

# EFFICACY OF PERMETHRIN-IMPREGNATED BED NETS ON MALARIA CONTROL IN A HYPERENDEMIC AREA IN IRIAN JAYA, INDONESIA

## III. ANTIBODIES TO CIRCUMSPOROZOITE PROTEIN AND RING-INFECTED ERYTHROCYTE SURFACE ANTIGEN

I Sutanto<sup>1</sup>, W Pribadi<sup>1</sup>, AL Richards<sup>2</sup>, Purnomo<sup>2</sup>, H-J Freisleben<sup>3</sup>, S Atmoesoedjono<sup>2</sup>,  
R Bandi<sup>4</sup> and P Deloron<sup>5</sup>

<sup>1</sup>Department of Parasitology and <sup>3</sup> Graduate Study Program Biomedical Sciences, Faculty of  
Medicine, University of Indonesia; <sup>2</sup>US Naval Medical Research Unit No. 2, Jakarta,  
Indonesia; <sup>4</sup> Health Research Institute and Development, Department of Health,  
Jakarta, Indonesia; <sup>5</sup> INSERM, Paris, France

**Abstract.** A two years intervention study was carried out using permethrin impregnated bed nets in a hyperendemic area, in Irian Jaya, Indonesia. To assess the influence of this intervention on natural immunity, concurrent immunological studies to determine levels of antibodies to the circumsporozoite (CS) and ring-infected erythrocyte surface antigen (RESA) proteins were conducted. Prevalence and titers of immunoglobulins (Ig)G and IgG subclasses were periodically measured in 138 individuals (30 children under the age of ten and 108 villagers ten years old and older). In the younger group, seropositivity of total IgG against CS fluctuated according to the parasite infection rates; however, IgG seropositive reaction against RESA gradually increased. In the older age group, seropositivity of both kinds of antibodies was stable during the whole study period. Nevertheless, the geometric mean titers of total IgG against CS and RESA were significantly reduced in this latter group in individuals who contained these antibodies before and after intervention. The geometric mean titer of IgG3 subclass against RESA was decreased at a highly significant level ( $p=0.0005$ ), and that of IgG4 against the same antigen was also decreased although to a lesser extent ( $p=0.02$ ).

### INTRODUCTION

Malaria vector control has become the subject of intensive investigation in the last few decades, especially the use of insecticide-impregnated bed nets (Rozendaal, 1989; Sexton, 1994). Although some studies reported on successfully reduced malaria infection, morbidity and mortality (Alonso and Lindsay, 1993; Binka *et al*, 1996; Sutanto *et al*, 1999a,b), it has been suggested that in areas of high endemicity the advantage of vector control programs would only be temporary. In addition,

effective vector control would hamper the development of natural immunity, so that disease and severe cases leading to death might only be postponed and not prevented. If so, vector control with impregnated bed nets should not offer a real advantage to malaria control programs. For this reason, it was important to conduct a study on malaria control with impregnated bed nets in parallel with the study of the immune response of villagers who had been living under this condition for a period of time.

Our study with permethrin-impregnated bed nets in Irian Jaya showed a sustained efficacy of malaria control by lowering endemicity of the area from high to low meso-endemic and reduced parasite infection rates both for *P. falciparum* and *P. vivax* (Sutanto

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Correspondence: I Sutanto, Department of Parasitology, Faculty of Medicine, University of Indonesia, Salemba Raya no. 6, Jakarta 10430, Indonesia.  
Tel : 0062-21-310 2135; Fax: 0062-21-391 4607  
E-mail: inge\_sutanto2@yahoo.com

*et al*, 1999a,b). Now, we evaluated the humoral immune response including IgG subclasses against the below antigens of inhabitants who utilized impregnated bed nets.

Antibodies to two malaria antigens from different stages of the plasmodium life cycle that have been used as important epidemiological markers, namely circumsporozoite (CS) protein and ring-infected erythrocyte surface antigens (RESA) were studied. Antibodies against the (NANP)<sub>n</sub> epitope containing the conserved tetra peptides repeat region of the CS protein have been shown to be the predominant anti-sporozoite antibodies in polyclonal antisera from geographically diverse endemic populations (Brown *et al*, 1989). Anti-(NANP)<sub>n</sub> CS antibody is produced by most naturally infected individuals and has a short serum half-life of less than 30 days (Webster *et al*, 1987). Although this antibody was reported not to play an important role in natural immunity (Hoffman *et al*, 1987; Beck *et al*, 1994), its position as a serological marker of *P. falciparum* transmission has been demonstrated (Webster *et al*, 1992). Antibodies to RESA have also been shown to be important in malaria control. They have been shown to inhibit merozoite invasion *in vitro* (Wahlin *et al*, 1984). Moreover, in monkey trials protection was shown by passive immunization (Berzins *et al*, 1991) with antibodies to RESA, but in other trials this could not be confirmed (Collins *et al*, 1991). RESA does not exhibit antigenic heterogeneity among different isolates of *P. falciparum* (Perlmann *et al*, 1987). In endemic areas, IgG to RESA increases with age, and the majority of adults have high antibody titers against this antigen; controversial evidence exists that this IgG antibody is protective (Wahlgren *et al*, 1986; Petersen *et al*, 1989; Chougnet *et al*, 1990; Riley *et al*, 1991; Mvondo *et al*, 1992; Al-Yaman *et al*, 1995). In Papua New Guinea, Beck *et al* (1995) showed with recombinant RESA that it was not the total IgG level, but the IgG3 subclass that was related to clinical immunity. For this reason, to obtain more accurate information IgG subclasses were evaluated in parallel with total IgG levels in our study.

## MATERIALS AND METHODS

### Study area and population

The study was conducted within the framework of a malaria intervention study with permethrin impregnated bed nets from April 1993 until April 1995 in East Mimika district, south-central Irian Jaya, Indonesia (Pribadi *et al*, 1998; Sutanto *et al*, 1999a,b). The area is highly endemic for malaria and transmission is perennial with seasonal rainfall fluctuation. Rainy season generally lasts from April to September. For the serological investigation, inhabitants were recruited from the treated village, Hiripau, which had 657 villagers and 158 households with 237 children under ten years of age (Sutanto *et al*, 1999a,b). About 90% of the population were local indigenous Irianese from the Kamoro group and the remaining inhabitants originated from islands outside Irian Jaya (Maluku, Sulawesi and Java). In the serological study only indigenous villagers were included.

### Blood collection

Nine cross-sectional epidemiological surveys were carried out in the communities for two years (one survey every two to four months) to evaluate the malaria intervention program. Parasitological examinations with Giemsa stain and spleen examinations based on the Hackett grading system were conducted. The results of the surveys corroborated the sustained efficacy of impregnated bed nets for the two years, in that the high endemic area became low mesoendemic (Sutanto *et al*, 1999a,b).

The assessment of humoral immune response was performed every six months (April 1993, October 1993, April 1994, October 1994 and April 1995) in the beginning and at the end of rainy seasons, respectively. For this purpose, 3-5 ml of venous blood were taken from selected individuals by venipuncture into SST tubes (Becton Dickinson, Franklin Lakes, NJ). In the field, blood samples were centrifuged to obtain sera and stored on ice. In the laboratory in Jakarta, sera were recentrifuged and aliquoted for 0.5 ml and stored at -20°C until used.

## Antigens

Synthetic peptides were utilized for all assays. For CS protein, we used NANP<sub>5</sub> obtained commercially from Emory University, Microchemical Facility, Winship Cancer Center, Atlanta, GA, USA. RESA peptide, EENV<sub>4</sub>-BSA, was kindly donated by INSERM, Paris, France. Antigens were diluted in sterile PBS pH 7.2 before use.

## Sera for antibody assays

At the end of the study in April 1995, all sera collected from each individual were grouped and assayed simultaneously in one ELISA plate to avoid plate variation.

Sera selected to be analyzed from this collection were based upon the limitations of both sera and antigens and also due to the compliance of participants: 138 sera were collected before intervention (April 1993) and analyzed for immunoglobulins to CS and RESA and a relationship of these antibody levels with malariometric parameters and different age groups. Seventy-seven inhabitants who participated in sera collections on five occasions (April 1993, October 1993, April 1994, October 1994 and April 1995) were evaluated for total IgG against NANP<sub>5</sub>, whereas 108 inhabitants who took part in yearly sera collections (April 1993, April 1994 and April 1995), were analyzed for their total IgG against EENV<sub>4</sub>-BSA. One hundred and thirty-eight villagers who participated in two blood collections, *ie* before and after two years of intervention (April 1993 and April 1995), were analyzed for changes in total IgG against NANP<sub>5</sub> and EENV<sub>4</sub>-BSA. From the latter group, those who showed positive results on both occasions were further evaluated to measure the levels of antigen specific IgG subclasses. Twenty-five participants were evaluated for IgG subclasses against NANP<sub>5</sub> and 68 villagers were evaluated for IgG subclasses against EENV<sub>4</sub>-BSA.

## ELISA for IgG against NANP<sub>5</sub> and EENV<sub>4</sub>-BSA

Briefly, 100 µl of NANP<sub>5</sub> solution (20 µg/ml) and EENV<sub>4</sub>-BSA solution (5 µg/ml) were

diluted in PBS pH 7.2 and coated into 96-well flat bottom (Organon®) microtiter plates overnight. These methods were based on that of Guidice *et al* (1987) and Deloron *et al* (1989) for NANP<sub>5</sub> and EENV<sub>4</sub>-BSA, respectively. The next day, plates were blocked for one hour with 5%-skim milk and 3% goat serum in PBS with 0.1%-Tween 20 (Sigma Chemical Company, St Louis, MO). Sera were diluted starting from 1:25 for NANP<sub>5</sub> and 1:100 for EENV<sub>4</sub>-BSA for total IgG in blocking solution and incubated for 1 hour. After washing three times with PBS-Tween, goat anti-human IgG labeled with horseradish peroxidase (TAGO, Camarillo, CA, USA) was added as the second antibody to detect total IgG. The enzymatic reaction was determined spectrophotometrically at 405 nm one hour later by the use of ABTS (2,2'-azino-bis-(3-ethyl-benzthiazoline 6-sulfonic acid) (Kirkegaard and Perry Laboratories, Gaithersburg, MD). Each serum sample was tested against antigen-coated and uncoated wells. In each assay, one plate contained one positive sample and three negative sera to determine cut-off value. Samples were considered positive when the OD with antigen minus the OD without antigen (blank) was greater than the cut-off value. Cut-off value for each antigen was determined based on the mean of cut-off values of daily assays plus three standard errors. The cut-off value for IgG NANP<sub>5</sub> was 0.320, that for IgG EENV<sub>4</sub>-BSA was 0.300.

## ELISA for IgG subclasses

The antigen concentration used for IgG subclass determinations was two times higher than that used for total IgG determinations (40 µg/ml for NANP<sub>5</sub> and 10 µg/ml for EENV<sub>4</sub>-BSA). Dilutions of sera were 1: 12.5 for both antigens. Mouse monoclonal antibodies against human IgG1-IgG4 (Serotec MCA514, MCA515, MCA516 and MCA 517) were used to detect immunoglobulin subclasses. As the third antibody, rabbit anti-mouse IgG F<sub>ab</sub> fragment labeled with horseradish peroxidase was used (Serotec STAR-43 or STAR-39). The enzymatic reaction was revealed between 60-90 minutes later after the addition of ABTS. Cut-off values for IgG subclasses against NANP<sub>5</sub>

and EENV<sub>4</sub>-BSA, respectively, were 0.05 and 0.07 for IgG1, 0.05 and 0.15 for IgG2, 0.03 and 0.1 for IgG3 and 0.08 and 0.05 for IgG4.

**Statistical analysis**

For the cross-sectional analysis,  $\chi^2$  test was used for the proportion of seropositive persons with  $p < 0.05$  as a limit of significance and  $p < 0.001$  as that of highly significant difference. Comparison before and after intervention used McNemar test and paired  $t$ 'test for total IgG towards NANP<sub>5</sub> and EENV<sub>4</sub>-BSA, qualitatively and quantitatively. For IgG subclasses, to see the changes of antibody level based on geometric mean of positive titers, Wilcoxon pairs signed test was used for each antigen with the same  $p$ -values.

**RESULTS**

***P. falciparum* infection rates in groups tested with two different peptides**

The percentage of people infected with *P. falciparum* significantly decreased in the group tested for antibodies to synthetic sporozoite ELISA antigen after six months using bed nets (April 1993: 18.2% = 14/77, October 1993: 1.3% = 1/77)(Fig 1a). Although the infection rate increased again at the end of the rainy season in October 1994 (11.7% = 9/77) (Fig 1a), this figure was still below the base line data. This fluctuation was also reflected in the two different age groups (Group < 10 years from April 1993 until April 1995: 28.6% = 4/14, 7.1% = 1/14, 0% = 0/14, 14.3% = 2/14, 0% = 0/14; group  $\geq$  10 years from April 1993 until April 1995: 15.9% = 10/63, 0% = 0/63, 1.6% = 1/63, 11.1% = 7/63, 1.6% = 1/63) (Fig 1a).

Furthermore, in the group assayed with RESA peptide, prominent reduction of *P. falciparum* infection was evident every year (April 1993: 16.7% = 18/108; April 1994: 2.8% = 3/108 and April 1995: 0.9% = 1/108)(Fig 1b). This pattern was also confirmed in the two different age groups (Group < 10 years from April 1993 until April 1995: 33.3%

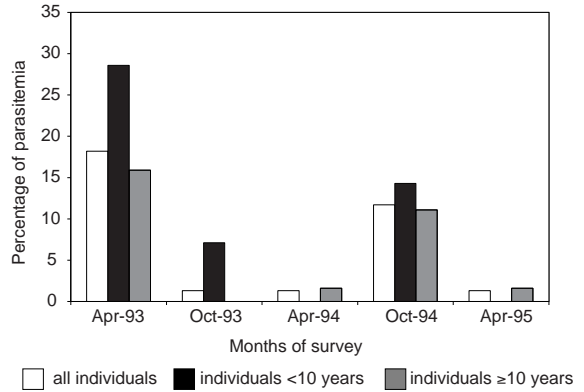


Fig 1a—*P. falciparum* infection rate in groups tested with CS peptide; both age groups showed a fluctuation pattern of infection rates which still below the base-line data (April 1993).

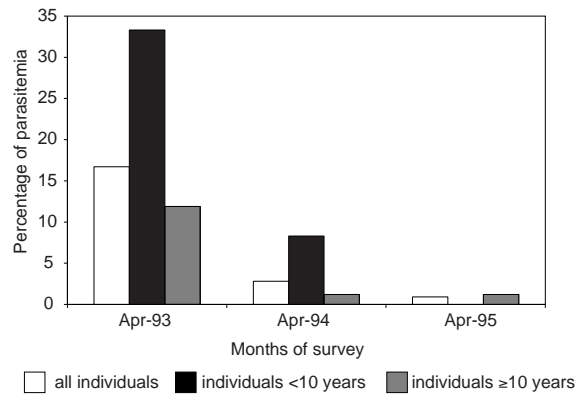


Fig 1b—*P. falciparum* infection rate in groups tested with RESA; both age groups demonstrated a marked reduction of infection rate every year.

= 8/24, 8.3% = 2/24, 0% = 0/24; group  $\geq$  10 years from April 1993 until April 1995: 11.9% = 10/84, 1.2% = 1/84, 1.2% = 1/84) (Fig 1b).

**Distribution of total IgG and IgG subclasses against NANP<sub>5</sub> and EENV<sub>4</sub>-BSA at the beginning of the study**

During the first survey in April 1993, only 29.7% (41 villagers out of 138) showed IgG against NANP<sub>5</sub>, whereas 64.5% (89 villagers) had IgG against EENV<sub>4</sub>-BSA (Fig 2a, 2b). Moreover, when analysis was carried out based on age differentiation (under and over ten years),

seropositive and geometric mean titers of total IgG against these two peptides were all significantly higher in the older group (Table 1). Significant difference in total IgG between the two age groups was not reflected at all by the response of any IgG subclass against the two antigens either qualitatively or quantitatively (data not shown). Distribution of IgG subclasses in both age groups showed dominance of IgG3 and IgG4 (50-75%), whereas proportions of IgG1 and IgG2 were found to be only between 11% and 25%. This distribution was similar for both peptides. Moreover, no association was found between total IgG and all of IgG subclasses to both peptides with malarimetric parameters, before intervention commenced (data not shown).

**Changes in total IgG to NANP<sub>5</sub> and IgG to EENV<sub>4</sub>-BSA during two years of intervention**

In total, 77 individuals (14 from the age group < 10 years and 63 from the age group ≥ 10 years) were followed up for their total IgG to NANP<sub>5</sub> for two years on five occasions. During this period of time, the prevalence of parasitemia in these individuals from both age groups was high after a rain peak and low at the beginning of the rainy season (Fig 1a). In the younger group, the seropositive reaction of IgG to NANP<sub>5</sub>, followed the pattern of parasitemia prevalence and rainfall (From April 1993 until April 1995: 21.4% = 3/14, 7.1% = 1/14, 0% = 0/14, 21.4% = 3/14, 7.1% = 1/

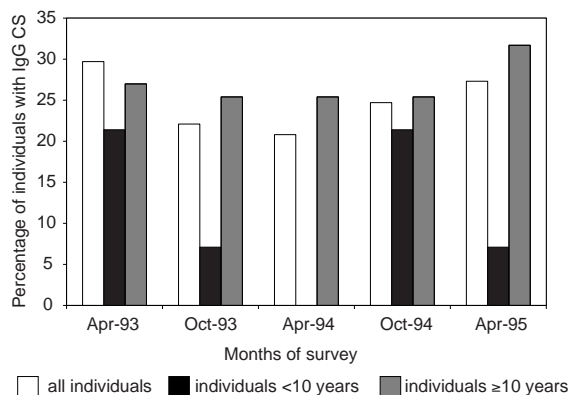


Fig 2a—Seropositive prevalence of IgG against CS by months of survey; individuals <10 years showed a fluctuation pattern, but individuals ≥10 years showed a constant one.

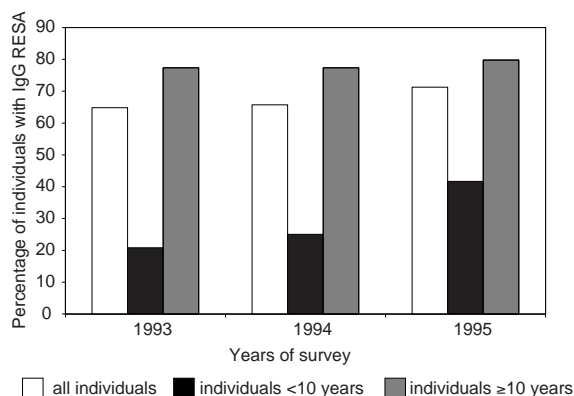


Fig 2b—Seropositive prevalence of IgG RESA by year of survey; individuals <10 years showed a gradual increase of seropositive rate, but individuals ≥10 years showed a constant rate.

Table 1  
IgG NANP<sub>5</sub> and IgG EENV<sub>4</sub>-BSA in different age groups before intervention.

Immunoglobulins	< 10 years	≥ 10 years	p-value
IgG NANP <sub>5</sub>			
Seropositive	13.3% (4/30)	34.3% (37/108)	0.0283 <sup>a</sup>
GMT <sup>c</sup>	19.3 ± 3.4	32.1 ± 4.8	0.0407 <sup>a</sup>
IgG EENV <sub>4</sub> -BSA			
Seropositive	20% (6/30)	76.9% (83/108)	0.0000 <sup>b</sup>
GMT <sup>c</sup>	75.9 ± 2.7	343.8 ± 5.5	0.0000 <sup>b</sup>

<sup>a</sup>significantly different (p < 0.05).

<sup>b</sup>highly significant (p < 0.001).

<sup>c</sup>GMT = Geometric mean of positive titers.

Table 2  
Geometric mean of positive titers of total IgG and IgG subclasses against NANP<sub>5</sub> before and after two years of intervention.

Immunoglobulins (N)	April 1993	April 1995	p-value
IgG total (25)	279 ± 4.2	131.9 ± 2.8	0.006 <sup>a</sup>
IgG3 (13)	38.3 ± 2.5	29.3 ± 1.9	0.2489
IgG4 (12)	14.9 ± 1.5	14 ± 1.3	0.6547

N = number of examined individuals.

<sup>a</sup>significantly different ( $p < 0.05$ ).

14) (Fig 2a). By contrast, in the older age group, although the proportion of those with parasitemia fluctuated, the percentage of total immunoglobulin to CS was constant (around 25%) (From April 1993 until April 1995: 27% = 17/63, 25.4% = 16/63, 25.4% = 16/63, 25.4% = 16/63, 31.7% = 20/63) (Fig 2a).

The follow up of total IgG to EENV<sub>4</sub>-BSA in these two years of intervention was carried out yearly with 108 villagers (24 from age group < 10 years and 84 from age group ≥ 10 years). The percentage of parasitemia in these individuals decreased in both age groups, markedly in the younger group and more slowly in the older group (Fig 1b). Unlike the total IgG to NANP<sub>5</sub>, which fluctuated with parasitemia, the proportion of seropositive IgG to EENV<sub>4</sub>-BSA was increased slightly in the younger group (April 1993: 20.8% = 5/24, April 1994: 25% = 6/24, April 1995: 41.7% = 10/24). In the older group, the seropositive reaction of total IgG showed again a constant pattern as in the case of NANP<sub>5</sub> peptide (Fig 2b). When analysis was further carried out with children under ten, IgG3 subclass was detected in 8 out of 10 children with positive IgG EENV<sub>4</sub>-BSA in April 1995, whereas the other subclasses (IgG1, IgG2 and IgG4) were found only in 2-4 children. By contrast, in April 1993, distribution of IgG subclasses showed no difference in these children (3-4 out of 5 children had positive reaction for each subclasses).

#### Comparison of total IgG and IgG subclasses before and after two years of intervention

One hundred and thirty-eight individuals

who attended surveys before and after two years of intervention were evaluated for changes of their total IgG and IgG subclasses against the two antigens. For both peptides, only a smaller proportion of individuals (around 20%) showed a seroconversion either from positive IgG to negative IgG or vice versa. All other individuals remained either IgG positive (57.2% for IgG to EENV<sub>4</sub>-BSA) or IgG negative (58.7% for IgG NANP<sub>5</sub>).

Analysis was then carried out only with individuals who were IgG positive on the two occasions, to look for changes of antibody levels before and after intervention. For the NANP<sub>5</sub> peptide, 25 individuals and for the EENV<sub>4</sub>-BSA peptide, 68 individuals were evaluated. The results showed a significant decrease in levels of total IgG to both peptides after intervention (NANP<sub>5</sub>: 279 ± 4.2 vs 131.9 ± 2.8, paired *t*-test,  $p = 0.006$  and for EENV<sub>4</sub>-BSA: 744.9 ± 4.3 vs 543 ± 4.3, paired *t*-test,  $p = 0.046$ ) (Table 2, 3).

Investigation of these two groups was further carried out on the IgG subclasses. The data revealed only a small proportion of those individuals (not more than 20%) who showed seroconversion from IgG subclass positive to IgG subclass negative or vice versa. About 80% remained IgG subclass positive (IgG3 and IgG4) or remained IgG subclass negative (IgG1 and IgG2). The level of changes of all immunoglobulin isotypes was then examined only in the individuals who remained positive, before and after intervention. For the NANP<sub>5</sub> peptide, only IgG3 and IgG4 were evaluated in 13 and 12 persons, respectively. For the EENV<sub>4</sub>-BSA

Table 3  
Geometric mean of positive titers of total IgG and IgG subclasses against EENV<sub>4</sub>-BSA before and after two years of intervention.

Immunoglobulins (N)	April 1993	April 1995	p-value
IgG total (68)	744.9 ± 4.3	543 ± 4.3	0.046 <sup>a</sup>
IgG1 (17)	52.1 ± 2.6	33.3 ± 2.3	0.0665
IgG2 (25)	34.9 ± 1.9	33.9 ± 2	0.4216
IgG3 (38)	72 ± 2.6	36.7 ± 2.6	0.0005 <sup>b</sup>
IgG4 (42)	28.1 ± 1.7	15.5 ± 2.7	0.0208 <sup>a</sup>

<sup>a</sup>significantly different ( $p < 0.05$ ).

<sup>b</sup>highly significant ( $p < 0.001$ ).

<sup>c</sup>N = number of examined individuals.

peptide, 17, 25, 38 and 42 individuals were analyzed for IgG1, IgG2, IgG3 and IgG4, respectively. Wilcoxon pairs signed test revealed a highly significant decrease in IgG3 to EENV<sub>4</sub>-BSA ( $p=0.0005$ ) after two years of intervention and a significantly reduced titer of IgG4 to EENV<sub>4</sub>-BSA ( $p=0.0208$ ) (Table 3). Assays of IgG3 and IgG4 to NANP<sub>5</sub> did not show any significant decrease after intervention for IgG3 ( $p=0.2489$ ) and IgG4 ( $p=0.6547$ ) (Table 2).

## DISCUSSION

The skeptic consideration of the declining immune response to malaria in populations sleeping under impregnated bed nets has long been a controversy among public health professionals. If this phenomenon really happens, the application of bed nets and similar intervention needs to be reevaluated. However, sufficient evidence of association between intervention and immune response has hitherto not been available.

A five-year study in Nigeria, West Africa, utilizing residual insecticide spraying and periodic mass drug administration compared parasitological and serological parameters of protected versus unprotected populations during the study period. Using crude antigens, it was shown that during intervention antibodies against *P. falciparum* and *P. malariae* declined

only quantitatively and this phenomenon was prominent at the end of the intervention period (Brögger *et al*, 1978).

Our study utilized base-line data of a protected population before intervention for evaluation of bed nets influence on the villagers' immune response to specific malaria antigens (NANP and RESA). Application of impregnated bed nets successfully reduced malaria infection rate, taking into account that the fluctuation of parasitemia prevalence was always under base-line data in both age groups throughout the study (Fig 1a, 1b). Unlike the study in Nigeria, changes in the immune response were detected either qualitatively or quantitatively in two different age groups. In children under the age of ten, qualitative changes were demonstrated (Fig 2a), where percentage of IgG seropositive to circumsporozoite peptide (NANP) fluctuated in parallel with the parasitemia (Fig 1a, 2a). The role of this antibody as a transmission marker has been reported in previous studies (Webster *et al*, 1992; Kremsner *et al*, 1992; Ramasamy *et al*, 1994) either in prevalence or titer. However, due to the small number of tested children (only 14 individuals), our results may not totally reflect the younger group's immunological response. Nevertheless, this data illustrated that some children responded normally to the exposure, *ie*, high in rainy and low in dry seasons during the intervention period. By contrast, no significant qualitative changes of IgG to NANP<sub>5</sub> were

detected in the older group during the two-year study (Fig 2a). Nevertheless, considerably decreasing titers of IgG against this peptide were detected at the end of intervention period only in a certain group of people who remained positive before and at the end of the intervention study (Table 2).

Alteration of the immune response against the asexual stage of *P. falciparum* was assessed with RESA antigen. In the older group, again, no qualitative changes were noted in terms of seropositive percentage. Other studies have indicated an influence of genetic factors on the hosts' immune response to this antigen (Björkman *et al*, 1990; Petersen *et al*, 1990; Riley *et al*, 1991; Rooth *et al*, 1991). This may explain the significant decrease in total IgG, in addition to IgG3 and IgG4 titers (the two dominant subclasses in the study) only in a certain group of people who remained positive before and at the end of the intervention (Table 3). The role of IgG3 subclass in malaria control has been reported to be substantial in terms of managing plasmodium infection (Beck *et al*, 1995; Aribot *et al*, 1996; Ferreira *et al*, 1996; Taylor *et al*, 1998). Furthermore, short-lived IgG3 depends on continuous malaria transmission; therefore, breaking parasite transmission due to seasonal changes and control activities might affect the longevity of anti-malarial immunity in adults (Ferrante and Rzepcyk, 1997). A longitudinal follow-up study could not be performed but this would have been helpful to determine the affect of discontinuing control activities in our study population, especially for those individuals 10 years of age or older, considering that an increase in susceptibility to re-infection has been reported to appear after one year's cessation of intervention in another study (Brögger *et al*, 1978).

In the younger group, individuals showed qualitative changes in terms of gradual increase in seropositive reaction to RESA antigen in parallel with increasing IgG3 prevalence. This is in line with Björkman *et al* (1991), who reported higher levels of antibodies to asexual stages in children with long-term chemoprophylaxis compared to children without preven-

tion therapy. This phenomenon was associated with the elimination of immune suppression due to lower parasite burden. Malaria parasites produce toxins that could induce immune suppression in their host. Consequently, by decreasing parasite burden, toxin levels would be diminished and immune response normalized. The possibility of existing immune-suppression in Irian Jaya, was reported by other investigators who demonstrated a low cellular response to circumsporozoite antigen in their study (Campbell *et al*, 1988). Our data suggested that this phenomenon also occurred among the younger individuals of our study. Therefore, the application of impregnated bed nets would not necessarily have a harmful consequence among children, but rather the reduced immune suppression would be an additional positive effect of lowering malaria parasite burden.

The results of our study showed the impact of impregnated bed nets on the immune response of population of villagers exposed to hyperendemic malaria. Moreover, the effects observed differed between children under the age of ten and those individuals 10 years old and older. At least two aspects of the impact of bed nets on the immune response to malaria are considered important: the influence of genetic factors that determines the host's immune response and the level of malaria endemicity in the study area. Clearly, in our study, people who responded to malarial antigens (CS and RESA) diminished their immune response during intervention. Whether this is a functional change in the immune response or not, must be determined before the consequences of bed nets utilization can be fully ascertained.

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