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A pilot safety and immunogenicity study of the malaria vaccine SPf66 in Gambian infants

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SUMMARY

A pilot safety and immunogenicity trial of the malaria vaccine SPf66 has been undertaken in 150 Gambian infants. No significant systemic side effects were recorded but modest local reactions were seen after the administration of a third 1·0 mg dose. SPf66 produced in Colombia was more immunogenic than SPf66 produced in the USA and a 1·0 mg dose of each vaccine gave higher antibody levels than a 0·5 mg dose. However, antibody levels fell rapidly after administration of the third dose of vaccine and showed little change over the following malaria transmission season. The incidence of clinical malaria was higher among children who received SPf66 than among children who received inactivated polio vaccine, the effect being most marked among children who received 1·0 mg Colombian SPf66. As the trial was not designed to measure the effect of SPf66 on morbidity from malaria, the significance of this finding is uncertain.

Keywords *malaria,* Plasmodium falciparum, *SPf66, vaccine, safety, immunogenicity*

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INTRODUCTION

The malaria vaccine SPf66 has given about 40% protection against clinical attacks of *Plasmodium falciparum* malaria in South America (Tanner, Teuscher & Alonso 1994) and about 30% protection in children in a highly endemic area of Tanzania (Alonso *et al.* 1994). In the Tanzanian study, SPf66 was given to children aged 1–4 years so that the youngest children in the trial were aged about 18 months when they had been fully immunized. In many parts of Africa, a substantial portion of the morbidity and mortality caused by malaria has taken place by this age. Thus, we have explored whether SPf66 is immunogenic and safe when given to African children under the age of one year. Two formulations of SPf66 were tested in a double-blind study. One was produced at the Instituto de Inmunologia, Bogota, Colombia (lot 0693-A) and the other was manufactured for the US Department of Defense under contract to Chiron Multiple Peptide Systems, San Diego, CA, USA, and formulated by the Salk Institute GSD, Swiftwater, PA, USA (lot 1-1-93). Both preparations underwent investigations for toxicity, pyrogenicity and sterility (D'Alessandro *et al.* 1995).

One hundred and fifty Gambian infants aged 6–11 months resident in villages near to Basse, The Gambia were allocated randomly to one of five groups. Twenty-five children received 1·0 mg Colombian SPf66, 25 0·5 mg Colombian SPf66, 25 1·0 mg American SPf66, 25 0·5 mg American SPf66 and 50 inactivated polio vaccine (IPV) (Pasteur Merieux, Lyon, France) (lot J0665). There were no significant differences in the age, sex or ethnic group distribution of children who received SPf66 and those who received IPV. The first dose of vaccine was administered in August 1993 at the start of the annual malaria transmission season, thus it is likely that the majority of these children had not been exposed to natural infection

prior to vaccination. Each child received three doses of vaccine on days 0, 30 and 180 given by subcutaneous injection into alternate thighs. Children were kept under close observation for 6 h after each vaccination and were visited at home 1, 2 and 6 days later when a side effects questionnaire was administered and the site of vaccination examined. Mothers of study children were encouraged to bring their child to the MRC clinic at Basse whenever the child was unwell. In addition, children were visited at home every two weeks during the period between the administration of second and third doses of vaccine and twice a week during the 1994 malaria transmission season. At home visits during the rainy season, the axillary temperature was recorded and a blood film obtained from any child with a temperature $\geqslant 37{\cdot}5°C$. A clinical attack of malaria has been defined as an illness accompanied by a temperature of 37·5°C or higher and an asexual *P. falciparum* parasitaemia >=6000 per μl. This threshold was determined on the basis of parasite densities in symptomatic and asymptomatic children (Armstrong-Schellenberg *et al.* 1994). Blood samples were obtained three weeks after administration of second and third doses of vaccine and five and nine months after administration of the last dose of vaccine. Antibody levels to SPf66 polymer were measured by an ELISA and antibody titres calculated as described elsewhere (D'Alessandro *et al.* 1995). Side effects data were analysed using SPSS. Differences between groups were assessed using chi-squared tests with continuity correction or two sided Fisher's exact test. Incidence rates for malaria were calculated by dividing the number of first or only episodes by the period of child-days at risk: following an episode of malaria the child was withdrawn from both the numerator and denominator. Children who were absent at follow-up visits were excluded for that period. Those who withdrew or died were included up to the day of the event. The overall incidence in each group was calculated by dividing the total number of episodes of malaria by the total number of child-days at risk. Following an episode of malaria a child was excluded for 28 days. A Poisson regression analysis was used to combine morbidity information from all vaccine groups; this also allowed for any variation in malaria incidence between villages. The study was approved by the Gambia Government/MRC Ethical Committee.

Only three children failed to complete the six-month course of immunization. One child died from pneumococcal meningitis after having received low dose Colombian vaccine, another child in the high dose American group was withdrawn after he was noted to have congenital cataracts and developmental delay and a third child in the same group was withdrawn by her family. One hundred and thirty-eight of the 147 vaccinated children (94%) completed the full period of surveillance; one child died, four moved away and four were withdrawn by their families.

No child had an allergic reaction to vaccination. General complaints were noted frequently during the first week after vaccination but their prevalence did not differ significantly between groups. Local reactions, tenderness or induration >5 mm, were recorded significantly more frequently after the third injection of 1·0 mg doses of Colombian and American SPf66 (8/25 and 6/23) than after a third dose of IPV (0/50) ($P<0{\cdot}01$). No persisting nodules were noted. Antibody titres before and after vaccination are shown in Figure 1. Colombian SPf66 was significantly more immunogenic than

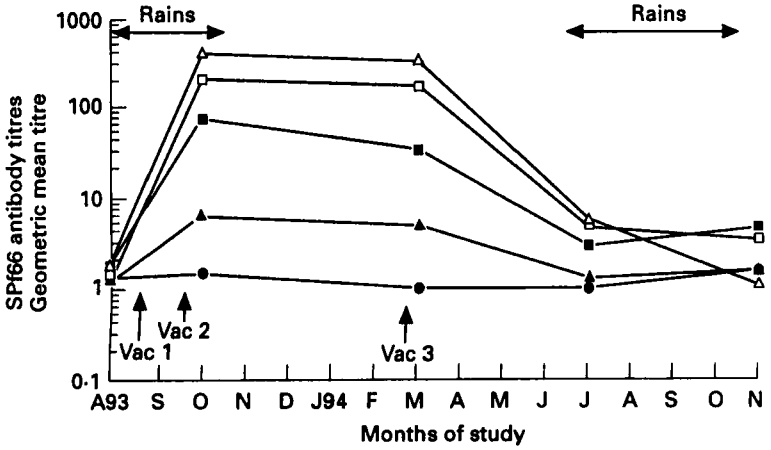

Figure 1 Antibody titres to SPf66 polymer as measured by an ELISA at various times after immunization with SPf66 vaccine produced either in Colombia or in the United States and given at a dose of either 1·0 mg or 0·5 mg. ■ 1 mg American; ▲ 0·5 mg American; △ 1 mg Colombian; □ 0·5 mg Colombian; ● polio.

American SPf66 and for each vaccine 1·0 mg doses were more immunogenic than 0·5 mg doses. Percentages of vaccine responders, defined as children with an antibody titre of 1 : 100 or greater after three doses of vaccine, were 96% 1 mg Colombian SPf66, 88% 0·5 mg Colombian SPf66, 65% 1·0 mg American SPf66, 36% 0·5 mg American SPf66 and 2% IPV. No increase in titre was seen after administration of a third dose of vaccine; it is possible that antibody titres fell between second and third doses and subsequently rose. Antibody levels fell rapidly after administration of the third dose of vaccine and showed little increase during the following rainy season, although 55% of children experienced an attack of malaria during this period. The higher immunogenicity of SPf66 produced in Colombia compared with that produced in the USA may reflect differences in the alum used in vaccine formulation but the significance of these differences is uncertain as no correlation has been found between antibody levels to SPf66 as measured by ELISA and biologically active antibodies (Rocha *et al.* 1992). Nevertheless, it is a matter of concern that ELISA antibody levels fell so rapidly after immunization.

Evaluation of the effect of SPf66 on the incidence of malaria was not an objective of the pilot study. However, as a safety measure, records were kept of all clinic attendances by study children. During a routine analysis of these data by the trial monitoring committee it was noted that significantly more children in the 1·0 mg Colombian SPf66 group had presented with a clinical attack of malaria than had children in the IPV group (7/25 *vs* 4/50) ($P = 0·04$). This finding led the trial monitoring committee to recommend that children in the trial should be kept under close surveillance during the 1994 malaria transmission season. During this period one child in the IPV group died from an undiagnosed acute illness. Two children presented with severe anaemia (PCV < 15%), one in the 0·5 mg American SPf66 group and one in the IPV group; no child developed cerebral malaria. Similar proportions of children who had received SPf66 (11/94) or IPV (4/48) were admitted to a health centre with a clinical diagnosis of malaria.

The incidence of clinical malaria, detected either by attendance at a clinic or by active surveillance, was higher in each group of children who received SPf66 than in the group who received IPV (Table 1). This effect

Table 1 Incidence of clinical attacks of malaria, determined as episodes per 1000 days at risk ×1000, in children who received SPf66 or IPV. Numbers of episodes and days at risk are shown in parentheses. Rate ratios (95% CI) for children who received SPf66 in relation to children who received IPV are shown also

	Colombian SPf66		American SPf66			
Between 2nd and 3rd dose	1·0 mg	0·5 mg	1·0 mg	0·5 mg	SPf66 combined	IPV
First episode						
Incidence	2·26	0·88	1·26	1·19	1·38	0·57
	(7/3095)	(3/3415)	(4/3177)	(4/3354)	(18/13041)	(4/6992)
Rate ratio	3·95*	1·54	2·20	2·08	2·30	
	[1·16, 13·51]	[0·34, 6·86]	[0·55, 8·80]	[0·52, 8·34]	[0·78, 6·81]	
Total number of episodes						
Incidence	1·93	0·84	1·20	1·10	1·27	0·55
	(7/3624)	(3/3567)	(4/3324)	(4/3645)	(18/14160)	(4/7252)
Rate ratio	3·50	1·52	2·18	2·00	2·41	
	[1·03, 11·96]	[0·34, 6·81]	[0·55, 8·72]	[0·50, 8·00]	[0·82, 7·13]	
After 3rd dose†						
First episode						
Incidence	8·48	7·46	5·95	7·89	7·37	4·92
	(13/1533)	(15/2011)	(12/2018)	(16/2029)	(56/7591)	(22/4472)
Rate ratio	1·72	1·52	1·21	1·60	1·57	
	[0·87, 3·42]	[0·79, 2·92]	[0·60, 2·44]	[0·84, 3·05]	[0·78, 3·16]	
Total number of episodes						
Incidence	12·24	8·22	7·70	8·12	8·91	5·03
	(25/2043)	(21/2555)	(19/2467)	(21/2586)	(86/9651)	(26/5169)
Rate ratio	2·43*	1·63	1·53	1·61	1·67	
	[1·40, 4·21]	[0·92, 2·90]	[0·84, 2·76]	[0·91, 2·87]	[0·94, 2·95]	

*$P = <0·05$. † Between July 25 and December 2, 1994.

was most marked and statistically significant in those who received 1·0 mg Colombian SPf66. A Poisson regression that took account of the village of the recipient and adjusted for overdispersion showed the overall difference between children who received SPf66 and those who received IPV to be non-significant ($P = 0{\cdot}11$ and 0·13 for first and total episodes between the second and the third doses and $P = 0{\cdot}20$ and 0·08 after third dose respectively), and no significant difference between the SPf66 groups due to dose or source of vaccine.

A similar pattern was observed during a cross-sectional survey of 138 of the study children undertaken at the end of the malaria transmission season when *P. falciparum* parasite rates were as follows—1·0 mg Colombian SPf66 30%, 0·5 mg Colombian SPf66 27%, 1·0 mg American SPf66 18%, 0·5 mg American SPf66 16% and IPV 13%.

The significance of these parasitological findings is uncertain. The pilot trial was not designed to investigate the effects of SPf66 on morbidity from malaria and they may have arisen by chance, for example if a higher proportion of children with a low risk of malaria had been randomised to the IPV group. However, there are examples of disease potentiation by vaccination (Grayston, Woolridge & Wang 1962, Fulginiti *et al.* 1967, Kim *et al.* 1969) so that this possibility cannot be excluded. Fortunately, no enhancement of either disease prevalence or severity was found during a larger trial of Colombian SPf66 (D'Alessandro *et al.* 1995). However, because of the timing of the first vaccination relative to the malaria transmission season, most of the children in this pilot study received SPf66 before they had been naturally exposed to malaria whilst this was not the case for the children in the efficacy trial and this could account for these different results.

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Footnote

Detailed breakdowns of side effects and immunogenicity data are available from the authors.

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