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Failure of malaria vaccination in mice born to immune mothers

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SUMMARY

Female BALB/c mice were vaccinated against blood stage *P. yoelii* (17XL strain), infected 2 weeks later and after recovery mated to normal C57B1/6 males. Control matings were with normal BALB/c females. The (C57B1/6 × BALB/c)F_1 progeny were vaccinated at 4, 6, 8 or 10 weeks of age and infected 2 weeks later with lethal *P. yoelii*. All control mice were fully protected, but in the offspring of immune mothers mortality was 100, 87, 50, and 0% respectively. Mice in which the protective effect of vaccination had been abolished showed greatly reduced specific IgG and delayed hypersensitivity (DH) responses to challenge with parasite antigen. Results indicate that this failure of vaccination is due to the transmission of maternal IgG to the offspring which acts to suppress both priming by the vaccine and the generation of specific T helper cells involved in IgG production, as measured by the response to TNP-*P. yoelii*.

INTRODUCTION

Recent encouraging results of immunoprophylaxis in experimental malaria indicate that blood stage merozoites constitute the most promising form of vaccine for potential use in a human trial (Cohen, 1979). However, malaria is unusual in that in endemic areas, virtually all babies have high titres of maternal antibody (McGregor *et al.*, 1965; Molineaux *et al.*, 1978). While this may confer valuable protection against infection during the early months of life (Cohen, McGregor & Carrington, 1961) it might also impair the effectiveness of vaccination, since very small amounts of maternal antibody have been shown in other infections, such as poliomyelitis (Perkins, Yetts & Gaisford, 1979) and measles (Greenwood & Whittle, 1981), to completely prevent protection by vaccination. We now report that in a mouse model, infants born to immune mothers fail to be protected by subsequent vaccination against blood stage malaria, and that this appears to be due to the transmission of specific maternal IgG antibody in the milk, which interferes with both priming and T helper (T_H) cell function.

MATERIALS AND METHODS

Mice. The (C57B1/6 × BALB/c)F_1 mice were bred in our laboratory from parental strains supplied by the National Institute for Medical Research, London.

Parasites. The origin of our lethal *Plasmodium yoelii* (PY) has been described previously

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(Playfair, De Souza & Cottrell, 1977). Briefly, the lethal PY is a variant which developed during passage of a non-lethal PY (17X strain). The parasite was subsequently cloned and has remained consistently lethal, killing all mice within 22 days.

Vaccination with formalin fixed PY (FFPY). The preparation of the vaccine has been described previously (Playfair, De Souza & Cottrell, 1977). Briefly, blood with a parasitaemia of 50% or more is washed and lysed with 0·1% saponin, and the washed parasites fixed overnight at 4°C in 0·06% formalin. The fixed parasites are thoroughly washed and injected intravenously in a volume of 0·2 ml. For routine protection, a dose corresponding to 5×10^7 FFPY is combined with 10^8 *Bordetella pertussis* organisms (Pertussis vaccine, Wellcome).

Measurement of antibody titres. Total anti-parasite antibody was measured by the slide fluorescence method (Voller & O'Neill, 1971), using parasitized blood from an early infection as the antigen. Two-fold dilutions of serum were applied to the antigen at 20°C for 30 min and the slides were washed and treated with fluorescein conjugated (FITC) polyvalent rabbit anti-mouse immunoglobulin (Ig) for another 30 min. The fluorescence was read in an u.v. incidence light microscope. The end point was taken as the last serum dilution showing clear fluorescence.

To estimate the antibody in the different Ig classes, the polyvalent FITC anti-mouse Ig was replaced by rabbit antisera highly specific for mouse IgM, IgG and IgA (Litton Bionetics, Maryland, USA). The slides were then washed, treated with FITC goat anti-rabbit Ig and scored as before.

Test for delayed hypersensitivity (DH) to parasite antigens. DH to malarial antigens was assessed in the mice 2 weeks after vaccination by subcutaneous challenge in the right pinna with 10 μl of the eliciting antigen (Cottrell, Playfair & De Souza, 1978). One day following challenge, the mice were injected with 1×10^7 ^{51}Cr-labelled bone marrow cells (BMC), which were harvested from femur washings of syngeneic mice. The BMC were washed and incubated with 100 μCi of ^{51}Cr (Sodium chromate, Amersham) per 10^8 cells for 45 min at 37°C, and were subsequently washed prior to intravenous injection. Twenty-four hours later, the mice were killed by cervical dislocation, the pinnae cut off at the hairline and counted in a LKB Wallac gamma counter. The BMC-homing to the experimental pinnae was calculated as the percentage homing (PH) to the right pinna compared to the left control, thus:

$$\text{PH (\%)} = \frac{\text{(counts in right pinna)} - \text{(counts in left pinna)} \times 100}{\text{(counts in left pinna)}}$$

The results are presented as the percentage increases in homing of the vaccinated groups, compared to control groups of non-vaccinated mice.

TNP coating of parasites. A modification of the method of Rittenberg & Pratt (1969) was used and is described in detail elsewhere (Playfair *et al.*, 1977). Briefly, blood with at least 20% parasitaemia was washed and the parasites freed from the erythrocytes by the saponin method described above, and washed, 2,4,6,trinitrobenzenesulphonic acid (TNBS) at a concentration of 5 mg/ml in 0·28 M cacodylate buffer (pH 6·9) was added to the packed parasites and the mixture stirred for 30 min at room temperature. The reaction was then stopped by the addition of cold barbital buffer and the TNP-coupled parasites (TNP-PY) washed in PBS until no free (yellow) TNP was visible in the supernatant. The TNP-PY were injected i.v. immediately after preparation.

Assay for plaque-forming cells (PFC). Four days after injection of the TNP-PY the mice were killed and the anti-TNP PFC in their spleens measured by the method of Cunningham & Szenberg (1968) using sheep erythrocytes coupled to TNP (TNP-SRBC) as described previously (Rittenberg & Pratt, 1969). To assess the PFC responses attributable to each IgG subclass, highly class-specific rabbit anti-mouse Ig reagents, as described above, were used as developers. In Table 3, the PFC are recorded as the geometric mean and the standard error of the log transformed data.

Statistics. Tests for significance were performed using the Students *t*-test.

RESULTS

Protection by vaccination against P. yoelii *(PY) in offspring of immune mothers*

Eight week old female BALB/c mice were vaccinated as above, infected 2 weeks later with 10^4 lethal

Table 1. Protection by vaccination against *P. yoelii* in offspring of immune mothers

Age of mice at vaccination (weeks)	Result of challenge		
	Number recovered/total	%	Mean recovery time (days)
4	0/8	0	—
6	2/16	12·5	23·0
8	6/12	50·0	23·3
10	10/10	100	8·0
Controls	20/20	100	7·9

Mice born to immune mothers were vaccinated at 4, 6, 8 or 10 weeks of age and infected 2 weeks later.

Offspring of control (non-immune) mice were similarly treated and the data pooled.

PY and shortly after recovery, when their serum contained high titres of anti-malarial antibody, they were mated with normal C57BL/6 males. Control matings were with BALB/c females immunized with formalin fixed uninfected red cell ghosts.

The (C57BL/6 × BALB/c)F_1 progeny of the above matings were weaned at 3 weeks, vaccinated at 4, 6, 8 or 10 weeks and infected 2 weeks later. Tables 1 and 2 summarize the results of six experiments involving the immunization and challenge of 180 offspring of vaccinated-recovered (VR) mothers. As assessed by survival (Table 1), the effectiveness of vaccination among offspring of VR mothers was greatly impaired in the 4, 6 and 8 week old groups, with mortalities of 100, 87, and 50% respectively; where mice did survive, recovery was greatly delayed relative to the controls. It is interesting to note that this is not a dose-related phenomenon, as no decrease in mortality was observed when the dose of vaccine administered was increased four-fold (data not shown). By 10 weeks of age, vaccination was completely protective with the mice clearing their infections within 7 days. All control mice, irrespective of age, recovered within 8 days of infection.

Previous results have shown that in successfully vaccinated mice there is a good correlation

Table 2. Responses to *P. yoelii* in offspring of immune mothers

Age of mice at vaccination (weeks)	Responses to infection			
	IFA titre (day 10 after infection)			DH as % of unvaccinated control
	Total	IgM	IgG	
4	1/1,000–1/2,000	1/256	1/256–1/1,000	272±27*
6	1/1,000–1/2,000	1/256	1/256–1/2,000	304±38*
8	1/1,000–1/4,000	1/512	1/512–1/4,000	392±63
10	1/32,000	1/1,000	1/8,000–1/16,000	533±21
Controls	1/32,000	1/1,000	1/8,000–1/16,000	525±42

Offspring of immune mothers were vaccinated as in Table 1. Control mice were age matched and the data pooled.

IFA titres were tested 10 days after infection; the values quoted are range of titres for a group of eight mice.

DH measurements are expressed as the mean ± 1 s.e.

* $P < 0{\cdot}01$.

Table 3. T helper cell responses to *P. yoelii* in offspring of immune mothers

Age of mice (weeks)	Challenge	Anti-TNP PFC per spleen			
		IgM	IgG1	IgG2a	IgG2b
4	10^5 TNP-PY	53,164 (48,430–58,300)	1,673* (1,200–2,335)	715* (569–899)	2,195* (1,289–3,737)
6	10^5 TNP-PY	31,340 (30,318–32,396)	4,908* (3,836–62,791)	2,249* (1,335–3,789)	1,491* (955–2,328)
8	10^5 TNP-PY	73,378 (67,856–79,349)	1,393* (750–2,587)	1,200* (778–1,853)	2,564* (1,479–4,444)
10	10^5 TNP-PY	50,671 (49,884–52,522)	20,952 (18,985–23,123)	19,087 (17,724–20,555)	25,860 (23,496–28,461)
Controls	10^5 TNP-PY	51,531 (43,170–61,510)	23,105 (20,313–26,281)	26,356 (24,165–28,747)	25,996 (23,360–28,929)

Offspring of immune mothers were vaccinated as in Table 1 and challenged 2 weeks later with 10^5 TNP-PY. Age matched control mice were similarly treated and the data pooled.

Each group consisted of at least eight mice.

Results are expressed as the geometric mean ± s.e.

* $P < 0.001$.

between the antibody (especially IgG) and delayed hypersensitivity (DH) responses to challenge and protection, under a variety of experimental conditions (Cottrell *et al.*, 1978; Playfair & De Souza, 1979). To determine if either of these responses was impaired, offspring of immune mothers were vaccinated as before, at 4, 6, 8 or 10 weeks, and their responses to challenge assessed. DH was measured as the increased accumulation of ^{51}Cr-labelled BMC to the ear 48 hr after challenge and the immunofluorescent antibody (IFA) titres tested 10 days after infection with 10^4 lethal PY. Typical of previous results, successfully vaccinated mice developed high titres of antibody when challenged and also displayed strong DH skin reactions to parasite antigens. However, mice in which the protective effect of vaccination had been abolished showed greatly reduced specific IgG and DH responses to malarial antigens; responses to an unrelated antigen, sheep erythrocytes, were normal in all groups (data not shown).

T helper (T_H) cell responses to PY in inhibited offspring

In view of the marked reduction in specific IgG responses compared to IgM (Table 2) and the fact that IgG responses are known to be particularly thymus dependent (Dresser & Popham, 1979), we considered the possibility that T_H cells were the principal target of maternal antibody. Therefore, we

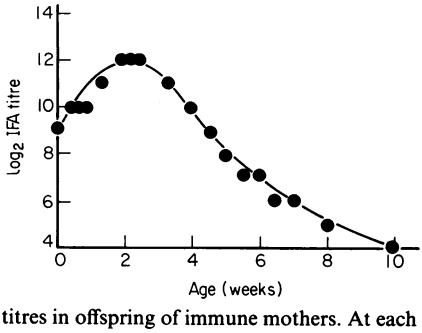

Fig. 1. Specific serum antibody titres in offspring of immune mothers. At each point, five mice were randomly selected from 12 litters and their sera pooled. The lowest titre considered to be parasite-specific was four.

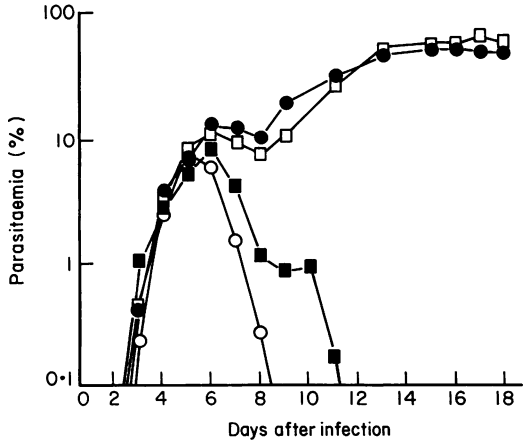

Fig. 2. Pre-natal or post-natal transfer of inhibition. Mice born to normal (○; □) or immune (●; ■) mothers were either suckled on their own mother (○; ●) or cross-fostered (□; ■). Results show the mean parasitaemia of groups of six mice followed either until death of the last mouse in the group, or recovery.

again vaccinated offspring at 4, 6, 8 or 10 weeks and, using the approach of coupling a standard hapten (TNP) to the free parasite, measured the anti-TNP PFC response to challenge with 10^5 TNP-PY: in this way carrier recognition of the parasite can be quantitated (Playfair *et al.*, 1977). The results (Table 3) show that T_H cell responses were indeed markedly impaired up to 8 weeks and that the deficiency was largely confined to the IgG component of the PFC response, which was reduced to 5–10% of control values. This suggests that the specific IgM B cells were relatively unaffected.

Pre-natal or post-natal transfer of inhibition

Mice born to and nursed by immune mothers had significant serum antibody levels at birth, which increased gradually with suckling time to a peak titre of 1:4,000 within 14–18 days after birth and declined slowly thereafter (Fig. 1). There was no detectable transmission of IgM or IgA from the mother to the circulation of the neonate. This suggested, in accordance with previous reports (Brambell, 1959), that mice acquired antibodies from their mothers largely through the milk. Therefore, to determine if the inhibition of vaccination was similarly transferred, offspring of immune and control females were either suckled on their own mothers or cross-fostered within 30

Table 4. Transfer of immunity from mother to offspring

Mother	Age of mice (weeks)	Dose of *P. yoelii* injected (i.v.)	% survival	Mean day of death	Mean day of recovery
VR	4	10^4	0	16·4	—
Control	4	10^4	0	8·3	—
VR	4	10^3	0	26·8	—
Control	4	10^3	0	8·3	—
VR	4	10^2	75·0	21·0	31·3
Control	4	10^2	0	9·5	—

Offspring of vaccinated–recovered (VR) and control mothers were infected at 4 weeks of age with graded numbers of lethal *P. yoelii*.

Each group consisted of at least six mice.

Table 5. Inhibition of vaccination by passive antibody

Serum IgG titre at vaccination	Number recovered/total	Mean day of recovery (days)	Mean day of death (days)
1:256	1/5	26·0	11·25
1:128	6/10	22·6	26·0
1:64	8/8	18·3	—
1:32	8/8	8·0	—
Normal mouse serum	8/8	7·4	—

Control mice were injected with graded amounts of serum (taken from syngeneic 3 week old offspring of immune mothers) one day prior to vaccination. Serum IgG titres in the recipients were then measured 1 hr before vaccination.

min of parturition. All mice were weaned at 3 weeks and vaccinated when 6 weeks old. As Fig. 2 shows, the interference with protection was only found in mice suckled by immune mothers, confirming that transfer of inhibition takes place through the milk.

Transfer of immunity from mother to offspring

Although we have demonstrated that neonatal uptake of IgG is involved in the inhibition of vaccination with FFPY, we were prompted to investigate whether this antibody might also confer a degree of passive protection against a primary infection with lethal PY, similar to that found in other host–parasite relationships (Bruce-Chwatt & Gibson, 1956; Desowitz, 1973; Palmer, 1978). Accordingly, offspring of control and VR mothers were weaned at 3 weeks and infected 1 week later with graded numbers of PY parasitized RBC. As assessed by recovery (Table 4), the transfer of immunity from VR mothers to their offspring was only found in those mice infected with 10^2 parasites, where mortality was reduced to 25%. In those offspring innoculated with 10^3 or 10^4 parasites, mortality was 100%, although some degree of protection was transferred, as evidenced by their significantly longer survival times relative to their controls. All control mice died within 10 days, irrespective of the size of the innoculum.

Inhibition of vaccination by passive antibody

Since the ability of antibody to inhibit an immune response is dependent on the ratio of antigen to specific antibody (Uhr & Baumann, 1961), we investigated whether this inhibition of vaccination could be attributable solely to the presence of maternally derived IgG. Accordingly, normal 6 week old (C57B1/6 × BALB/c)F_1 mice were given various amounts of 'immune serum' (taken from syngeneic 3 week old offspring of VR mothers) i.v. 1 day before vaccination. They were then challenged 2 weeks later with 10^4 PY. As Table 5 shows, the effectiveness of vaccination is greatly impaired when the specific serum IgG titre immediately prior to vaccination exceeds 1:128, where the mortality is 40%. When the specific antibody falls below 1:128 there is no impairment of vaccination in terms of mortality, although recovery is delayed in some cases. These results confirm that maternally derived IgG plays an important role in the abolition of protection by vaccination.

DISCUSSION

In this paper, our results clearly demonstrate that offspring of immune mothers fail to be protected by vaccination against *P. yoelii* until at least 8 weeks of age. The mortality appears to correlate best with the greatly impaired generation of IgG memory, as shown by the poor responses to a challenge infection (Table 2). Furthermore, the transfer of inhibition occurs post-natally via specific maternal IgG in the milk (Fig. 2).

The development of immunity as assayed by T_H cell activity to challenge was markedly reduced

(Table 3). Indeed the IgG component of that response was reduced to below 10% of control values even until 8 weeks of age. Therefore, as IgG production is particularly T cell dependent (Dresser & Popham, 1979), we propose that mortality among vaccinated offspring of immune mothers is due to passively derived maternal antibody which acts to suppress the T_H cell response. This results, upon challenge, in the virtual inability to produce sufficient parasite-specific IgG to ensure survival.

It has been postulated that when antigen is injected i.v. into an animal which already has IgG present in the circulation, the active production of antibody can be suppressed either by rapid elimination of the antigen from the circulation (Solomon, 1971) or by a feedback mechanism which prevents proliferation of the antibody synthesizing cells (Moller & Wigzell, 1965). If maternal IgG, in this case, is acting solely via these mechanisms to abolish the protective effects of vaccination, then transfer of serum from inhibited to control animals should result in a similar mortality at comparable antibody titres. Comparison of Fig. 1 and Table 1 with Table 5 indicates that this is not the case. In offspring of immune mothers, the mortality at 6 weeks and at 8 weeks is 87% and 50% respectively, while transfer of identical titres to control animals 1 day prior to vaccination resulted in 40% and 0% mortality (Table 5). Evidently maternal IgG plays a dominant but not a unique role in this inhibition. It is possible that contributory inhibition could result from the prolonged exposure of neonatal cells to maternal antibody, with the consequent induction of T suppressor (T_S) cells. Two variants of this theme can be envisaged. T_S cells generated by a feedback suppressor circuit and possessing anti-idiotypic receptors could directly counteract the action of the T_H cells in the absence of antigen, via idiotype–anti-idiotype interactions (Janeway, 1980). Alternatively, idiotype-specific T_S cells could act indirectly to antagonize the action of T_H cells in the presence of antigen (Eichmann, 1975), possibly via an antigen bridge. Either of these T_S cells could be induced by the transmission of maternal antibody to the offspring. In the former case transmission of idiotype positive IgG or, in the latter case transmission of maternal antibody bearing anti-idiotypic receptors. Interestingly, our preliminary data, which will be published in due course, suggest the presence of a specific T suppressor cell which is generated by the presence of high titres of maternal antibody. Furthermore, this dependence on the transmission of high titres of maternal IgG for the suppression of vaccination may explain why a similar finding was not reported recently with sporozoite vaccination where the antibody titres transferred were eight-fold lower (Orjih, Cochrane & Nussenzweig, 1981).

In areas of holoendemic malaria, the section of the population most at risk to the serious consequences of infection are young children under the age of 5 years. The results of these experiments indicate that young experimental animals acquire a degree of protective immunity against a primary infection (Table 4) through the high levels of maternal IgG, which paradoxically serves to abolish the generation of acquired immunity through vaccination. It is interesting to note that similarly high titres of parasite-specific IgG are transferred from mother to infant in endemic areas (Molineaux *et al.*, 1978; McGregor *et al.*, 1965) and that a similar suppression has been noted in human infants immunized against poliomyelitis (Perkins *et al.*, 1959), *B. pertussis*, measles (Greenwood & Whittle, 1981) and diptheria toxoid (Barr, Glenny & Randall, 1950). Our results imply that the conditions for vaccinating neonatal humans in endemic areas may have to be chosen with great care, and that further studies in larger animals, and with natural host–parasite combinations, are required. Maternal antibody may also be a problem with other vaccines against chronic parasitic diseases.

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