; Jarrett and Urquhart, 1971; Jarrett, 1973). Such anti-parasitic reagins persist in the circulation for long periods and the levels are elevated following reinfection.

It has been shown moreover, that helminth infection in the rat, in addition to evoking a parasite-specific IgE response may cause non-specific potentiation of a variety of unrelated IgE responses (Orr and Blair, 1969; Jarrett, 1972; Jarrett and Stewart, 1972) thereby providing a means of inducing high serum titres to some conventional antigens.

During the course of investigations into the mechanism of this potentiated response in Hooded Lister rats, we observed that very high levels of IgE antibody to egg albumin could also be obtained in some circumstances as a secondary response to the antigen. This led us to perform a series of experiments to determine the conditions of antigen presentation which would consistently produce these high reagin levels.

This paper describes the effect of variations of the dose of antigen on the development and magnitude of primary and secondary IgE responses.

MATERIALS AND METHODS

Rats

The experiments were performed with female Hooded Lister rats (Animal Suppliers (London) Ltd), weighing 150–200 g.

Antigen preparations and antibody determinations

Stock solutions of egg albumin (Sigma Grade V) and keyhole limpet haemocyanin (Calbiochem A grade) at a concentration of 10 mg/ml were prepared in 0·15 M saline. The different doses of antigen were then obtained by making appropriate dilutions of these solutions. Each dose of antigen in 0·1 ml saline was injected intraperitoneally immediately after dilution. For initial immunization the antigens were injected together with a *Bordetella pertussis* suspension (Wellcome Biological Reagents) containing 1×10^{10} organisms. Subsequent doses of antigen were injected without adjuvant.

Rats were bled from the tail vein by cutting off the tip of the tail and manually 'milking' the tail into a test tube. Approximately 1 ml of blood was collected in this way. Occasionally blood was obtained by heart puncture; no more than 2 ml was withdrawn if the rats were to be kept.

The level of circulating reagins was estimated by passive cutaneous anaphylaxis (PCA) tests (Ovary, 1964) performed on Hooded Lister rats. Thus 0·1 ml quantities of saline dilutions of test serum were injected intradermally and each injection was duplicated on different test animals. The animals were injected intravenously 48–72 hours later with 2·5 mg of either egg albumin (EA) or keyhole limpet haemocyanin (KLH), together with 0·5 ml of 1 per cent Evans Blue. The skin reactions were examined after 20 minutes. The reaginic titre recorded is the greatest dilution that gave skin reactions with a diameter of greater than 5 mm.

An attempt was also made to detect heat-stable (IgGa) homocytotropic antibody by performing PCA tests with a 2–4 hour latent period using sera heat-inactivated at 56°. The period of heat inactivation required to eliminate PCA activity due to reaginic (IgE) antibody was found to depend on the reaginic titre of the serum, e.g. 30 minutes for low titre and 2–3 hours for high titre sera.

For passive haemagglutination, formalinized human red blood cells were tanned and coated with EA or KLH according to the method described by Herbert (1967). These cells were used in the titration of haemagglutinating antibodies using Microtitre equipment. Antibody titres were determined on sera first diluted 1:8 in the absorption process. The titres are expressed as the last well demonstrating haemagglutination. In some experiments sera were treated with an equal volume of 0·2 M mercaptoethanol to obtain an estimate of the relative proportions of IgM (mercaptoethanol-sensitive) and IgG (mercaptoethanol-insensitive) haemagglutinating antibodies.

Statistical analysis

Means and standard errors were calculated using logarithmically transformed antibody titres. Results from appropriate groups were compared by analysis of variance or Student's *t*-test. Animals which did not produce detectable antibody were not included in the calculations. The number of such non-reacting animals in each group is shown in the Results section.

RESULTS

THE PRIMARY REAGIN RESPONSE OVER A RANGE OF DOSES OF ANTIGEN

Six groups of six rats were injected with EA in the doses shown below together with *B. pertussis* as adjuvant.

Group	Dose of EA (μg)
1	100
2	10
3	1
4	0·1
5	0·01
6	0·001

The animals were bled 12 and 26 days after immunization. Table 1 shows that on day 12 most of the animals in the first three groups were producing reaginic antibody, but that reagins could be detected in only one animal of Group 4. Reaginic antibody was not detected in any animal of Group 5 or 6. On day 26 after immunization no additional

animals were producing reaginic antibody and the mean titre of those which were had not changed significantly (Student's *t*-test).

Analysis of variance carried out on the reagin titres of the first three groups on both day 12 and day 26 gave F ratios below the critical value. The differences in reagin titres between the 3 groups on each of these days are therefore below the significance limit ($P > 0{\cdot}05$).

TABLE 1

PRIMARY REAGINIC ANTIBODY RESPONSES OVER A RANGE OF DOSES OF EGG ALBUMIN (EA)

Rat number	PCA titres on days 12 and 26 after immunization with these doses of EA*							
	100 μg (Group 1)		10 μg (Group 2)		1 μg (Group 3)		0·1 μg (Group 4)	
	Day 12	Day 26	Day 12	Day 26	Day 12	Day 26	Day 12	Day 26
1	256	256	128	128	4	8	0	0
2	2	32	128	32	16	128	0	0
3	2	4	256	1024	512	256	0	0
4	0	0	64	512	128	1024	2	1
5	16	512	64	128	0	0	0	0
6	32	64	128	64	16	8	0	0

* EA injected i.p. together with 1×10^{10} *B. pertussis* organisms.

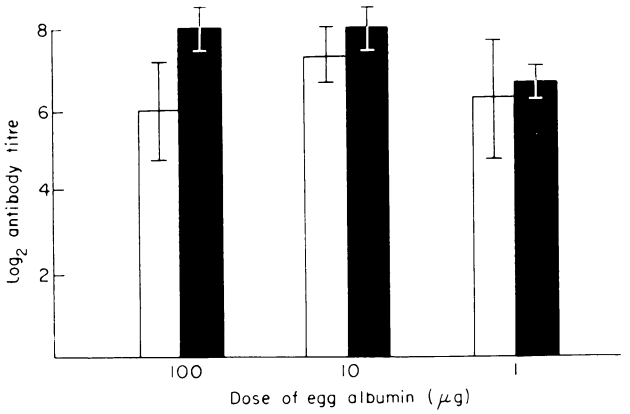

FIG. 1. (□) Reaginic and (■) haemagglutinating antibody levels 26 days after immunization with a range of doses of egg albumin. The bars show ±s.e.

The results of this experiment indicated that variation of the dose of EA between 100 and 1 μg did not significantly effect the level of the primary reaginic antibody response. When this was present on day 12 after immunization it was maintained without decline at least until day 26.

Haemagglutinating antibodies were not detectable in any sera on day 12 after immunization but were present on day 26 in five out of six animals of Group 1, six out of six animals of Group 2 and three out of six animals of Group 3. Fig. 1 compares the levels of reaginic and haemagglutinating antibodies of the first three groups on day 26 after immunization.

SECONDARY REAGIN RESPONSE

On day 30 of the experiment the rats of the first three groups were challenged with the same dose of antigen that they had received previously and the secondary reagin response was studied. Thus Group 1 rats received 100 μg, Group 2 10 μg and Group 3 1 μg of EA without adjuvant. The animals were bled 4 days after the second dose of antigen as previous experiments had shown that this was when the maximum secondary response could be expected. From the results which are shown in Table 2 it can be seen that there was a marked difference in response to this second dose of antigen: greater and more

TABLE 2

SECONDARY REAGINIC ANITBODY RESPONSE OF THE FIRST THREE GROUPS OF RATS SHOWN IN TABLE 1 CHALLENGED WITH THE SAME DOSE OF ANTIGEN 30 DAYS AFTER THE FIRST INJECTION

PCA titres on day 4 after challenge with these doses of EA		
100 μg (Group 1)	10 μg (Group 2)	1 μg (Group 3)
512	1024	2
8	1024	1024
32	16,384	16,384
0	16,384	32,768
4096	2048	0
64	1024	1024

consistent secondary responses occurred in the rats of Groups 2 and 3 than in those of Group 1.

It appeared from these results that the secondary responses seen in Groups 2 and 3 were due to stimulation with doses of antigen which were relatively smaller than those administered to Group 1 rats. The results did not indicate whether it was the first or the second small dose of antigen which caused this response. A series of experiments was then designed to determine whether the magnitude of the secondary response was influenced by (a) the size of the first dose or (b) the size of the second dose of antigen.

EFFECT OF THE SIZE OF THE FIRST DOSE OF ANTIGEN ON THE LEVEL OF THE SECONDARY REAGIN RESPONSE

Three groups of rats were immunized as follows.

Group	Number of rats	Dose of EA
1	8	1 mg
2	10	1 μg
3	9	0·1 μg

The animals were bled 12 and 26 days after immunization and were challenged on day 29 with 0·1 μg of EA without adjuvant. The variable in this experiment therefore was the size of the immunizing dose, the challenge dose being constant.

As in the previous experiment it was found that reagin responses on day 12 and day 26 after immunization were similar. Table 3 shows the PCA titres of the rats on day 26 after the first dose and day 4 after the second dose of EA. Comparing the mean responses of the

TABLE 3

EFFECT OF THE FIRST DOSE OF ANTIGEN (EA) ON THE LEVEL OF THE SECONDARY REAGIN RESPONSE

	PCA titres before and after challenge*					
	Group 1†		Group 2		Group 3	
Rat number	1st dose (1 mg) pre-challenge	2nd dose (0·1 μg) post-challenge	1st dose (1 μg) pre-challenge	2nd dose (0·1 μg) post-challenge	1st dose (0·1 μg) pre-challenge	2nd dose (0·1 μg) post-challenge
1	128	256	16	128	0	0
2	32	512	8	4096	0	0
3	64	256	64	2048	0	256
4	32	256	64	2048	0	0
5	32	32	128	8192	2	2048
6	32	16	2	4096	0	0
7	128	1024	8	8192	0	256
8	0	0	0	0	0	256
9			32	2048	0	0
10			0	128		

* Serum taken 3 days before and 4 days after challenge.

† The rats of groups 1, 2 and 3 were immunized with 1 mg, 1 μg and 0·1 μg of EA respectively, and were all challenged 29 days later with 0·1 μg of EA.

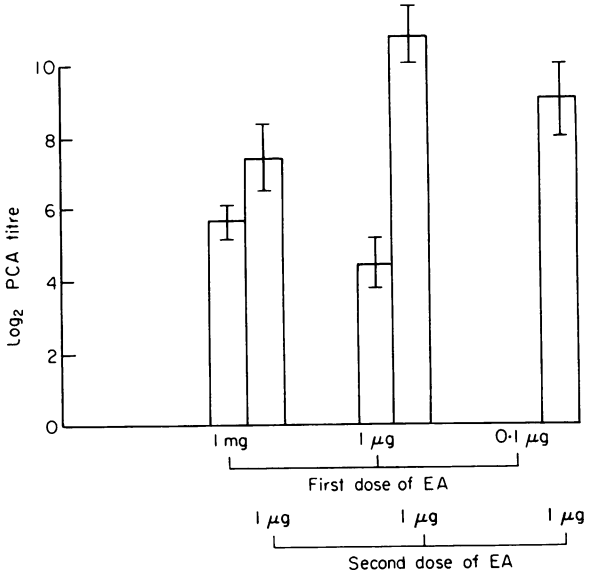

FIG. 2. Effect of the first dose of antigen on the level of the secondary IgE response: mean levels of reaginic antibody 26 days after a first and 4 days after a second dose of egg albumin (EA).

rats in Groups 1 and 2 (Fig. 2), it is evident that immunization with 1 μg followed by challenge with 0·1 μg of EA is far more conducive to the production of a good secondary reagin response than is immunization with 1 mg followed by the same challenge dose. A

comparison by Student's *t*-test of the $\log_2$ reagin titres of Groups 1 and 2 on day 4 after challenge gave a *P* value of 0·0045.

The response of Group 3 rats was interesting in that three animals which had not previously produced detectable circulating reagins did so after the second dose of EA. From this it would appear that although initial immunization with 0·1 μg of EA is not sufficient to evoke reaginic antibodies in most animals it may nevertheless have a priming effect so that a response occurs on subsequent contact with an equally small dose of antigen given without adjuvant. The animals of all the groups were bled again on day 12 after the second dose of antigen when it was found that their EA reagin levels had considerably declined. The geometric mean PCA levels were 13 in Group 1, 84 in Group 2 and 20 in Group 3.

In the checkerboard experiment described below the effect of varying the first and second dose of EA was simultaneously assessed. Thus two groups of rats were immunized with 1 mg and 1 μg of EA respectively. Their primary antibody response was examined by bleeding on days 12 and 26 after immunization, after which they were subdivided into four groups and challenged on day 29 with a second dose of antigen as shown below.

Dose of EA	Group	Amount of dose	Number of rats
First		1 mg	20
First		1 μg	18
Second	1	100 μg	10
Second	2	0·01 μg	10
Second	3	100 μg	9
Second	4	0·01 μg	9

From Fig. 3 it can be seen that, as in the previous experiments, the reagin levels on day 26 after the first dose of antigen were similar in the two groups despite the disparity in the size of the immunizing doses. However, it is clearly seen from the results in Table 4 and Fig. 3 that the size of the first dose did have a marked effect on the level of the secondary reagin response. Whereas the animals immunized with 1 mg of EA produced poor secondary responses on challenge (the highest titre being 512), approximately half of the animals immunized with 1 μg EA produced high titres of reagin (up to 16,384) following the second dose.

The results indicated moreover that the size of the second dose of antigen did not affect the level of the secondary reagin response. The responses of Groups 1 and 2 were very similar, as were those of Groups 3 and 4 (see Table 4). A comparison of the $\log_2$ reagin titres on day 4 after challenge gave the following results: comparison of Group 1 with Group 2 and of Group 3 with Group 4 gave *P* values of 0·44 and 0·47 respectively; comparison of Group 1 with Group 3 and of Group 2 with Group 4 gave *P* values of 0·009 and 0·008 respectively. The difference in the level of the secondary reagin response between the groups must therefore be due to variations in the first dose of antigen.

Levels of haemagglutinating antibodies were estimated for the four groups before and after challenge in order to evaluate their possible inhibitory effect on the development of the secondary responses. Haemagglutinins were present on day 26 in all animals immunized with 1 mg and in eleven out of eighteen animals immunized with 1 μg (Fig. 3). The mean levels of the reactor animals were significantly different ($P = 0·0002$). Haemagglutinating antibody levels were elevated as a result of the second dose of antigen.

Haemagglutinins were detected in all animals of Groups 1 and 2 and in eight out of nine animals of Group 3 and seven out of eight animals of Group 4. The levels were similar in all except the last group, in which less marked secondary responses were seen. It seemed

TABLE 4

EFFECT OF VARIATION OF SIZE OF THE FIRST AND SECOND DOSE OF EA ON THE MAGNITUDE OF THE SECONDARY REAGIN RESPONSE

	Immunizing dose of EA			
	1 mg		1 μg	
	Challenge dose of EA			
	(100 μg) Group 1	(0·01 μg) Group 2	(100 μg) Group 3	(0·01 μg) Group 4
PCA titres day 4 after second dose	128	32	4086	512
	64	128	64	32
	256	256	256	4096
	256	256	128	2048
	32	32	512	32
	64	256	2048	2048
	32	64	64	2048
	256	64	4096	512
	32	512	16384	2048
	512	64		

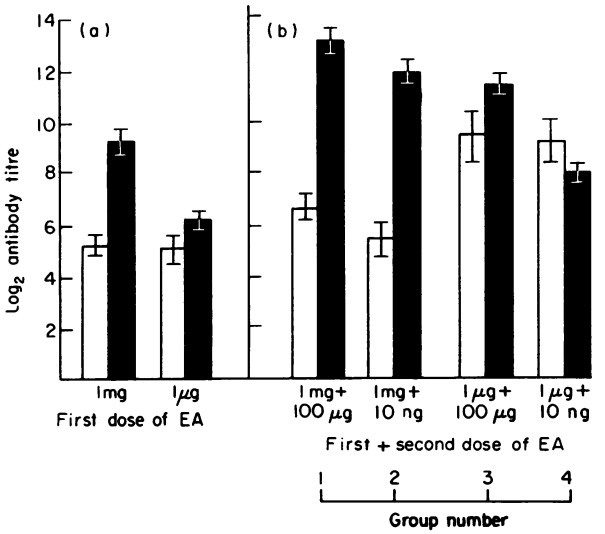

FIG. 3. Effect of varying the amount of the first and the second dose of antigen on the level of the IgE response. (□) Reaginic and (■) haemagglutinating antibody levels 26 days after a first and 4 days after a second dose of egg albumin (EA). (a) Pre-challenge antibody levels. (b) Post-challenge antibody levels. The bars show ±s.e.

noteworthy that in all groups the animals with the highest reagin levels usually also had the highest levels of haemagglutinating antibody. On this basis it seems unlikely that the poor or absent secondary response of Group 1 and 2 rats could be attributed to feedback inhibition by haemagglutinating antibodies.

All the sera that were tested for haemagglutinins in this experiment were titrated before and after mercaptoethanol treatment. In no sample was the titre decreased by more than 2 $\log_2$ units as a result of mercaptoethanol treatment. From this we conclude that the EA haemagglutinins which were detected were largely IgG antibodies.

An attempt was also made to detect heat-stable (IgG) homocytotropic antibodies by performing 2–4-hour PCA tests using heat-inactivated, pooled serum samples. Neither before nor after challenge nor in any other experiment described in this communication were we able to detect any PCA activity other than that attributable to IgE antibodies.

The rats of all groups were given a third dose of 1 μg EA 21 days after the second dose, and were bled 4 days later. On this occasion the EA PCA titre of all of the animals was below 256 both before and after challenge. It seemed that a tertiary booster reagin response could not be evoked under these circumstances.

THE EFFECT OF THE SIZE OF THE SECOND DOSE OF ANTIGEN ON THE LEVEL OF THE SECONDARY REAGIN RESPONSE

The following experiments were done to confirm the finding that the size of the second dose of antigen did not affect the level of the secondary reagin response.

Twenty-seven rats immunized with 1 μg of EA and *B. pertussis* were bled 26 days later to determine the level of the primary response and were challenged on day 34 with the following doses of antigen.

Group	Antigen dose (μg)
1	10
2	1
3	0·1
4	0·01
5	0·001

Table 5 shows the individual reagin titres on day 4 after the second dose of EA and Fig. 4 the mean $\log_2$ titres before and after challenge. An analysis of variance performed on the logarithmically transformed data of Table 5 gave an F ratio below the critical value,

TABLE 5

EFFECT OF VARIATION OF THE SIZE OF THE SECOND DOSE OF EA ON THE SECONDARY REAGIN RESPONSE

	Immunizing dose of EA (1 μg)				
	Challenge dose of EA				
	(10 μg) Group 1	(1 μg) Group 2	(0·1 μg) Group 3	(0·01 μg) Group 4	(0·001 μg) Group 5
PCA titres day 4 after second dose	4096	512	2048	64	512
	2048	2048	1024	8192	1024
	512	1024	1024	1024	4096
	4096	1024	8192	2048	2048
	1024	2048	4096	1024	1024
	512				

and indicated that the rats of Groups 1–5 could be regarded as having been drawn from the same population. It is quite clear therefore, that the level of the secondary reagin response is unaffected by variations in the second dose of EA between 10 μg and 1 ng.

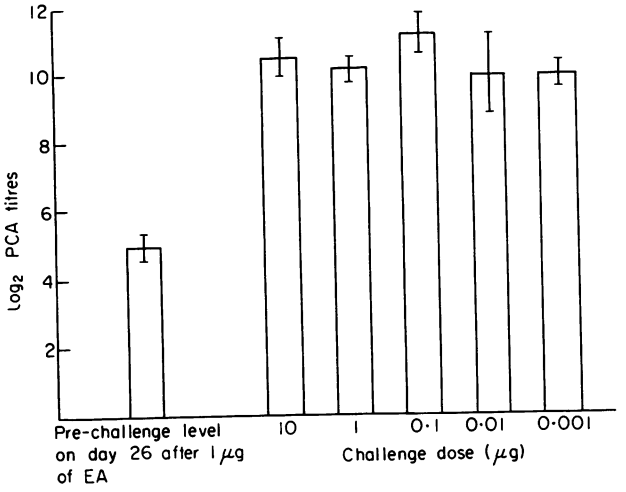

FIG. 4. Effect of the second dose of antigen on the level of the secondary IgE response: reaginic antibody levels 26 days after a first and 4 days after a second injection of egg albumin (EA).

Titration of haemagglutinating antibodies showed that on day 4 after challenge the levels of these antibodies were also similar for the different groups.

All the rats produced haemagglutinins and the levels had risen from a $\log_2$ mean of $9{\cdot}5 \pm 0{\cdot}29$ before challenge to the following levels after challenge.

Group	Haemagglutination levels after challenge ($\log_2$ mean $\pm$ s.e.)
1	$10{\cdot}7 \pm 0{\cdot}21$
2	$10{\cdot}8 \pm 0{\cdot}31$
3	$10{\cdot}2 \pm 0{\cdot}37$
4	$10{\cdot}4 \pm 0{\cdot}24$
5	$11{\cdot}5 \pm 0{\cdot}56$

DURATION OF THE SECONDARY RESPONSE

We had noticed in some of the previous experiments that the reagin titre of the secondary response fell quite rapidly to its prechallenge level. We wished to examine this decline following a variety of secondary doses of EA. Accordingly groups of five rats which had been immunized and challenged as shown below were bled on days 4, 11, 18 and 25 after the second dose of antigen.

Group	Immunization (μg of EA + *B. pertussis*)	Challenge
1	1	1 mg EA
2	1	100 μg EA
3	1	1 ng EA
4	1	No challenge

All the animals given 1 mg of EA as a second dose died within 1 hour, showing signs of anaphylactic shock, i.e. dyspnoea and complete prostration. The mean reagin titres of the animals in the remaining groups are shown in Fig. 5. Two points should be noted: first, the maximum level of the secondary response on day 4 after injection of antigen is similar in

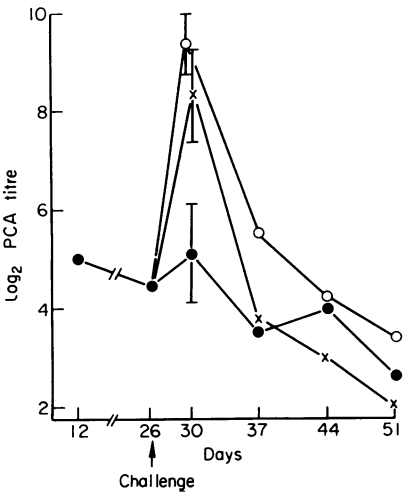

FIG. 5. Kinetics of the secondary IgE response. Rats immunized with 1 μg of egg albumin were given a second dose of 100 μg (×) or 0·001 μg (○) 26 days later. Their response is compared with that of unchallenged rats (●).

the rats challenged with 100 μg or 1 ng EA (Student's t-test, $P = 0{\cdot}2$) whereas the mean levels of both groups are significantly different from Group 4 rats which received no challenge (Student's *t*-test between Groups 2 and 4, $P = 0{\cdot}03$, and between Groups 3 and 4, $P = 0{\cdot}004$). Secondly, that the secondary responses in both Groups 2 and 3 declined rapidly to the level of the unchallenged rats. These were still producing low levels of reagin on day 51 after initial immunization.

SECONDARY RESPONSE WITH KEYHOLE LIMPET HAEMOCYANIN (KLH)

In order to determine whether the results which we had obtained with EA would also apply with another antigen a dose experiment using KLH was set up.

Three groups of six rats were immunized with 10 μg, 1 μg and 0·1 μg of KLH respectively together with *B. pertussis* and were given a second dose of 1 μg KLH 32 days later. The results in Table 6 show that only the rats of the first group (i.e. immunized with 10 μg KLH) produced detectable reagins as a primary response. Four days after the second

injection, however, the rats of both Groups 1 and 2 had circulating reagins, while the rats of Group 3 (injected with 0·1 μg of KLH on the first occasion) were still not producing detectable reaginic antibody. It can be seen that the secondary response of the Group 1 rats was considerably greater than that of Group 2 (Student's *t*-test, $P = 0{\cdot}008$) but

TABLE 6

PRIMARY AND SECONDARY REAGIN RESPONSES TO KEYHOLE LIMPET HAEMOCYANIN (KLH)

Rat number	PCA titres before and after challenge*			
	Group 1†		Group 2	
	1st dose (10 μg) pre-challenge	2nd dose (1 μg) post-challenge	1st dose (1 μg) pre-challenge	2nd dose (1 μg) post-challenge
1	32	2048	0	256
2	64	8192	0	4096
3	64	4096	0	32
4	4	1024	0	128
5	32	4096	0	512
6	64	512	0	32

* Serum taken 5 days before and 4 days after challenge.
† The rats of Groups 1 and 2 were immunized with 10 μg and 1 μg of KLH, respectively. They were given a second injection of 1 μg of KLH 32 days later.

TABLE 7

ATTEMPT TO PRODUCE A TERTIARY REAGIN RESPONSE IN RATS IMMUNIZED AND CHALLENGED WITH MINUTE DOSES OF EA

Rat number	PCA and haemagglutinating antibody titres					
	Day 27 after 1st dose (0·1 μg of EA)		Day 4 after 2nd dose (0·001 μg of EA)		Day 4 after 3rd dose (0·001 μg of EA)	
	PCA	Haemagglutination titre	PCA	Haemagglutination titre	PCA	Haemagglutination titre
1	0	< 16*	256	< 16	2	64
2	0	< 16	256	< 16	4	< 16
3	4	< 16	1024	256	16	< 16
4	0	< 16	128	< 16	256	< 16
5	0	< 16	2048	< 16	32	< 16
6	0	16	2	< 16	256	32
7	0	256	512	< 16	32	32
8	64	16	4096	256	64	32
9	0	< 16	128	< 16	32	16
10	0	< 16	512	32	32	64

* Lowest haemagglutinating antibody levels detectable are 1/16 since sera were diluted in absorption process.

nevertheless that the reagin level of some of the rats (notably rats number 2 and 5) in the latter group was higher than that usually seen in the first response to the antigen.

It appeared that with this preparation of KLH 10 μg was the lowest immunizing dose which would evoke circulating reagins and that 1 μg, although not evoking reagins in the primary response, sensitized the animals for a secondary response. This result is comparable with that seen for EA in Experiment 2 with the exception that the dose of EA required to achieve similar responses was ten-fold lower (i.e. 1 μg and 0·1 μg respectively).

ATTEMPTS TO PRODUCE A TERTIARY REAGIN RESPONSE

In a previous experiment we had found that a third dose of antigen given after the decline of the secondary response did not again cause elevation of the reagin levels. We considered the possibility that this might be due to a feedback effect by IgG (haemagglutinating) antibodies which were boosted to high levels after the second dose of antigen (see Fig. 3). An attempt was made, therefore, to induce a tertiary reaginic antibody response in rats given very small first and second doses of antigen in order to minimize the stimulation of haemagglutinating antibodies.

Ten rats immunized with 0·1 μg EA were challenged with 0·001 μg EA 29 days later and again with 0·001 μg EA 30 days after the second dose. The results of this experiment are shown in Table 7. It is evident that these rats produced only low or undetectable levels of haemagglutinating antibody. It can also be seen that, although the first dose of antigen (0·1 μg) was insufficient to stimulate a primary circulating reagin response in most animals, a secondary type of response was elicited after a second dose of 0·001 μg EA. Nevertheless, despite the low or absent haemagglutinating antibody levels, a tertiary response of similar magnitude was not seen after the third dose of antigen.

DISCUSSION

Reaginic antibodies in the rat belong to an antigenically and physicochemically distinct immunoglobulin class (Stechschulte, Orange and Austen, 1970), whose biological role in the production of immediate hypersensitivity reactions is comparable to that of human IgE. However, as a model for investigating the factors concerned in the induction of hypersensitivity states, rats appeared to have the major disadvantage of being poor producers of reaginic antibodies. In this respect it seemed that they were unlike allergic human beings who may persistently produce high levels of IgE antibodies against common environmental substances. Rather, they could be compared to normal individuals who may be induced to produce IgE antibodies only following exceptionally provoking stimuli such as parenteral immunization with certain antigens (Leskowitz and Lowell, 1961; Schwarz and Terr, 1971; Marsh, Lichtenstein and Norman, 1972) or following infection with helminth parasites (reviewed by Bennich and Johansson, 1971).

During our investigations of the role of parasitic infection in the biosynthesis of IgE we found that secondary IgE responses to EA occurred in Hooded Lister rats which had been immunized with EA and on which skin tests with EA were subsequently performed. The very small amount of antigen used in the skin tests had apparently acted as a booster for the evocation of high levels of reaginic antibody. (The effect of route of administration of antigen on IgE production will be the subject of a further report). As a result of this finding we performed a series of experiments to determine the conditions of antigen presentation which dispose to the production of IgE antibody in these rats.

In most of the experiments described here egg albumin (EA) was used as the antigen. For the induction of primary responses it was injected intraperitoneally together with *B. pertussis* suspension as adjuvant. In order to elicit secondary responses the antigen was injected intraperitoneally without adjuvant. In one experiment keyhole limpet haemocyanin (KLH) was used for comparative purposes.

Primary reagin responses could be induced with doses ranging from 1 mg to 1μg of EA (Tables 1–3). Circulating reagins could not be detected after the injection of 0·1 μg EA,

but it became evident that a proportion of animals receiving this dose were nevertheless primed for reagin production since a response could be elicited in them following a second even smaller dose of EA given without adjuvant (e.g. Table 7). On the other hand, animals receiving 0·01 μg EA or less as an immunizing dose did not produce reagins then, or on subsequent stimulations. With KLH it was found that the pattern of reagin production was similar, but that the dose had to be increased by a factor of 10. Thus 10 μg was the lowest dose which resulted in detectable circulating reagin and a dose of 1 μg primed the animals for response to a second dose. It may be that the degree of association of the KLH preparation has a bearing on the minimum dose; White and Holm (1973) have found that associated and dissociated forms of haemocyanin result in dissimilar patterns of reagin production in rats.

Hooded Lister rats are not the only strain of rats able to produce reaginic antibody following immunization with small doses of antigen. A reagin response has been reported to occur in Sprague–Dawley rats after inhalation of an estimated dose of 12·8 μg of EA (Van Hout and Johnson, 1972) or intraperitoneal injection of 10 μg of EA (Bloch, Ohman, Waltin and Cygan, 1973). In our own (unpublished) experiments we found, however, that Wistar or CFY rats do not produce reagins following doses of 1 μg, although they do so after immunization with 1 mg.

The level of the primary reagin response was unaffected by the amount of antigen given. The PCA titres in groups of animals given doses of EA ranging from 1 mg to 1 μg were similar and fell in the range of 2–256 with occasional animals having titres of 512–1024.

Whatever the dose it was found that the levels of reaginic antibody did not change significantly between days 12–26 after immunization. It was also established that following a dose of 1 μg of EA reagins were still detectable in the serum 51 days later (Fig. 5).

Secondary reagin responses could be induced with doses ranging from 100 μg to 0·001 μg of EA. Secondary responses can thus be induced with doses of antigen which are far smaller than that required to initiate reagin production in the first instance. We have found on the other hand that the injection of larger doses of antigen, e.g. 1 mg of EA, almost invariably results in fatal anaphylaxis.

The level of the secondary reagin response was determined by the amount of antigen given on the first occasion and was not influenced by the size of the second dose of antigen. Most animals immunized with 1 mg of EA had a poor secondary response or no secondary response at all. This was found to be true following a wide range of second doses of antigen (100–0·01 μg). By contrast many animals immunized with 1 μg of EA showed considerable secondary responses with PCA titres up to 16,384 in response to the same booster doses of antigen (Tables 3 and 4).

The results set out in Table 5 and Fig. 4 show that the amount of antigen given as a booster had no effect on the level of the secondary response since groups of animals, immunized with 1 μg EA and challenged with a variety of doses ranging from 10 μg to 0·001 μg EA produced similar levels of reaginic antibody.

The elevated reagin levels of the secondary response were found to decline within 1–2 weeks to the region of pre-challenge levels (Fig. 5), which were then maintained for periods of at least 1 month. As it has been shown that IgE antibody production in several species is subject to feedback control by IgG antibody directed against the same antigen (Strannegard and Belin, 1970; Tada and Okumura, 1972; Ishizaka and Okudaira, 1972), it is possible that the rapid decline in the reagin levels of the secondary response is caused by an inhibitory effect of post-formed IgG antibodies on IgE-synthesizing cells.

Such a mechanism might also be the cause of our failure to obtain repeated booster responses. Thus we have found that although rats were still producing low levels of reagin following the decline of their secondary response, it was not possible to re-elevate the levels by injecting a third dose of antigen.

Levine and Vaz (1970) obtained repeated booster reagin responses in some strains of mice with small (0·1 μg) doses of EA. In their experiments aluminium hydroxide gel was used as adjuvant and was given not only with the first dose of antigen but also with every booster dose. It may be, therefore, that the continued use of adjuvant is a critical requirement for the production of multiple booster responses.

The above authors also found that high levels of IgG homocytotropic antibody (see review on the heat-stable homocytotropic antibodies by Bloch and Ohman, 1971) were produced in the responding strains of mice following immunization with low doses of antigen. In contrast, we were unable to detect any heat-stable rat (IgG) homocytotropic antibody in the experiments described here.

Our system of immunization frequently led to the development of high titres of predominantly IgG haemagglutinating antibody, and this raised the possibility that these antibodies might be functioning as suppressors of IgE synthesis. Against this was the fact that haemagglutinins appeared during the primary response and since their presence did not apparently interfere with the development of the first booster response it seemed unlikely that it should do so with the second. To resolve the question we attempted to induce tertiary responses in rats given minute first and second doses of EA (0·1 μg and 0·001 μg respectively) in order to minimize the stimulation of IgG (haemagglutinating) antibodies. Although this objective was achieved in that the rats produced very low or undetectable levels of haemagglutinating antibody, a tertiary reagin response was still not obtained (Table 7). If our inability to produce tertiary reagin responses is caused by a feedback inhibition by IgG, then clearly this is not effected by those antibodies which we detect in the haemagglutination test. Another antibody, or alternatively another mechanism, must be sought to explain the lack of tertiary reagin responses.

There is now compelling evidence for the existence of a population of suppressor T cells which can specifically inhibit the response of B cells (Baker, Stashak, Amsbaugh, Prescott and Barth, 1970; Gershon and Kondo, 1971; Jacobson, Herzenberg, Riblet and Herzenberg, 1972; Okumura and Tada, 1971, 1973). Whether these are the same T cells which in other circumstances help B cells to produce antibody, or a different population, is not yet clear, but in any event it is possible that in our system such suppressor cells might be preferentially activated following large doses of antigen and/or following the second injection of antigen without adjuvant, so that the balance is tipped from a positive to a negative regulation of B-cell activity. This could then explain the inhibitory effect of large primary doses of antigen on the secondary response, the rapid decline of secondary responses where they occur, and our inability to produce tertiary responses.

It is also possible that a combination of IgG feedback effect and T-cell suppressor activity is involved in these phenomena.

Levine and Vaz (1970) and Vaz, Vaz and Levine (1971) have demonstrated that certain strains of mice may be induced to produce reaginic antibody by the injection of small doses of antigen, whereas other strains may not. The differences in antibody production were related to the histocompatibility (H-2) type of the strains (Vaz and Levine, 1970). The implications of their findings for the genetics of human allergic disease have been extensively discussed by these authors, who also suggest that the reason why reagin pro-

duction may be so effectively induced with small doses of antigen is that the precursors of IgE-forming cells may have a higher avidity for antigen than cells destined to produce immunoglobulin of other classes.

Our results support the general proposition that reagin production forms a prominent part of the immune response to minute doses of antigen in animals of suitable genetic predisposition. In addition, some interesting features of such responses have been revealed, the immunological implications of which can be subjected to experimental investigation.

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