Immunology, 1972, **23**, 749.

Potentiation of Rat Reaginic (IgE) Antibody by Helminth Infection

SIMULTANEOUS POTENTIATION OF SEPARATE REAGINS*

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(*Received* 5*th April* 1972)

Summary. Further evidence has been produced that rat reaginic antibody responses which have been programmed by the injection of antigen with *Bordetella pertussis* can be greatly potentiated by a subsequent infection with the helminth parasite *Nippostrongylus brasiliensis*. Potentiated responses to keyhole limpet haemocyanin and house dust extract have been demonstrated.

It is shown that the timing of the parasitic infection must take into account the kinetics of the reagin response to the particular antigen under test.

Simultaneous potentiation of two reagin responses could be demonstrated; occasionally selective potentiation of one reagin response was found to occur.

The possible relevance of these mechanisms for the development of clinically manifest immediate hypersensitivity is discussed.

INTRODUCTION

Rats can be programmed to produce reaginic antibody by injection of ovalbumin together with *B. pertussis* (Mota, 1964) and the response can be considerably augmented by subsequent infection with the nematode parasite *N. brasiliensis* (Orr and Blair, 1969). Similar potentiation of reagins to ovalbumin has also been demonstrated following infection of rats with the trematode parasite *Fasciola hepatica* (Jarrett, 1972).

While biosynthesis of IgE antibody against helminth antigens is a prominent result of infection with many helminth parasites (see reviews by Ogilvie and Jones, 1969; Jarrett and Urquhart, 1971), the above results establish an additional function of helminth infection in causing potentiation of the IgE antibody response to non-helminthic antigens, a mechanism which may have important implications for the development of allergic responses.

The applicability of the above as a general principle has been explored using other antigens (keyhole limpet haemocyanin and house dust extract). Preliminary experiments with these antigens proved unsuccessful if the original time scale was adhered to.

This paper also demonstrates that two already established reagin responses can be simultaneously augmented by *N. brasiliensis* infection.

* Departmental publication No. 7203.

MATERIALS AND METHODS

Animals

150–200 g female Hooded Lister rats were used in all the experiments.

Parasite

The strain of *N. brasiliensis* used has been maintained for several years by repeated subinoculation in rats in the Wellcome Laboratories for Experimental Parasitology, the University of Glasgow. Rats were infected by s.c. inoculation of 4000 larvae. The technique for culture of the parasite is described by Jennings, Mulligan and Urquhart (1963).

Antigen

Egg albumin (Sigma Grade V) and keyhole limpet haemocyanin (Calbiochem, Ltd A grade) were made up as solutions in 0·15 M saline. Rats were injected i.p. with 1 mg in 0·1 ml saline together with *B. pertussis* suspension. House dust extract (150 per cent) was kindly provided by Bencard, Brentford, Middlesex. Rats were injected i.p. with 0·2 ml of the extract together with *B. pertussis* suspension. *B. pertussis* suspension of heat killed bacteria (Wellcome Reagents, Ltd) was injected at a dose of 1×10^{10} organisms per rat.

A phenol extract of house dust mite (Dermatophagoides pteronyssinus), kindly provided by Dr J. Kerr, the Western Infirmary, Glasgow, was also used as antigen in the PCA titration of house dust reagins.

Collection and titration of reaginic sera

The level of circulating reagins was examined using serum collected by serially bleeding rats from the tail vein at strategic times after the various treatments, or by heart puncture for final exsanguination. The individual sera were titrated for reaginic activity by passive cutaneous anaphylaxis (PCA) tests in the rat. These were carried out by intradermal injection of saline dilutions (0·1 ml), each dilution being duplicated on separate test animals. 48–72 hours later the animals were injected intravenously with one of the antigens as follows: ovalbumin 2·5 mg, keyhole limpet haemocyanin 2·5 mg, house dust extract 0·25 ml or *N. brasiliensis* antigen 0·5 ml (saline extract of 1000 homogenized worms/ml) together with 0·5 ml of a 1 per cent Evans' blue solution. The reaginic titre recorded is the reciprocal of the greatest dilution which gave skin reaction sizes larger than 5 mm.

RESULTS

REQUIREMENTS FOR PROGRAMMING AND POTENTIATION OF REAGIN RESPONSE TO KEYHOLE LIMPET HAEMOCYANIN (KLH)

It had been found in an experiment involving twenty rats that a potentiated reagin response to KLH did not occur if the *N. brasiliensis* infection was given 10 days after injection of the antigen and *B. pertussis*. No reagins to KLH were present 12 days after the parasitic infection. The following experiment was therefore done to determine the time of appearance of circulating KLH reagins and to infect the animals with *N. brasiliensis* after this had occurred.

Twenty rats were injected with KLH and *B. pertussis* suspension (see Materials and Methods) and groups of five of these animals were bled from the tail vein 10, 17 and 24 days later. Assay of these sera by PCA showed that circulating reagins appeared in most

of the animals between day 17 and 24. All twenty rats were bled on day 26 and titration of the sera showed that fourteen rats had KLH reagin titres ranging from 4–128 (reactors) while six animals had no detectable reagins (non-reactors).

The group of reactor animals was divided into two and infection of one of these groups on day 28 with *N. brasiliensis* resulted in marked potentiation of the KLH reagin response in the majority of animals (Table 1). This did not occur in the uninfected rats.

TABLE 1

POTENTIATED REAGINIC ANTIBODY RESPONSE TO KEYHOLE LIMPET HAEMOCYANIN (KLH)

Treatment		Rat No.	Reciprocal of circulating KLH reagin titre	
			D26 after antigen injection	D40 after antigen (D.12 after *N. brasiliensis*)
Group 1	Day 0. 1 mg KLH and 1×10^{10} *B. pertussis* organisms i.p. Day 28. 4000 *N. brasiliensis* larvae s.c.	1	128	2
		2	4	16
		3	16	4096
		4	16	1024
		5	4	1024
		6	8	2048
		7	32	4096
Group 2	Day 0. 1 mg KLH and 1×10^{10} *B. pertussis* organisms i.p.	8	4	2
		9	4	2
		10	8	8
		11	64	2
		12	8	4
		13	64	2
		14	16	16

TABLE 2

POTENTIATED REAGINIC ANTIBODY RESPONSE TO HOUSE DUST EXTRACT

Rat No.	Reciprocal of circulating house dust reagin titre	
	D12 after antigen* injection	D27 after antigen (D12 after *N. brasiliensis*)
1	2	2048
2	2	128
3	128	512
4	0	0
5	128	2048
6	2	16
7	2	2048
8	32	4096
9	0	0
10	64	512

* Rats were injected with 0·2 ml house dust extract (Bencard 150 per cent) together with 1×10^{10} *B. pertussis* organisms i.p.

The non-reactor animals had not produced circulating reagins by the end of the experiment, nor was hypersensitivity to intradermally injected KLH demonstrable in these rats.

REQUIREMENTS FOR PROGRAMMING AND POTENTIATION OF REAGIN RESPONSE TO HOUSE DUCT EXTRACT (HDE)

Table 2 shows the results of an experiment in which the induction and potentiation of reaginic antibodies to house dust extract was achieved. Reaginic antibodies were present in the circulation of 8/10 rats 12 days after injection of the antigen with *B. pertussis*. Infection with *N. brasiliensis* 15 days after sensitization resulted in a potentiated response in six of these animals.

It was established using the phenol extract of cultured house dust mite (see Materials and Methods) as antigen in PCA titrations that the potentiation involved in particular reagins to the mite component of the crude Bencard extract which was used to sensitize the animals.

SIMULTANEOUS POTENTIATION OF REAGINS TO OVALBUMIN (OV) AND KEYHOLE LIMPET HAEMOCYANIN (KLH)

From the previous experience it might be expected that the nematode infection might potentiate the IgE component of any previously programmed immune response. The possibility that the infection could potentiate several reagin responses at the same time was explored.

A group of ten rats were sensitized to both OV and KLH by injection of a mixture of these antigens together with *B. pertussis*. On day 23 after injection when the majority of rats were producing reagins to both antigens they were infected with *N. brasiliensis*. The

TABLE 3

SIMULTANEOUS POTENTIATION OF REAGINIC ANTIBODIES TO OVALBUMIN (Ov) AND KEYHOLE LIMPET HAEMOCYANIN (KLH)

	Reciprocal of circulating reagin titres			
	Day 23 after injection of antigens*		D35 after antigens (D12 after *N. brasiliensis*)	
Rat No.	OV	KLH	OV	KLH
1	4	4	256	256
2	32	64	500	500
3	16	128	2048	2048
4	32	128	128	128
5	32	4	4096	2048
6	64	128	4096	2048
7	32	0	64	0
8	32	0	0	0
9	0	256	4096	4096
10	32	128	2048	2048

* 1 mg of both antigens injected together with 1×10^{10} *B. pertussis* organisms i.p.

results in Table 3 show that marked potentiation of both OV and KLH reagins occurred in five animals (rats 3, 5, 6, 9 and 10) with a lesser degree of potentiation in a further two (rats 1 and 2). The experiment was repeated (Table 4). On this occasion sera obtained 12 days after *N. brasiliensis* infection showed potentiated reagin responses for both antigens in all

the animals. On day 27 after *N. brasiliensis* the OV and KLH reagins were found to have declined to a titre of less than 16 while the reagins specific for the parasite had appeared and attained a high titre.

TABLE 4

SIMULTANEOUS POTENTIATION OF REAGINIC ANTIBODY RESPONSE TO OVALBUMIN (OV) AND KEYHOLE LIMPET HAEMOCYANIN (KLH) Exp. 2

	Reciprocal of circulating reagin titres				
	Day 12 after *N. brasiliensis*		Day 27 after *N. brasiliensis*		
Rat No.	OV	KLH	OV	KLH	*N. brasiliensis*
1	512	512	2	8	2048
2	512	512	0	4	2048
3	256	512	4	8	4096
4	2048	2048	16	2	1024
5	512	1024	2	8	1024
6	2048	2048	4	16	4096
7	1024	512	2	2	2048
8	2048	2048	4	16	2048
9	1024	1024	0	16	1204
10	1024	1024	2	2	512
11	512	512	4	0	4096
12	512	512	2	2	1024

The results of a further experiment set out in Table 5 show that simultaneous potentiation does not always occur. In this experiment ten rats were injected with a mixture of OV, KLH and HDE together with *B. pertussis.* Five of the animals produced reagins against all three antigens and those rats were infected with *N. brasiliensis.* It is evident that whereas the ovalbumin reagins were potentiated in all the rats, and in two rats to very high levels, potentiation of haemocyanin reagins occurred to a much lesser extent and to house dust extract not at all.

TABLE 5

REAGINIC ANTIBODY RESPONSE TO THREE ANTIGENS FOLLOWING *N. Brasiliensis* INFECTION

	Reciprocal of circulating reagin titres 12 days after *N. brasiliensis*		
Rat no.	OV	KLH	HDE
1	1024	1024	0
2	2045	128	0
3	512	0	0
4	16384	512	128
5	8192	512	128

DISCUSSION

The results reported here show that it is possible to induce reaginic antibodies to keyhole limpet haemocyanin (KLH) and to house dust extract (HDE) in the rat and to greatly potentiate the circulating levels of these antibodies by subsequent infection with the nematode parasite *N. brasiliensis.*

It is evident that the time of appearance of cirulating reagins after initial stimulation may differ for different antigens. Thus reagins to OV or HDE are present in the circulation 10–12 days after injection of these antigens together with *B. pertussis* whereas circulating reagins to KLH do not appear until 17–24 days after injection. This fact is an important consideration in the timing of the parasitic infection. KLH reagins could be potentiated if rats were infected 23 or 28 days after injection of the antigen, but not if they were infected 10 days after injection, the time at which OV reagins can be successfully potentiated. This emphasizes the previous finding (Jarrett *et al.*, 1972) that parasitic infection will not cause *de novo* synthesis of reagins to unrelated antigen but is capable only of potentiating an already existing reagin response.

The results shown in Tables 3 and 4 establish that the potentiating effect of *N. brasiliensis* infection may embrace more than one reaginic antibody response. Thus KLH and OV reagins were simultaneously potentiated in infected rats.

The potentiated responses were found to be of short duration, a result similar to that previously described for OV reagins in both *N. brasiliensis* (Orr, Riley and Doe, 1971) and *F. hepatica* infections (Jarrett, 1972). The rapid decline of such responses has been discussed in a previous paper (Jarrett, Orr and Riley, 1971) in which it was shown that it is probable that the decline in heterologous reagin titre is partly an apparent effect caused by the rise of homologous (*N. brasiliensis*) reagins which compete for mast cell sensitization sites in the PCA assay.

An attempt was made in these experiments to determine the effect of second injections of OV, KLH or *N. brasiliensis* in various permutations. This was frustrated by the high *N. brasiliensis* reagin levels which persisted in the rats for several months and whose presence interfered with the accurate assay of OV and KLH reagins by passive cutaneous anaphylaxis (Jarrett *et al.*, 1971). While the accurate assay of individual reagins in serum mixtures of reagins is clearly not possible by the *in vivo* method of passive cutaneous anaphylaxis an *in vitro* method such as the radio-allergo-sorbent test developed by Wide, Bennich and Johansson (1967) for the assay of human reaginic antibodies would be expected to achieve this successfully.

In the experiments involving two reagins it was a striking fact that both reaginic antibodies were usually potentiated to similar levels in individual rats (see Tables 3 and 4). This appeared to be in accord with the general hypothesis that a helminthic factor could stimulate the antibody-producing cells of any specificity previously programmed for IgE production. However, the results of the experiment in Table 5 and others unpublished showed that selective potentiation of a reagin response could occur. The precise circumstances responsible for this result are not yet clear.

An extrapolation of these results to the clinical situation suggests that in addition to causing an antiparasitic reagin response the role of a helminth infection could be that of elevating circulating levels of one or more existing IgE antibody responses without initiating their production.

ACKNOWLEDGMENTS

Dr Ellen Jarrett is in receipt of a Research Fellowship from the Wellcome Trust and the work was supported by grants from the Medical Research Council and Fisons Ltd, Pharmaceutical Division. We would like to thank Professor R. G. White for helpful discussion.

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