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The immune response to type III pneumococcal polysaccharide in mice with malaria

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SUMMARY

The immune response of BALB/c mice to type III pneumococcal polysaccharide (SIII), as measured by splenic PFC, was abolished at the height of an acute self-limiting attack of malaria caused by the murine plasmodium *P. yoelii*, over a wide range of antigen doses. The response to antigen, given at various times after clinical recovery, gradually reappeared, but did not reach normal levels until 12 weeks after the injection of the parasite. A second injection of *P. yoelii* given 1 hr before SIII caused a moderate degree of depression, although in this case the plasmodium does not multiply. In chronic malaria the response to SIII was also very poor. Short term under-nourishment was found to reduce only slightly the response to SIII.

INTRODUCTION

During malarial infection mice show depressed humoral immune responses to some antigens, but respond normally to others. (Salaman, Wedderburn & Bruce-Chwatt, 1969; Greenwood, Playfair & Torrigiani, 1971a; Barker, 1971; Voller, Gall & Manawadu, 1972).

Several theories have been advanced to account for this selective effect on antibody production. Loose and his colleagues (Loose, Cook & Di Luzio, 1972; Loose, Trejo & Di Luzio, 1971) and also Greenwood (1974), have suggested that certain aspects of macrophage function may be defective in murine malaria. The effect of malaria on T-cell function seems to be variable. Greenwood *et al.* (1971a) reported that contact sensitivity and allograft rejection were normal in *P. yoelii* infections. On the other hand, we found (Wedderburn, 1974) that a proportion of mice injected with *P. yoelii* on the day of grafting of allogeneic skin showed lengthened graft survival times.

It seems that infection with the lethal *Plasmodium berghei* may effect T-cell function more severely. Sengers, Jerusalem & Doesburg (1971) observed considerably lengthened graft survival times in mice undergoing prolonged infection with *P. berghei* and Jayawardena *et al.* (1975) found that contact sensitivity to oxazolone was depressed in this disease, although not in *P. yoelii* induced malaria.

The response of mice to type III pneumococcal polysaccharide (SIII) has been considered to be thymus-independent (Howard *et al.*, 1971b). We report here the effect of an acute and also of a chronic malarial infection on the response to this antigen.

MATERIALS AND METHODS

Groups of five inbred female BALB/c mice were used. *Plasmodium yoelii* (17X) and *Plasmodium berghei* (Anka strain) were maintained as described (Salaman *et al.*, 1969). These agents were referred to previously as *Plasmodium berghei yoelii* and *Plasmodium berghei berghei* respectively. (see Killick-Kendrick, 1974). Mice were infected with 10^6 parasitized erythrocytes, i.p.

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SIII (Wellcome Reagents Ltd, Beckenham) was reconstituted and either stored at 4°C and used for up to 4 weeks, or re-lyophilized in smaller aliquots which were reconstituted on the day of use. This preparation had an optimal immunizing dose of 100 μg, given i.v., with peak splenic PFC responses 4–6 days later. A purified batch of SIII, kindly donated by Dr G. H. Christie, was used in one experiment; the optimal immunizing dose was 5 μg.

The procedure for PFC assays was that of Howard, Christie & Courtenay (1971a). Spleen cells were assayed in duplicate by the method of Askonas, Farthing and Humphrey (1960).

RESULTS

Evaluation of the response to SIII

When the splenic PFC response to SIII is measured as described, uninfected unimmunized mice always have a background count of about 1000 PFC. This is raised in *P. yoelii* infections. When infected mice are immunized with SIII, the number of PFC in their spleens usually falls below background levels. Since PFC are estimated by subtracting PFC to normal SRBC from PFC to SRBC coupled to SIII, the estimated value for individual mice may fall below zero. This in fact occurred in the immunized infected group. We obtained the mean PFC for such groups by assigning the value of 1 to all negative PFC values and taking the geometric mean. PFC values assessed in this way have not been given a standard deviation, as this would be of doubtful value; it should be noted that the arithmetic means of such PFC values are often higher than the geometric although the former take account of negative PFC values.

Responses to SIII in P. yoelii infection

P. yoelii causes a self-limiting infection in BALB/c mice, with peak parasitaemias on days 7–9, and $<0{\cdot}1\%$ parasitized erythrocytes by 17 days.

When 100 μg SIII was injected i.v. during such an attack, infected mice had lower PFC counts than their unimmunized counterparts (Table 1). In view of this lack of response, the experiment was repeated using 5 μg of purified SIII (see the Materials and Methods section); infected animals failed to respond. To ensure that this result was not due to different kinetics between the two groups, normal and 7 day *P. yoelii* infected mice were immunized with 100 μg SIII, and assayed over a period of 8 days. No response occurred in infected mice during this time (Fig. 1).

To test whether *P. yoelii* lowered the threshold of tolerance to SIII, normal and infected mice were immunized with doses of from 1–1000 μg, and assayed for PFC 5 days later. There was a direct relationship between dose and response in normal mice over the range 1–100 μg SIII. 1000 μg SIII produced a smaller response than 100 μg. Infected animals did not respond (Fig. 2).

Responses to SIII in chronic malarial infection

Chronic malaria was established by infecting mice with *P. berghei* when they had recovered from an attack of *P. yoelii* induced malaria (Wedderburn, 1974; Wedderburn, Turk & Hutt, 1975). Mice treated in this way experience a non-fatal attack following *P. berghei*, after which parasitaemias fall to very low levels.

Table 2 shows the PFC response to SIII, injected at intervals after the second infection, compared with those of mice which had received *P. yoelii* only, and with those of uninfected mice. The response after a single acute attack caused by *P. yoelii* only returns to normal after 12 weeks; by 16 weeks however, it was higher ($0{\cdot}02 > P > 0{\cdot}01$) than that of controls. Mice immunized 1 and 5 weeks after *P. berghei* had undetectable responses. These mice had parasitaemias detectable by blood smear. Twelve weeks after *P. berghei* the response to SIII was still significantly reduced, compared with uninfected mice ($P < 0{\cdot}01$), even though at this time, parasitaemias were not detectable by blood smear, although a comparable group were all patent as shown by transfer of blood to uninfected animals. Chronically infected mice, therefore, show depressed responses for considerable periods, even when parasitaemia is very low.

The effect of two injections of the same or of different plasmodia on the response to SIII

BALB/c mice are immune to reinfection with *P. yoelii* after a primary infection (Topley, Bruce-

TABLE 1. Effect of *P. yoelii* on the PFC response to SIII in immunized and non-immunized mice

Day of *P. yoelii* injection*	SIII	PFC/spleen†	× ÷ s.d.
—	−	1410	2·2
—	+	34,400	1·8
−4	−	2860	2·0
−4	+	232	—
−7	−	2070	1·2
−7	+	134	—
−14	+	2440	1·3

* Relative to SIII on day 0. PFC assayed on day 5.
† Geometric means. For numbers without sd, see text.

Chwatt & Dorell, 1970). A second injection of *P. yoelii* was given at various times relative to SIII, following a primary infection 5 weeks earlier. Splenic PFC after a single infection 5 weeks before SIII were depressed compared with controls. Table 3 shows that there was a further reduction in the response when the second *P. yoelii* injection was given between 7 days before, and 5 hr after, SIII, which was most marked when the interval between the second *P. yoelii* and the SIII injections was 1 hr. However, the same number of either parasite given 1 hr before SIII to a previously uninfected animal does not depress the response. The same was true when *P. berghei* was given 1 hr before SIII, following an earlier infection with *P. yoelii*. In fact, these three regimens tend to enhance the response (Table 4). It appears, therefore, that only secondary stimulation with an identical plasmodium is suppressive ($0{\cdot}02 > P > 0{\cdot}01$) 1 hr before antigen; it is interesting that *P. berghei* following *P. yoelii* does not have this effect, since there is presumably some protective cross immunity, and some cross-reacting antibody has been demonstrated (Cox & Turner, 1970).

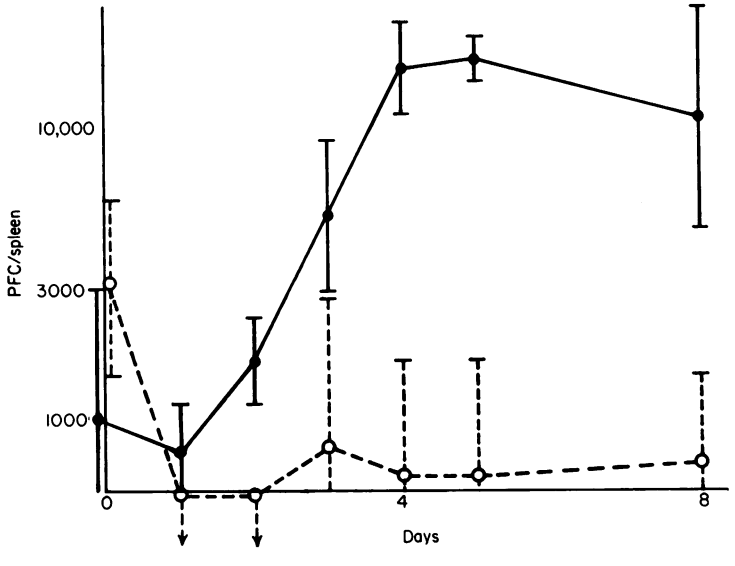

FIG. 1. The response to SIII in uninfected mice and in mice infected with *P. yoelii* 7 days before immunization. Uninfected (●—●); *P. yoelii* infected (○ – – – ○).

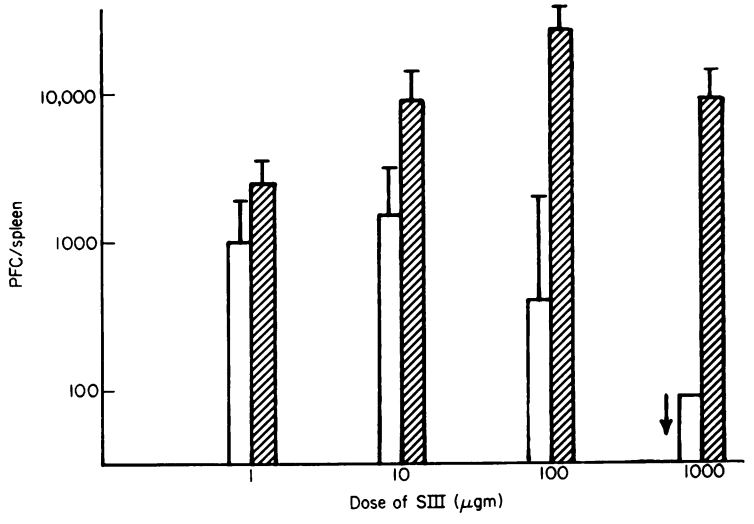

FIG. 2. The effect of *P. yoelii* infection on the response to various doses of SIII. Uninfected mice (▨); *P. yoelii* infected (□).

Response to SIII in mice undergoing short term undernourishment

Passwell, Steward & Soothill (1974) have reported that protein restriction can affect the amount and affinity of the antibody formed by mice against human serum transferrin. There is also a decrease in antibody affinity in murine malaria (Steward & Voller, 1973). We investigated the possibility of temporary non-selective undernourishment playing a part in the depression of the response to SIII seen in this disease. We found that the food intake of mice given *P. yoelii* was significantly lower than that of controls for 3–4 days at the height of the disease, but the body weights of the two groups did not diverge significantly. However, there was a significant loss in weight during a *P. berghei*-induced attack. A group of uninfected mice were given 60% of their normal food intake, starting nine days before SIII injection and continuing throughout the experiment. Undernourished mice lost 18%, and controls gained 7%, of their original body weight. Spleen weights were lower in undernourished mice: PFC per spleen were somewhat reduced in these mice; PFC/10^6 nucleated cells were not.

TABLE 2. The response to SIII in chronic malarial infection

Plasmodial injection, and time (weeks)			
P. yoelii	*P. berghei*	PFC/spleen × ÷	s.d.
—	—	39,500	1·4
−5	—	14,800	2·6
−9	—	18,500	1·5
−12	—	33,300	1·4
−16	—	82,100	1·4
−5	−1	1160	1·5
−9	−5	100	—
−16	−12	8520	2·4

TABLE 3. The effect of a second injection of *P. yoelii* at various times relative to SIII on the PFC response

Time of second *P. yoelii* injection*	PFC/spleen	× ÷ s.d.
−7 days	9330	1·5
−4 days	9330	1·9
−1 hour	6880	1·7
+5 hours	8130	2·7
+1 day	18,200	3·0
—	17,000	2·1
—†	34,000	1·4

* Relative to SIII on day 0. First injection of *P. yoelii* at −5 weeks.

† Controls, no *P. yoelii* at any time.

TABLE 4. The effect of various regimens of plasmodial injections on the response to SIII

Plasmodial injection		PFC/spleen	× ÷ s.d.
−5 weeks	−1 hr		
—	—	33,600	1·3
—	*P. yoelii*	65,600	1·5
—	*P. berghei*	42,400	1·4
P. yoelii	—	19,500	1·6
P. yoelii	*P. yoelii*	5890	1·9
P. yoelii	*P. berghei*	32,400	1·8

DISCUSSION

We have shown that responses to SIII over a wide dose range are abolished at the height of an attack of malaria. PFC numbers often fall below background levels, particularly after high doses of antigen. The SRBC response is also very low in infected mice, and fails to rise above background at certain antigen doses (Wedderburn, 1974). In contrast, infected mice respond more or less normally to keyhole limpet haemocyanin and to human serum albumin plus pertussis vaccine (Greenwood *et al.*, 1971a). Since certain antigens can induce normal responses, it seems unlikely that B-cell function is non-specifically affected in malaria.

There is some evidence that the SIII response is macrophage-dependent, at least *in vitro* (Aaskov & Halliday, 1971). Additional *in vivo* evidence comes from studies with Biozzi mice: low responders, originally selected for their poor responses to SRBC, also respond less well to SIII and are more susceptible to high dose tolerance than are high responders (Howard *et al.*, 1972). The low responders which, like BALB/c mice infected with malaria, have high background PFC, show increased macrophage activity. While the intracellular digestion of antigen is faster in low responders, less antigen is available at the macrophage surface, and the clearance of SIII from the circulation during the first week after immunization is more rapid than in high responders (Wiener & Bandieri, 1974).

Malarial mice also have a faster rate of clearance of carbon, and of chromium-labelled SRBC, from the circulation (Greenwood *et al.*, 1971b; Loose *et al.*, 1972; Loose & di Luzio, 1976). On the other hand, the detoxification of endotoxin is reduced in the livers of *P. berghei*-infected mice (Loose *et al.*, 1971).

Loose *et al.* (1972) also incubated peritoneal macrophages with SRBC *in vitro* and then transferred

them to syngeneic recipients, whose spleens were subsequently examined for PFC. Both normal and malarially-infected recipients of macrophages from infected mice produced equally low responses. Since macrophages from infected mice appeared to contain normal numbers of SRBC after incubation (and did not transmit malaria to non-infected mice: Loose L.D., personal communication), it is possible that their antigen processing/presenting capability (as distinct from antigen uptake) was compromised by the infection. However, although infected recipients of normal macrophages produced more PFC than the two former groups, they responded less well than normal recipients of normal macrophages, so that it would appear either that the environment of the infected host adversely affected the functioning of the donor macrophages, or that there is a disfunction in additional co-operating cell types in the infected host.

Recent work indicates that responses to a greater number of thymus-independent antigens than was formerly supposed may in fact be dependent on 'accessory cells' (Lee *et al.*, 1976). The response to SIII seems to be independent of helper T cells (Howard *et al.*, 1971b; Manning, Reed & Jutila, 1972), but the marked enhancing effect of ALS on the response in normal mice indicates a role for suppressor T cells (Baker *et al.*, 1970b). These same workers observed that congenitally athymic (nude) mice responded only marginally better than their euthymic littermates, and concluded that 'amplifier' T cells were also involved in the SIII response.

However, Howard *et al.* (1971b) showed, and we have confirmed (Wedderburn & Dracott unpublished), that mice given a lethal dose of X-rays and reconstituted with bone marrow still respond very poorly to SIII 3–5 months later, although they can respond normally to SRBC. It has recently been shown that both nude, and irradiated, bone marrow restored mice have 'activated' macrophages as assessed by resistance to *listeria*, and in the former case, tumoricidal activity also. (Cheers & Waller, 1975; Meltzer, 1976). One possibility, therefore, is that some of the effects, to explain which a T-amplifier cell was postulated, may in fact be due to variations in macrophage activity. However, this is clearly not the only possible explanation of the relatively poor responsiveness of nude compared with normal ALS-treated, and of X-irradiated bone marrow restored mice, compared with normal mice. It is of interest in this connection that Droege (personal communication) has found that certain T-cell lineages, present in normal adult mice, fail to reappear in irradiated, marrow-restored mice, but do so in mice restored with foetal liver. It might be illuminating to compare the SIII responses of marrow and foetal liver-restored mice.

Clearly, the roles of the various types of thymus-processed lymphocyte in the SIII response have yet to be unequivocally defined. The situation is further complicated by the fact that the characteristics of the response measured with chromic chloride-coupled SIII on SRBC may differ from those measured with SRBC passively coated with *Diplococcus pneumoniae* culture filtrate (Baker & Prescott, 1975). The latter method, which we used, is probably the less satisfactory of the two in that it may measure antibodies directed to more than one antigen. In addition, the involvement of T cells is less easily demonstrated, and the enhancing effect of ALS less apparent (Warr, Ghaffar & James, 1975; Baker *et al.*, 1970a).

Evidence for altered T-cell function in malaria is equivocal and appears to depend partly on the infecting strain. In *P. berghei* malaria almost complete thymic atrophy is seen shortly before the mice die (Krettli & Nussenzweig, 1974), whereas in *P. yoelii* malaria, there is only a transient loss of about 30% of thymus weight which quickly returns to normal (Wedderburn, unpublished observations). There is no direct evidence for effects on helper T cells, and a comparison of SRBC responses after infection and after neonatal thymectomy shows that the former induces a far more profound suppression. This could be considered as an indication that infection affects other categories of cells besides helper T cells, and activation of suppressor T cells cannot be excluded. (Jayawardena, personal communication).

Greenwood *et al.* (1971a) found that mice infected with *P. yoelii* either 5 or 10 days before allogeneic skin grafting had normal rejection times; we have observed prolonged allograft rejection times in some, but not all, mice infected with *P. yoelii* on the day of grafting (Wedderburn, 1974), whereas Sengers *et al.* (1971) observed considerably lengthened rejection times for skin xenografts on mice with artificially prolonged *P. berghei* infections. In general, this pattern is seen in the results of other workers: for example, oxazolone sensitivity, and transformation by PHA, were reduced by *P. berghei* but not *P. yoelii* (Greenwood *et al.*, 1971a; Jayawardena *et al.*, 1975).

Byfield, Christie & Howard (1973) showed that there is a transient period in the SIII response during which it is enhanced by a GVH reaction, followed by a prolonged period during which such reactions cause profound suppression. Our results were similar to theirs in that *P. yoelii* given together with SIII enhanced the response, which was suppressed when antigen was given later in the disease. We also showed that a second dose of *P. yoelii*, given with SIII, was suppressive, while a first injection of either *P. yoelii* or *P. berghei*, or an injection of the latter following the former, were all slightly enhancing at this time (Table 4). The only suppressive regimen in this experiment is also the only one of the four in which the parasite does not multiply during the 5 days before PFC are estimated, (Topley, Bruce-Chwatt & Dorell, 1970), and since we also showed that in chronic malaria considerable immune suppression can co-exist with very low parasitaemia, it is clear that large numbers of parasites are not necessary for at least a moderate depression of the response.

Finally, since it has been reported that antibody of low affinity is produced both during malaria, and in protein restriction (Steward & Voller, 1973; Passwell, Steward & Soothill, 1974), it seemed necessary to determine whether malarial mice, by eating less, may alter their immune responses. Our results indicate that this is not the case, at least when SIII is the antigen.

Immune suppression of several weeks duration follows a single acute infection with *P. yoelii*, while a short-lived and moderate suppression follows a second injection of this plasmodium, although the animal is protected against growth of the parasite and clinical symptoms by the first infection. In addition, a much more prolonged lowering of the immune response occurs in chronic malarial infection. This suggests that in areas of holoendemic malaria, a degree of immune suppression may be more or less continous.

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