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# Time course studies on rat IgE production in *N. brasiliensis* infection

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## SUMMARY

We present here a study of the relationship in time between the elevation of total serum IgE, the parasite-specific IgE response, and the potentiated IgE response to unrelated antigen which occurs in rats following infection with the worm parasite *N. brasiliensis.*

During a first infection the potentiated IgE response (to egg albumin) and elevation of total IgE occur synchronously rising to a peak on days 12–14 after infection, with the fastest rate of increase occurring between days 8 and 10. *N. brasiliensis*-specific IgE rises to a peak some 2–3 weeks later when both total IgE and the potentiated response have largely declined.

A strain difference is shown in that Wistar rats produce far lower levels of total and parasite-specific IgE than Hooded Listers.

Events following reinfection differ in that total IgE rises more rapidly, very high levels being reached 6 days after reinfected together with a secondary specific IgE response to *N. brasiliensis.* The total IgE level, however, rises by a far greater factor than parasite-specific IgE and declines rapidly while the parasite-specific response declines slowly over many weeks. The egg albumin response is not repotentiated.

It is proposed that the total IgE response and the potentiated IgE response which forms a small component of it results from the release of a non-specific IgE-stimulating factor produced by *N. brasiliensis*-specific T cells. In this scheme the same or similar cells are involved in the production of *N. brasiliensis*-specific IgE through a separate specific helper function.

## INTRODUCTION

Infection with helminth parasites is, in all species, an exceptionally potent stimulus for the production of IgE. In rats infected with the nematode *Nippostrongylus brasiliensis* the IgE response shows itself in three ways: the production of a high level of parasite specific reaginic antibody (Ogilvie, 1964), the potentiation of reaginic antibody responses to unrelated antigens (Orr & Blair, 1969), and a great elevation of total serum IgE (Jarrett & Bazin, 1974).

Here we present a study of the relationship in time between these three responses and incorporate the findings into our present understanding of the mechanism of stimulation of IgE production in helminth infection.

## MATERIALS AND METHODS

*Animals.* One hundred and fifty to 200 g outbred female Hooded Lister rats (Animal Suppliers Ltd., London), were used in all the experiments. In one, an additional group of Wistar (Carworth CF) rats of similar weight was incorporated to study the effect of strain difference.

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*Parasite. N. brasiliensis* is maintained in this laboratory by repeated sub-inoculation in Hooded Lister rats. The method for culture is described by Jennings, Mulligan & Urquhart (1963). Rats were infected by subcutaneous inoculation of 4000 larvae in 1 ml saline.

*Immunization and IgE antibody determinations.* These methods have been fully described in a previous publication (Jarrett & Stewart, 1974). Briefly, rats were immunized by intraperitoneal injections of egg albumin (EA-Sigma grade V) together with $10^{10}$ *Bordetella pertussis* organisms (Wellcome Biological Reagents *B. pertussis* suspension). The animals were bled from the tail or occasionally by heart puncture, at appropriate times after immunization and parasitic infection. The level of circulating reagins was determined by passive cutaneous anaphylaxis (PCA) tests (Ovary, 1964) performed on Hooded Lister rats. 0·1 ml of dilutions of test serum in saline were injected intradermally into each of duplicate rats. After 48–72 hr each rat was inoculated intravenously with 2·5 mg EA or 0·5 ml *N. brasiliensis* antigen (saline extract of 1000 homogenized worms/ml) together with 0·5 ml 1% Evans blue. Titres of reaginic antibody are expressed as the greatest dilution of serum giving a PCA reaction of 5 mm or more.

*Estimation of total serum IgE* was by the radioactive single radial-diffusion technique of Rowe (1969). The method of preparation of the specific immunological reagents used in this assay, i.e. purified rat IgE, goat anti-rat IgE, rabbit anti-goat IgG ($^{125}$I-labelled), and reference preparation of known IgE content, have been fully described elsewhere (Jarrett & Bazin, 1974, Bazin *et al.*, 1973, 1974).

Dilutions of anti-rat IgE in the agar from 1/100 to 1/4000 could be used. The last dilution gave the greatest sensitivity enabling estimation in the range of 0·69–22·24 μg/ml of IgE. The reference preparation (IR162 Temoin) was estimated to contain 5·7 mg/ml of rat IgE.

## RESULTS

### *Time course of elevation of total IgE and N. brasiliensis-specific IgE during infection*

We have previously shown that total serum IgE is greatly elevated in Hooded Lister rats 12 days after infection with *N. brasiliensis*, whether or not they have been previously immunized with egg albumin and before the time when *N. brasiliensis* (*N.b.*)-specific reagins have appeared in the serum (Jarrett & Bazin, 1974). In the first experiment described here, the progress of the total IgE response was followed in a group of rats bled repeatedly over a period of 80 days with and without a reinfection. The results in Table 1 show that total mean IgE rose between days 6 and 12 after infection from 1·28 to 247 μg/ml and thereafter declined, at first rapidly and then more slowly to reach a level of 25 μg/ml on the 80th day after infection. *N.b.* reagins appeared in the serum and rose in level during the decline of the total IgE

TABLE 1. Total IgE and parasite-specific IgE in the serum of Hooded Lister rats infected with 4000 *N. brasiliensis* larvae

| Days after infection | Total IgE (μg/ml) mean ± s.e. | *N. brasiliensis* PCA titre–GM (range)† |
|---|---|---|
| 6* | 1·28 ± 0·34 | — |
| 12* | 247 ± 42 | — |
| 18* | 104 ± 8·0 | 548 (256–1024) |
| 24* | 46 ± 4·7 | 723 (256–1024) |
| 34 | 82 ± 18 | 890 (512–1024) |
| 40 | 62 ± 8·3 | 675 (64–2048) |
| 80 | 25 ± 5·4 | 147 (64 ± 512) |
| Days after reinfection | | |
| 6 | 376 ± 26 | 2350 (1024–4096) |
| 12 | 364 ± 29 | 891 (512–1024) |
| 40 | 29 ± 3·9 | 294 (128–1024) |

* Group of ten rats. On D twenty-eight rats divided into two groups of five and one group reinfected. The same rats were bled repeatedly on the days shown.

† GM = geometric mean.

response so that the peak of the parasite-specific response occurred 2–3 weeks after the peak of the total IgE. After a second infection total IgE rose again (376 μg/ml), this time by the 6th day after reinfection, together with an increase in *N.b.*-specific reagin levels.

Table 2 shows the results of an experiment in which Wistar and Hooded Lister rats were compared for IgE production during *N.b.* infection. The total IgE response of the Wistar rats was strikingly less than that of the Hooded Listers, although quite high levels were reached especially after a second infection. The Wistar rats also produced lower levels of *N.b.*-specific reagins.

The kinetics of the total IgE response were studied over the period when IgE levels are rising. Fig. 1 shows that the fastest rate of increase in total IgE occurred between days 8 and 10 after infection. Thereafter, the rate declined each day until day 13 when the IgE level began to fall.

TABLE 2. Total IgE and parasite-specific IgE in serum of Wistar and Hooded Lister rats during *N. brasiliensis* (*N.b.*) infection

| Days after infection | Total serum IgE (μg/ml) | | *N.b.* PCA titre GM (range) | |
|---|---|---|---|---|
| | Wistar* | Hooded Lister† | Wistar | Hooded Lister |
| 13 | 50±16 | 226±42 | — | — |
| 17 | 15±2·6 | 96±16 | 54 (8–256) | 63 (1–256) |
| 24 | 8·7±2·1 | 45±7·7 | 30 (1–512) | 76 (32–512) |
| 34 | 3·9±1·7 | 11±1·5 | 26 (1–1024) | 511 (32–1024) |
| Days after reinfection | | | | |
| 6 | 163±42 | 452±72 | 1096 (128–4096) | 2042 (1024–4096) |
| 12 | 76±22 | 353±111 | 548 (128–8192) | 776 (128–2048) |
| 34 | 7·4±1·8 | 22±2·6 | 97 (16–512) | 512 (512) |

* Ten rats; † five rats bled repeatedly on days shown.

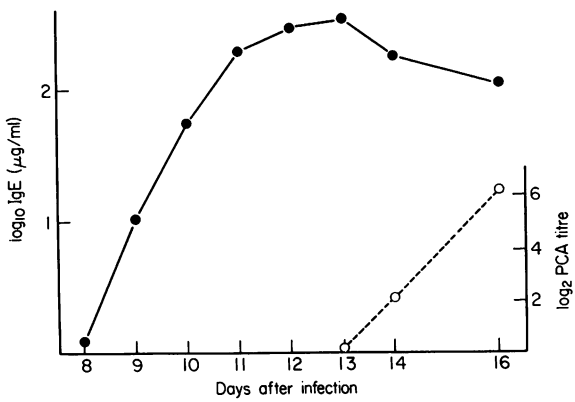


FIG. 1. Kinetics of total IgE production (●——●) in *N.b.*-infected rats, studied by bleeding out groups of five rats on the days shown. *N.b.*-specific IgE (○ - - - ○) first detected on day 14 after infection.

*The potentiated response and total IgE*

We have carried out several time course experiments on rats immunized with egg albumin (EA) and infected subsequently with *N. brasiliensis* in order to relate both EA- and *N.b.*-specific IgE responses to total serum IgE.

The results of two of these experiments, shown in Table 3 and in Fig. 2, can be summarized as follows: following a first infection, EA reagins and total IgE rise to peak together at 12–14 days after infection. These two responses also decline together and during this decline *N.b.*-specific reagins appear and rise to high levels in the serum. The *N.b.*-specific response declines only slowly over many weeks.

The events following a second infection are different, in that total IgE rises within 6 days to its former high peak or even higher. *N.b.*-specific reagin levels are also boosted at the same time, but EA reagins on the other hand decline to very low levels or disappear entirely from the serum. We have not found it possible to cause re-elevation of the EA response by a second *N. brasiliensis* infection.

TABLE 3. Relationships in time of potentiated, parasite-specific and total IgE responses in *N. brasiliensis* infection (five rats)

| Days after infection | Total IgE mean ± s.e. (μg/ml) | EA PCA titre GM (range) | *N.b.* PCA titre GM (range) |
|---|---|---|---|
| 6 | 1·44 ± 0·05 | 12 (2–64) | — (0) |
| 9 | 12·3 ± 3·18 | 193 (64–1024) | — (0) |
| 14 | 334 ± 69 | 1350 (1024–2048) | — (0) |
| 17 | 117 ± 34 | 387 (64–1024) | 147 (8–1024) |
| 21 | 108 ± 16 | 97 (32–256) | 387 (128–1024) |
| 55 | 48 ± 8 | 24 (16–32) | 511 (128–1024) |
| 75 | 26 ± 6 | 9 (1–16) | 293 (16–1024) |
| Days after reinfection | | | |
| 6 | 227 ± 87 | (0) | 1551 (1024–2048) |
| 12 | 332 ± 67 | (0) | 1023 (512–2048) |

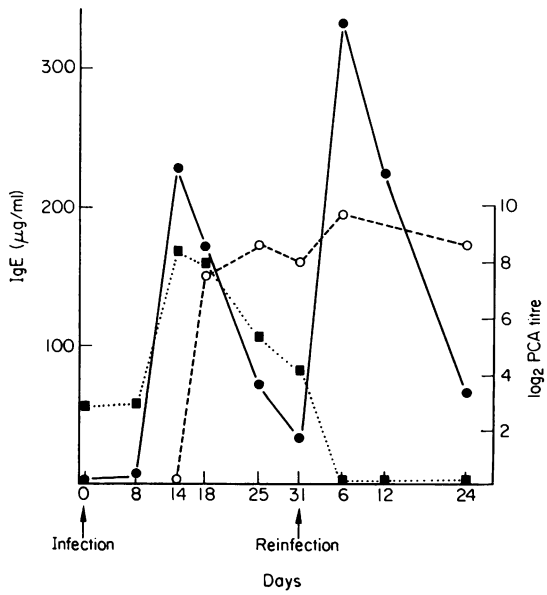


FIG. 2. Relationship in time between total IgE and egg albumin and *N.b.*-specific IgE responses in a first and second *N.b.* infection of ten rats bled repeatedly on the days shown. (●———●) Total IgE; (■ · · · ■) EA reagins; (○ – – – ○) *N.b.* reagins.

## DISCUSSION

We have previously shown that infection of the rat with *N. brasiliensis* leads to a great elevation of total serum IgE and that the bulk of this IgE is surplus to concentrations expected against known antigens (Jarrett & Bazin, 1974).

The time course studies reported here, show that while the peak of the total IgE response occurs on days 12–14 after infection, the fastest rate of increase in IgE levels occurs earlier, between days 8 and 10. The indications are that the particular activity of the parasite which stimulates IgE production occurs to maximum effect shortly before or during this latter period, and thereafter declines. The decline may be partly due to the progressive reduction in the number of worms in the intestine, since *N. brasiliensis* infection is terminated by an immunological expulsion of worms starting 10–11 days after infection and proceeding exponentially over several days (Jarrett, Jarrett & Urquhart, 1968a). Other results results suggest, however, that an additional factor may operate. Thus, infection of rats before 6 weeks of age results in a form of diminished immunological responsiveness which shows itself in a reduced ability to expel worms (Jarrett, Jarrett & Urquhart, 1966, 1968b). In such rats, total serum IgE levels rise and fall at the same time as in adult rats, despite the continuing presence of a sizeable unexpelled worm burden (our unpublished results).

Previous experiments involving modification of the life cycle of the parasite in the host (Jarrett & Stewart, 1973a) have shown that it is the adult worm in the intestinal lumen, rather than the migrating larval stage, which exerts the greatest IgE-potentiating effect. The stimulus for IgE production is presumably a soluble factor secreted by the parasite and absorbed across the intestinal epithelium. Adult worms affected by immunity show structural damage particularly of the gut cells which appears before worms are expelled (Ogilvie & Hockley, 1968). The worms present in young rats show similar morphological changes even although they are not subsequently expelled. It could be, that 'damaged' worms cease to secrete the IgE-stimulating factor and that IgE levels therefore fall.

In a first infection with *N.b.*, the EA-potentiated reagin response and the total serum IgE response occur together in time; almost certainly they are both the result of the same stimulus (Table 3, Fig. 2) and the potentiated reagin response could be regarded as an indicator system within a larger phenomenon.

*N.b.*-specific reagins, on the other hand, are not detectable in the serum until 16 days or so after infection, by which time both potentiated and total IgE responses are declining. We know, however, that parasite-specific reagins are being produced in the infected rat long before this time. Their presence can be demonstrated as early as 10 days after infection by the occurrence of immediate skin reactions following intradermal, or anaphylactic shock following intravenous injection of *N.b.* antigen (Jarrett & Stewart, 1973b). The possibility has not yet been ruled out that *N.b.*-specific IgE is present in the circulation before 16 days after infection, but that it is rendered undetectable in the PCA test by complexing with worm antigen.

Events following reinfection are rather less straightfoward. We do not know why the EA reagin response is not re-potentiated after a second *N.b.* infection. Perhaps a suppressor mechanism is activated, or alternatively the cells involved are no longer in a state of susceptibility to the potentiating effect.

Total serum IgE, on the other hand, rises again to even greater levels than in a first infection, this occurring by the 6th day after infection synchronous with the *N.b.*-specific secondary IgE response. However, since total IgE rises by a far greater factor than *N.b.*-specific IgE the antibody content of a large proportion of this total IgE remains to be accounted for. We have postulated previously that the total IgE response after a first infection consists at least in part of a number of potentiated reagin responses to miscellaneous unknown antigens to which the animal has become naturally sensitized (Jarrett & Bazin, 1974). This is a reasonable assumption, since the known IgE responses are potentiated following a first infection (Jarrett & Stewart, 1972). This is not the case following reinfection and while it is possible that the EA responses is an exception and that other naturally occurring responses are re-potentiated, it is less easy to be sure that the total IgE is largely constituted of multiple potentiated responses on this occasion. This point must be explored further.

Finally, what can be deduced from the earlier rise of total IgE levels in reinfection? We know that T cells are essential in *N. brasiliensis* infection both for the occurrence of the potentiated reagin response (Jarrett & Ferguson, 1974) and for the elevation of total serum IgE (Jarrett & Ferguson, unpublished results). The fact that a reinfection stimulates a massive total IgE response at the same time as the secondary *N.b.* response, indicates that it is *N.b.*-specific T cells which are activated by the parasite to produce the IgE-stimulating factor. This concept is in agreement with recent findings of Kojima & Ovary (1975) in IgE potentiation experiments in mice.

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