

MALARIA TRANSMISSION AND DEVELOPMENT OF ANTI-SPOROZOITE ANTIBODIES IN A RURAL AFRICAN COMMUNITY

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The circumsporozoite protein (CS) of P. falciparum and its recently described synthetic repetitive epitope, offer a promising basis for future malaria vaccine development. Baseline information about the dynamics of the antibody response to sporozoites in relation to entomological and clinical data may allow one to assess the role of anti-sporozoite antibodies in the development of resistance against malaria and help to define strategies of vaccination trials in endemic areas. The immune response against CS was assessed in individuals living in a hyperendemic area of rural Tanzania. A cohort of 132 children from one month to 15 years of age was followed for three consecutive years. Comprehensive clinical, parasitological and anthropometric data were collected each year. Serum samples were tested for the presence of antibodies to P. falciparum blood-stage antigens by IFAT and to the synthetic CS peptide (Asn-Ala-Asn-Pro)₄₀ by ELISA. Antibodies to the peptide increased with age. Antibody levels against the synthetic CS peptide did not correlate with antibodies against P. falciparum blood-stage antigens. There was a significant negative correlation between antibodies to the peptide and both parasitaemia and spleen enlargement among these children. Parasite-free or weakly parasitized (< 0,1%) children and those without spleen enlargement had higher median antibody levels against the synthetic CS peptide, but comparable median levels of antibodies against blood-stage antigens. Entomological studies within the same community showed that all the children were exposed to similar levels of infectious mosquito bites. The results of this study suggest that the appearance of anti-sporozoite antibodies may contribute to the development of resistance against malaria in hyperendemic areas.

The recent advances in malaria research have shown that the circumsporozoite proteins (CS) can play a role in the protection against malaria infection in rodent, monkey and human malarias. CS proteins are stage- and species-specific and represent protective antigens. They consist of polypeptides which cover the surface of the sporozoite. The presence of CS proteins on sporozoites is related to the infectivity (reviewed by Cochrane, Nussenzweig & Nardin, 1980; Nussenzweig & Nussenzweig, 1985; Zavala et al., 1985). The immuno-dominant epitope of *P. falciparum* CS protein consists of repetitive units of four amino acids (Asn-Ala-Asn-Pro=NANP, Dame et al., 1984; Enea et al., 1984; Zavala et al., 1985). Monoclonal and polyclonal antibodies to sporozoites react with (NANP)₃ and can neutralize the infectivity of sporozoites *in vitro* (Hollingdale et al., 1984; Mazier et al., 1986). *In vivo*, protective immunity was shown to be associated with the presence of antibodies to CS proteins (Clyde et al., 1975; McCarthy & Clyde, 1977; Cochrane, Nussenzweig & Nardin, 1980).

Antisporozoite antibodies could be demonstrated in sera of individuals living in malaria endemic areas like The Gambia, Thailand and Gabon, who had been repeatedly exposed to infectious mosquito bites (Nardin et al., 1979, 1981; Tapchaisri et al., 1983, 1985; Del Giudice et al., 1986a). All these studies showed that the appearance of antisporozoite antibodies is age-related. However, little is known about the contribution of these antisporozoite antibodies to the development of resistance or concomitant immunity against malaria although Sinton (1940) suggested the importance of antisporozoite immunity, on the basis of his clinical studies with *P. ovale*. Comparisons of the sporozoite-specific immune response with entomological, parasitological and

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clinical data are scarce. An initial attempt was made by comparing levels of antisporezoite antibodies between patients with cerebral and uncomplicated malaria in Thailand (Tapchaisri et al., 1985).

The present report intends to review our recent findings with regard to antisporezoite antibodies and immunity against malaria in endemic areas. The data reviewed originate from a longitudinal study in a rural community of Tanzania (East-Africa). The original comprehensive data underlying this report are being published (Tanner et al., 1986a,b; Del Giudice et al., 1986b).

COMMUNITY BASED STUDIES IN AN ENDEMIC AREA OF TANZANIA

A community-based project based on primary health care was initiated in Kikwawila village (Morogoro region, southeastern Tanzania) in 1982. The project aimed at a study of interactions between nutrition, infection and immunity (Tanner et al., 1987a,b). The village is situated in the Kilombero riverplain at 270 meters above sea level. The area is hyperendemic for malaria as revealed by the malarialogical parameters for the children between 2 and 9 years of age (Fig. 1). The data for this cohort, which was followed for three consecutive years, indicate the well known development of semi-immunity as observed in hyper- and holoendemic areas. More than 90% of the malaria cases were due to *P. falciparum*. Besides malaria, many other parasitic infections were present (*Necator americanus*, *Strongyloides*, *Schistosoma haematobium* and *Giardia lamblia* were the most prevalent, Tanner et al., 1982, 1987b). For example, only 7% of the children were parasite-free in 1982.

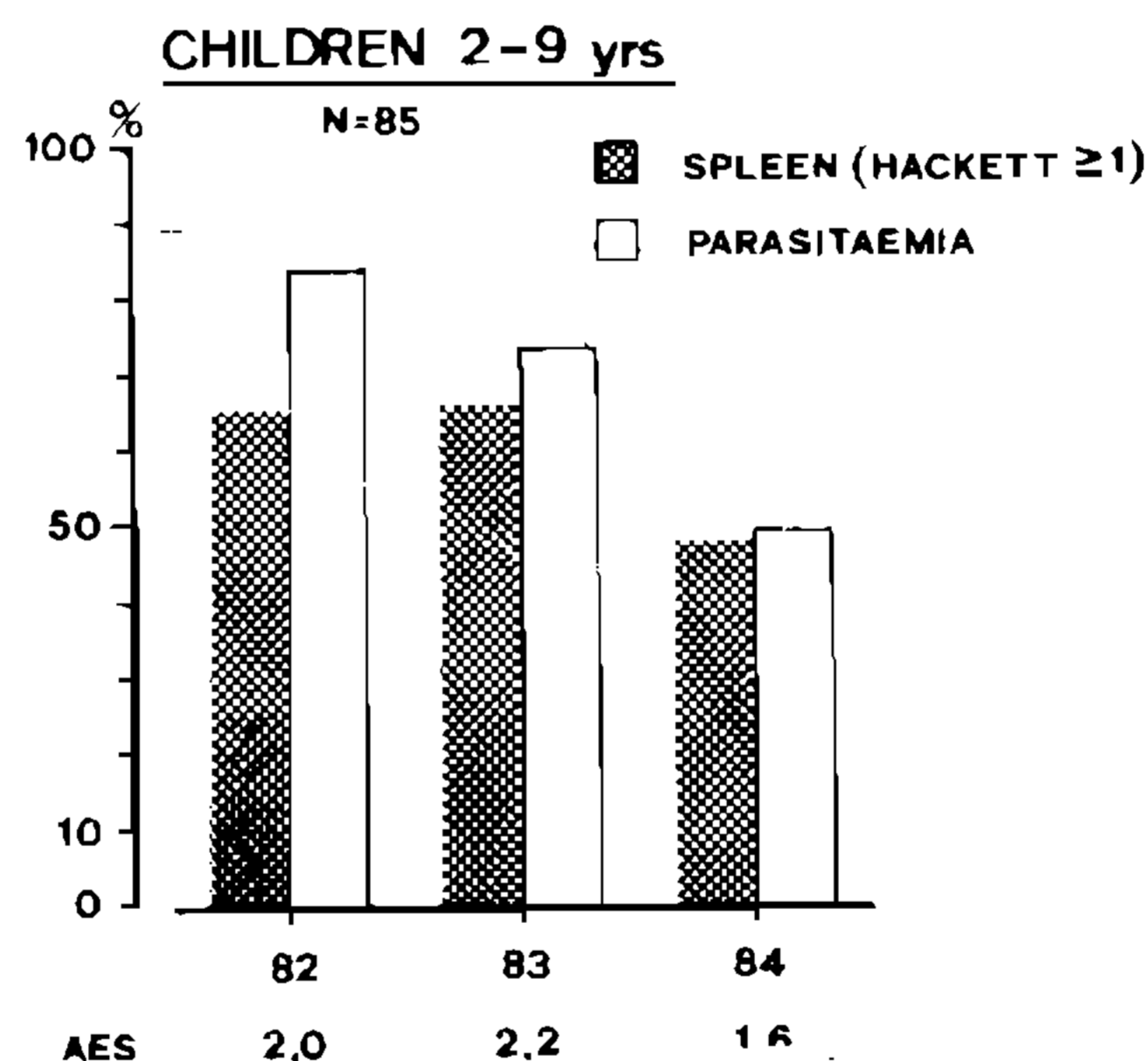


Fig. 1: Spleen rate and parasite rate of 85 children from Kikwawila village followed for three consecutive years. AES = average enlarged spleen according to the Hackett classification.

Children and adults underwent comprehensive clinical, parasitological, serological and anthropometric examinations once per year in October. A representative (compared to the population census of 1982) cohort of 132 children from one month to 15 years of age could be followed for three consecutive years. In the course of an in-depth study on malaria transmission entomological data were collected for nine months in 1983 and 1984 and led to the identification of the most important vectors for malaria in this community: *A. gambiae sensu stricto* and *A. funestus* (Biro, 1987). The entomological data also allowed an estimation of the entomological inoculation rate (EIR=infectious bites/man/night, MacDonald, 1957) faced by the community over the year (Fig. 2). The EIR was seasonal (with a peak of nearly three infectious bites/man/night in June, i.e. just after the rainy season) and different in the two vectors (*A. gambiae s.l.*, *A. funestus*). These findings are a reflection of the pattern of vector densities in the community before, during and after the rainy season (Biro, 1987). The EIR gives an indication of the infection load encountered in the study area, but it does not provide a measure of the amount of antigen inoculated. The number of sporozoites per infected mosquito was not assessed in our study.

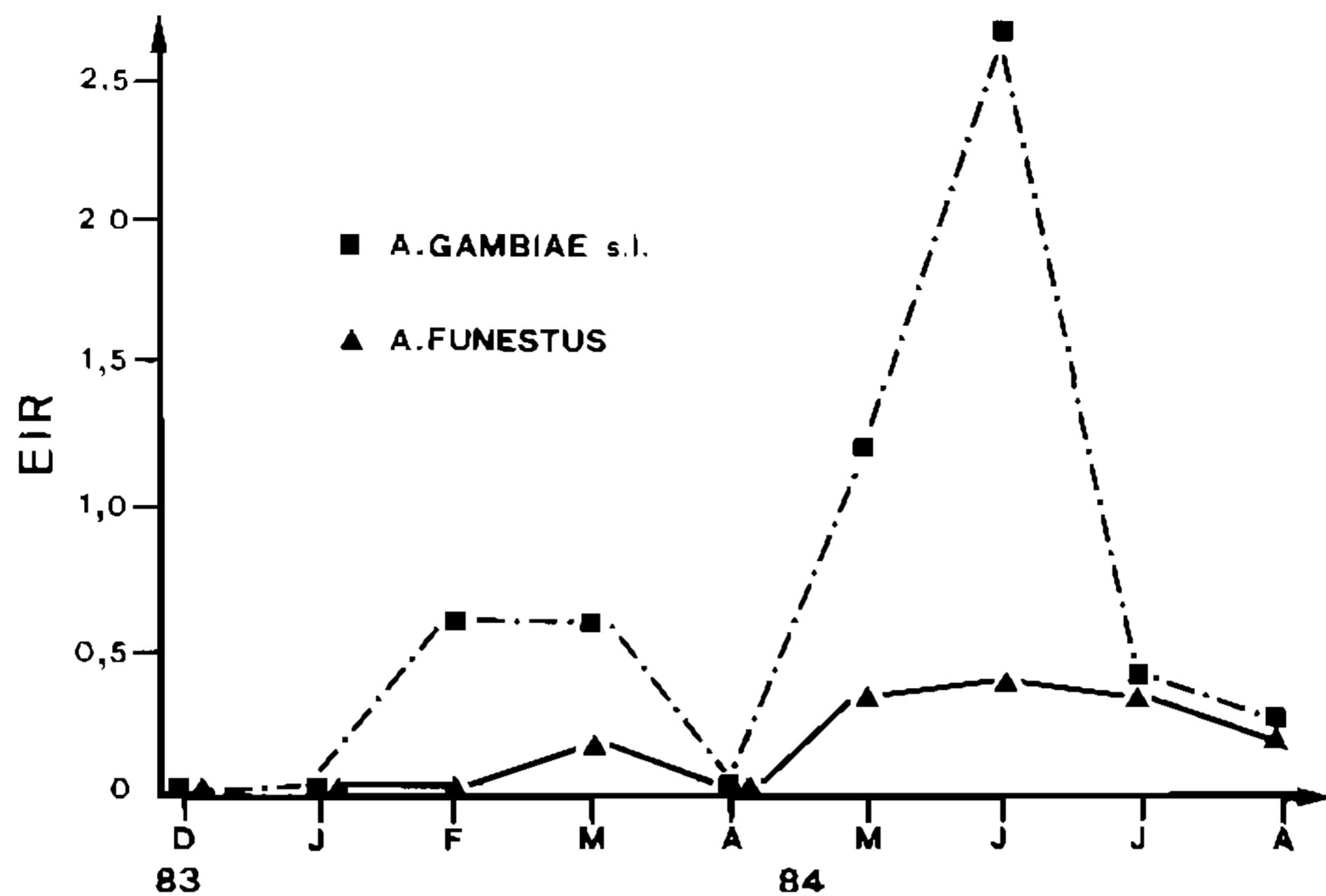


Fig. 2: Entomological inoculation rate (EIR, infectious bites per man and night) in Kikwawila village 1983/84 for the two most important vector species *A. gambiae* s.l., *A. funestus* (graph adapted from Biro, 1987).

Antibodies to sporozoites were determined by an ELISA assay using the synthetic CS polypeptide (NANP)₄₀ as antigen. Ig subclasses and isotypes were not differentiated in our initial studies. This ELISA test system has recently been developed by Del Giudice et al. (1986a). Antibodies against *P. falciparum* blood-stage antigens were detected by IFAT (Ambroise-Thomas, 1974).

Fig. 3 shows the percentage of individuals with antibodies to (NANP)₄₀ as well as the median OD values from the ELISA assay in each age group in 1982. As shown in earlier studies (Nardin et al., 1979; Tapchaisri et al., 1983; Del Giudice et al., 1986a), the frequency of anti-sporozoite antibodies increased as a function of age, reaching values of 100% in adults > 40 years old. The cohort of 132 children from one month to 15 years of age was followed for three consecutive years. In 1982, 65/132 (49%) were positive; in 1984, 59% were positive. The median OD values of anti-sporozoite antibodies tested in ELISA increased within each age group and from one age group to the next (Fig. 3, Del Giudice et al., 1986b). The study also allowed us to look at the individual patterns of seroconversion from 1982 to 1984. Twelve out of 53 (23%) children in the age-range between one month to 5 years converted from negative to positive, compared with only 9% (7/79) among children between 6 and 15 years. The proportion of children converting from positive to negative was similar in both groups (9% vs. 5%). The younger children (1 month-5yrs) had a significantly higher degree of variation in the patterns (e.g. pos. → neg. → pos. or neg. → pos. → neg.) when compared to children aged 6-15yrs (15% vs. 9%). Consequently, children with a positive serology for (NANP)₄₀ during all three years were predominantly found among the older (6-15yrs) ones (54% vs. 25%). Interestingly, the proportion of children with no detectable anti-sporozoite antibodies during all three surveys was similar in both age groups (28% vs. 23%). These findings suggest that one fourth of the children had no or low exposure to infectious bites and/or may have been unable to raise anti-sporozoite antibodies. The latter is indicated by an in-depth individual analysis at the household level (Del Giudice et al., 1986b). Two households with comparable indoor resting mosquito densities, the same house construction (mud wall, corrugated iron sheets) and no mosquito nets in use, had children with both similar spleen enlargement and antibody levels to *P. falciparum* blood-stages. However, a striking difference was observed with regard to anti-sporozoite antibodies (Del Giudice et al., 1986b). While four out of five children of one household had developed antibodies to (NANP)₄₀, only one child out of four of the other household was positive once during the three years follow-up. These observations raise the question of genetic control of the immune response to CS proteins as indicated by recent studies in mice (Del Giudice et al., 1986c).

Anti-sporozoite antibodies correlated positively with age, and a negative correlation with the parasite- and spleen rate was observed. There was no correlation of antibodies to *P. falciparum* blood-stage antigens either with age, parasite rate, spleen enlargement or anti-sporozoite antibodies. Table I summarizes these correlations obtained from the data for the cohort of 132 children for 1982.

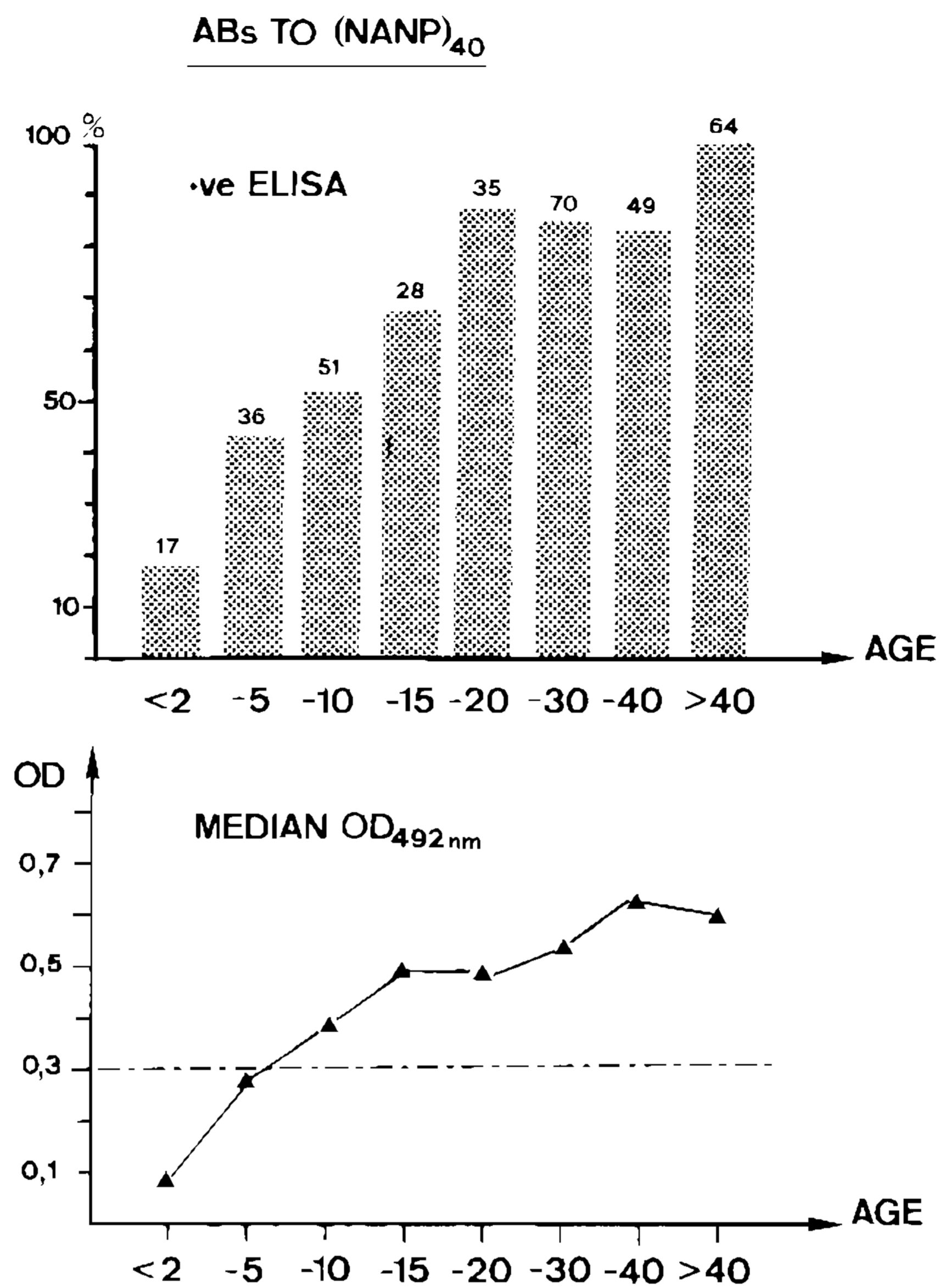


Fig. 3: Frequency of antibodies (abs) to the synthetic CS peptide (NANP)₄₀ among residents from the Ifakara area in 1982. A. Proportion of positive tests in each age group (N on top of each column). B. Median absorbance in ELISA at 492nm for each age group; cut off for pos. ≥ 0.30 (- - -).

TABLE 1

Malaria among children (1 month - 15 years) in Kikwawila Village 1982: Correlations (Spearman rank) between antibody levels and parasitological and clinical parameters

	Spleen Rate	Parasite Rate	Antibodies to: (NANP) ₄₀ ELISA	<i>P.f.</i> bloodst. IFAT
Age	-	---	+++	NS
Spleen Rate		NS	--	NS
Parasite Rate	NS		-	NS

+ = positive correlation

- = negative correlation

+ /

++ / - -

+++ / ---

$P < 0.01$

$P < 0.001$

$P < 0.0001$

NS = not significant, $P < 0.01$.

The longitudinal study in Kikwawila village provided evidence that anti-CS-antibodies (in our case detected with NANP₄₀ as antigen) may be involved in the development of resistance to infection, or concomitant immunity. Fig. 4 summarizes the results from this longitudinal study. Children with no or scarce ($< 0.1\%$) malaria parasites in a thick smear had higher median antibody

levels to (NANP)₄₀ when compared to those with a concurrent *P. falciparum* blood-stage infection (thick smear > 0.1%). A similar picture was obtained for spleen enlargement. Children (2-9 yrs) with no enlargement of the spleen (Hackett classification) had higher median antibody levels to (NANP)₄₀. The mean and median age was not significantly different (U-test) among the two groups (8 vs. 6 yrs). This was not the case for the mean ages of the two groups classified on the basis of parasitaemia. As described in Del Giudice et al., 1986b, the differences in median antibody levels were statistically significant (U-test) for 1982 and 1983 between the groups which were classified on the basis of parasitaemia and spleen enlargement. In contrast, all groups had similar antibody levels to *P. falciparum* blood stage antigens.

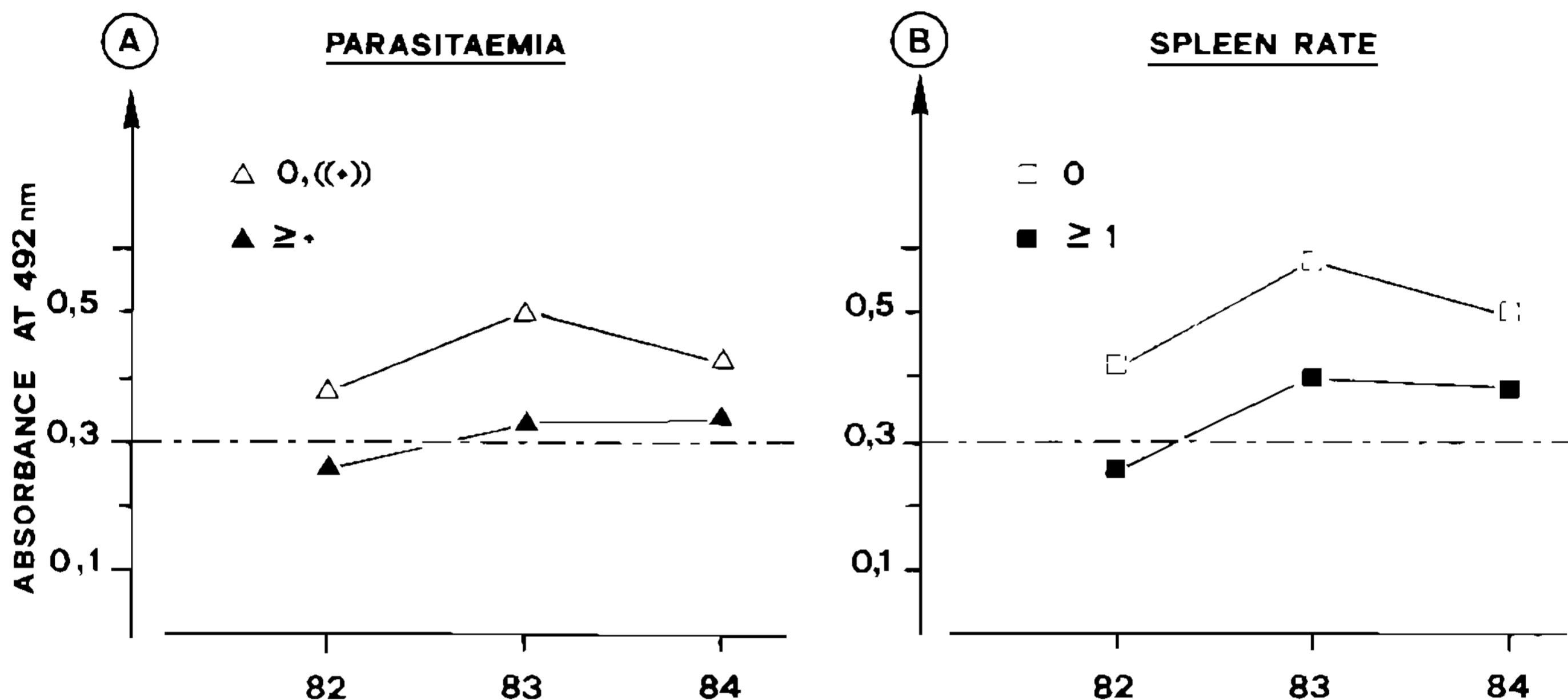


Fig. 4: ELISA with the synthetic CS peptide (NANP)₄₀ as antigen; Median absorbance values at 492nm (cut off pos. ≥ 0.30) among:
 A. 65 children (1m-15yrs) not or weakly (<0.1%) parasitized (Δ) compared to 65 parasitized children (>0.1%, \blacktriangle).
 B. 51 children (2-10yrs) with spleen enlargement (Hackett ≥ 1 , \blacksquare) compared to 25 children without spleen enlargement (\square).
 Results from three consecutive years 1982-1984. (Summarized after Del Giudice et al., 1986n.)

CONCLUSIONS

The limited number of studies carried out to date with sera from malaria endemic areas have shown that the development of antibodies to CS proteins is age-related (Nardin et al., 1979; Tapchaisri et al., 1983; Del Giudice et al., 1986a, b). Antisporozoite antibody levels increase with age leading one to assume a parallel increase in protection to reinfection. Nardin et al. (1979) showed that the increase of antisporozoite antibody levels is also reflected in the number of positive circumsporozoite precipitations (CSP) in sera from The Gambia. Although the significance of the CSP reaction is not yet fully established, the reaction may be associated with protection. Sporozoites which have experienced a CSP reaction lose their infectivity (Cochrane, Nussenzweig & Nardin, 1980; Nussenzweig & Nussenzweig, 1985). On the other hand, Tapchaisri et al., 1983 could not observe CSP reactions in 120 sera from endemic areas of Thailand although 11% of the children and 53% of the adults had antibodies to sporozoites. Following experimental immunization with irradiated sporozoites in man, CSP reactions correlated with protection (McCarthy & Clyde, 1977). CSP testing with sera from our field study in Tanzania could support our findings indicating that antisporozoite antibodies play a significant role in the development of immunity in endemic areas. However, *in vitro* assays with human hepatoma cells (Hollingdale et al., 1984) may be more sensitive as the inhibition of sporozoite invasion can already be detected in the presence of low concentrations of antisporozoite antibodies (Zavala et al., 1985; Mazier et al., 1986).

Immunity to malaria develops by non-specific and specific responses to blood-stage antigens and possibly to sporozoites (Perrin & Dayal, 1982; Nussenzweig & Nussenzweig, 1985). In endemic areas, resistance to infection is dependent on the degree and the duration of exposure (Mcgregor, 1978). In view of the recent findings concerning a blood-stage antigen which shares determinants of the *P. falciparum* CS protein (Hope et al., 1984; Coppel et al., 1985), interrelations between the immune response to blood-stages and sporozoites appear to be even more complex. It remains to be established if the lower anti-CS-antibody levels among children with a concurrent

blood-stage infection is due to absorption of anti-CS antibodies to crossreacting blood-stage antigens. With the same reasoning one could explain the instability of seroconversion patterns among young children (1 month-5 years, s. above) who have more frequent and higher parasitaemia compared to children ≥ 6 years. The contribution of ant sporozoite antibodies to the development of immunity to malaria in endemic areas would appear difficult to establish on immunological grounds alone. The comparison of the immunological findings with clinical, parasitological and entomological data collected during low and high transmission seasons may help to elucidate the involvement of ant sporozoite antibodies in protection, as indicated in this report (Fig. 4, Del Giudice et al., 1986b). Comparative in-depth studies among households with similar and different transmission (especially with regard to EIR, i.e. the number of infectious bites/man/night) will show the dynamics of ant sporozoite antibodies in relation to malaria attacks and spleen enlargement. It may also show whether the presence of ant sporozoite antibodies can be used as an indicator for monitoring therapy and malaria control programmes as proposed by Tapchaisri et al. (1985). Moreover, such household studies could elucidate whether genetic control occurs with regard to the immune response to CS proteins. This hypothesis is supported by recent studies in mice showing H-2 restriction for the immune response to the synthetic CS peptide (NANP)₄₀ (Del Giudice et al., 1986c). If a genetic control were to be identified, it would also be possible to assess the role of ant sporozoite antibodies in protection. The number of malaria attacks over time could be compared with immunological, clinical and parasitological data among responders and non-responders in the same endemic setting.

Responsiveness to CS protein should also be assessed against the background of nutrition. It is well established that nutritional disorders can impair both humoral and cellular immune responses (reviewed by Beisel, 1982). Malaria endemic areas often face problems in food production and food availability leading to malnutrition, which may affect morbidity irreversibly or seasonally. Especially with regard to malaria important interrelations have been described (Murray et al., 1978) and were critically reviewed by McGregor (1982).

The fact that individuals with demonstrable ant sporozoite antibodies still suffer from malaria attacks does not rule out a role for these antibodies in protection. The short exposure of sporozoites to the immune system of the host after an infectious mosquito bite and the varying number of sporozoites injected (Collins et al., 1984) make it possible that single sporozoites may escape and initiate exo- and erythrocytic parasite development. These possibilities make it likely that immunization with CS proteins, or synthetic or genetically engineered CS peptides may not lead to sustained sterile immunity. However, a decreased load of malaria attacks due to immunization with sporozoite and/or blood stage antigens would definitely reduce morbidity and mortality. The results summarized in this paper again confirm the need to assess any future immunization strategies on the basis of the conditions prevailing in the different endemic settings.

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