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Brief communication

Follow-up of Gambian children recruited to a pilot safety and immunogenicity study of the malaria vaccine SPf66

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SUMMARY

A pilot safety and immunogenicity trial of the malaria vaccine SPf66 was undertaken in The Gambia in 1993. One hundred and fifty infants aged 6–11 months were immunized with either 0·5 mg or 1·0 mg of SPf66 produced either in Colombia or in the USA or with a control vaccine. Children who received SPf66 experienced more clinical attacks of malaria than did children in the control group during the first period of surveillance and the difference in incidence between children who had received high dose Colombian vaccine and the control children was statistically significant at the 5% level. During the 1995 malaria transmission season, 127 children from the original cohort of 150 were observed. During 18 weeks of intensive surveillance, the incidence of clinical malaria was again higher among children who had received SPf66 than among children who had received inactivated polio vaccine (6·23 vs *4·89 clinical attacks per 1000 days at risk), the effect being most marked among children who were in the high dose groups, but differences between groups were now no longer statistically significant*.

Keywords *malaria, vaccine, SPf66, Gambian children*

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INTRODUCTION

Trials conducted in several parts of South America have shown that the malaria vaccine SPf66 is safe and immunogenic and that it provides partial protection against malaria (Tanner, Teuscher and Alonso 1995). However, in Thailand SPf66 produced in the United States was ineffective (Nosten *et al.* 1996). In an area of intense malaria transmission in Tanzania, SPf66 gave 31% protection against first attacks of malaria in children aged 1–4 years (Alonso *et al*. 1994). Since, in Africa, malaria is primarily a disease of young children we have investigated the efficacy of this vaccine in Gambian infants. Initially, 150 infants, aged 6–11 months, were recruited to a safety and immunogenicity study which compared two doses of SPf66 (1·0 mg and 0·5 mg) and batches of SPf66 produced either in Colombia or in the USA (Leach *et al*. 1995). No significant systemic or local side effects were recorded. However, children who received SPf66 experienced more clinical attacks of malaria than children in the control group who were given inactivated polio vaccine (IPV). The effect was most marked, and just statistically significant, in those who received Colombian produced SPf66 at a dose of 1·0 mg (7/25 *vs* 4/50) ($P = 0{\cdot}04$). However, the significance of this apparent enhancement of malaria is uncertain as morbidity from malaria was not one of the primary end-points for the pilot trial and this effect was not seen during a larger Gambian trial which had clinical malaria as its main end-point (D'Alessandro *et al*. 1995). Nevertheless, children in the pilot trial have been followed during a further malaria transmission season (1995) to ensure that there were no long-term harmful effects of the vaccine and to ensure that there was no increased incidence of cerebral malaria among the vaccinated children as they approached the age at which

this complication of malaria is seen most frequently. The results of this further follow-up are presented here.

The vaccination procedure and details of the first year of follow-up have been described elsewhere (Leach *et al.* 1995). Briefly, during the period of August 1993 to February 1994, 150 infants aged 6–11 months who lived in villages near to Basse, The Gambia were randomized 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 IPV. Each child received three doses of vaccine on days 0, 30 and 180. One hundred and twenty-seven children from the original cohort of 150 children (85%) were traced and enrolled into a further follow-up study; most of the missing children had moved out of the study area. Children were visited at home once a week throughout the 1995 malaria transmission season by a project field assistant. The axillary temperature was recorded and two thick-blood films were prepared if the temperature was 37·5°C or higher. All children had unrestricted access to the MRC clinic at Basse and mothers were encouraged to bring their children to the MRC clinic whenever a child was unwell. Children in the trial who came to the MRC clinic had their axillary temperature taken and a blood film prepared if fever was reported during the preceding 24 h or if their temperature was 37·5°C. Two cross-sectional surveys were carried out, the first at the beginning of the rainy season and the second at the end of the rainy season. At these surveys each child was examined, a blood film obtained and the packed cell volume (PCV) was determined. Antibody levels of SPf66 were measured in 122 children at the time of the second cross sectional survey using an ELISA method described previously (D'Alessandro *et al*. 1995).

A clinical episode of malaria was defined as an illness associated with an axillary temperature of ≥37·5°C and *Plasmodium falciparum* parasitaemia at a density of ≥6000/μl, a cut-off value defined as described by Smith, Armstrong-Schellenberg & Hayes (1994). The estimated sensitivity and specificity for this case-definition were both 86%. Incidence rates for malaria were determined by dividing the number of first or only episodes by the period of child-days at risk. Children who were lost to follow-up, who withdrew or who died were included up to the date of the event. Similarly, children who were not seen during the weekly visits were excluded for the period during which they were not under observation. The overall incidence of malaria in each group was calculated by dividing the total number of episodes of malaria by the total number of child-days at risk. After each recorded episode and treatment, a child was considered not to be at risk for the next 28 days and he or she was removed from the numerator and denominator for that period. A Poisson regression analysis was used to adjust for vaccine dose and source, bednet usage and overdispersion (Leach *et al*. 1995).

During the 18 weeks of intensive surveillance, two children died of cerebral malaria at the MRC clinic; one was in the 1·0 mg American SPf66 group and the other in the 0·5 mg Colombian SPf66 group. One child in the IPV group received a blood transfusion for severe malaria anaemia. Seven children were admitted to the MRC clinic

Table 1 Incidence of clinical attacks of malaria, determined as episodes per 1000 days at risk, 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

	Vaccine Group					
	Colombian SPf66		American SPf66		SPf66 combined	IPV
	1·0 mg	0·5 mg	1·0 mg	0·5 mg		
	(*n*.24)	(*n*.22)	(*n*.18)	(*n*.22)	(*n*.86)	(*n*.41)
First episode						
Incidence	6·80	4·50	8·83	5·65	6·23	4·89
	(14/2060)	(10/2224)	(13/1473)	(11/1945)	(48/7702)	(19/3888)
Rate ratio	1·39	0·92	1·81	1·16	1·28	1·00
	(0·70, 2·77)	(0·43, 1·98)	(0·89, 3·65)	(0·55, 2·43)	(0·75, 2·17)	
Total number of episodes						
Incidence	7·81	6·19	8·27	7·45	7·38	5·43
	(20/2560)	(15/2425)	(15/1814)	(17/2282)	(67/9081)	(24/4418)
Rate ratio	1·44	1·14	1·52	1·37	1·36	1·00
	(0·80, 2·60)	(0·60, 2·17)	(0·80, 2·89)	(0·74, 2·55)	(0·85, 2·16)	

with malaria; 2/41 (4·9%) in the IPV group and 5/86 (5·8%) in the SPf66 group. The incidence of first episodes of clinical malaria detected either by active or passive surveillance was higher in three of the four groups of children who had received SPf66 than in the group who had received IPV and the rate of total episodes was higher in all four of the SPf66 groups than in the control group (Table 1). The incidence of first and of overall episodes of malaria was higher in the children who had received a higher rather than a lower dose of SPf66. However differences between groups were not statistically significant. Poisson regression used to adjust for bednet usage and for overdispersion showed no significant difference between those who had received SPf66 and those who received IPV ($P = 0{\cdot}49$ for first episode and $P = 0{\cdot}37$ for total episodes), and no difference between the SPf66 groups according to dose or source of vaccine.

One hundred and twenty-four children were seen during a cross-sectional survey undertaken at the end of the rainy season. No significant differences in any malaria indices were found between the children who had received SPf66 and those who had received IPV (data not shown).

Antibody levels to SPf66 in children who had received SPf66 two years previously had fallen to background levels. Reciprocal geometric mean antibody titres in high dose Colombian, low dose Colombian, high dose American, low dose American and polio groups were 147, 165, 228, 199 and 124 respectively.

Assessment of the effect of SPf66 on the incidence of malaria was not an objective of the pilot trial. However, as a safety measure, children in the trial have been kept under surveillance for a period of three years. During the initial period of follow-up, children who had received SPf66 had more malaria episodes than those who had received IPV. This pattern continued during a further year of follow-up, although the differences between the groups became less marked. For example the rate ratio for all episodes of malaria was 2·43 in the high dose Colombian vaccine group during the first period of follow-up but 1·44 during the second. How can these findings be explained? One possible explanation is that SPf66 vaccine induced an immune response that made children more susceptible to malaria, as has been observed following vaccination in other infectious diseases (Chin *et al*., 1969, Grayston, Woolridge & Wang 1962) but that this effect decreased with time. An alternative explanation is that the increased susceptibility to malaria in children who received SPf66 was a chance event following the randomisation process. In The Gambia, the risk of malaria varies substantially from village to village (Thompson *et al.* 1994) and within different parts of the same village (Greenwood 1989) so that, because of the small sample size, a higher proportion of children with low risk of malaria might have been randomised to the IPV group. Which of these explanations is correct is unlikely to be established and, fortunately, no enhancement of either disease frequency or severity was found in a much larger efficacy trial of Colombian SPf66 in Gambian children during a two-year period of follow-up (D'Alessandro *et al.* 1995, Bojang *et al*., in preparation). However, future trials of blood stage malaria vaccines must be carefully designed to detect any possible enhancement of clinical disease, especially in young children in areas of intense malaria transmission.

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REFERENCES

Alonso P.L., Smith T., Armstrong-Schellenberg J.R.M. *et al.* (1994) Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet* **344,** 1175–1181

Bojang K.A., Obaro S.K., D'Allesandro U.D. *et al.* (1997) An efficacy trial of the malaria vaccine SPf66 in Gambian infants—second year of follow-up. *Vaccine* (in press)

Chin J., Magoffin R.L., Shearer L.A. *et al.* (1969) Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a paediatric population. *American Journal of Epidemiology* **89,** 449–463

D'Alessandro U., Leach A., Drakeley C.J. *et al.* (1995) Efficacy trial of malaria vaccine SPf66 in Gambian infants. *Lancet* **346,** 462–467

Grayston J.T., Woolridge R.L. & Wang S. (1962) Trachoma vaccine studies on Taiwan. *Annals NY Academy of Science* **98,** 352–367

Greenwood B.M. (1989) The microepidemiology of malaria and its importance to malaria control. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83** (suppl), 595–598

Leach A., Drakeley C.J., D'Alessandro U. *et al.* (1995) A pilot safety and immunogenicity study of the malaria vaccine SPf66 in Gambian infants. *Parasite Immunology* **17,** 441–444

Nosten F., Luxemburger C., Kyle D.E. *et al.* (1996) Randomised double blind placebo controlled trial of SPf66 malaria vaccine in children in northwestern Thailand. *Lancet* **348,** 701–707

Smith T., Armstrong-Schellenberg J.A. & Hayes, R. (1994) Attributable fraction estimates and case definition for malaria in endemic areas. *Statistics in Medicine* **13,** 2345–2358

Tanner M., Teuscher T. & Alonso P.L. (1995) SPf66—the first malaria vaccine. *Parasitology Today* **11,** 10–13

Thomson M.C., D'Alessandro U., Bennett S. *et al.* (1994) Malaria prevalence is inversely related to vector density in The Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88,** 638–643