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Lymphocyte Proliferative Response and Subset Profiles during Extended Periods of Chloroquine or Primaquine Prophylaxis

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Immune suppression and disturbances of normal leukocyte populations are side effects attributed to many antimalarial drugs and were concerns during a recent year-long placebo-controlled trial that compared daily primaquine (0.5 mg of base per kg of body weight per day) with weekly chloroquine (300 mg of base one time per week) for malaria prophylaxis. The study took place in Irian Jaya, Indonesia, from July 1994 to August 1995 and enrolled 129 Javanese men with normal glucose-6-phosphate dehydrogenase function. Tests for lymphocyte function and subset composition were conducted blindly on a cross-section of subjects during weeks 10 ($n = 42$) and 48 ($n = 72$) of supervised prophylaxis. Lymphocyte function, measured as the proliferative response of peripheral blood mononuclear cells to a panel of mitogens (pokeweed mitogen, phytohemagglutinin, and concanavalin A) and antigens (purified protein derivative of *Mycobacterium tuberculosis* and *Clostridium tetani* toxoid) and expressed as a stimulation index, allowed for statistical comparison between groups and sampling times. The lymphocyte subset composition for each group and time point was based on flow cytometry profiling, and the results were expressed as the mean percentages of CD3 (total T cells), CD19 (total B cells), $CD4^+$ (T-helper and inducer cells), and $CD8^+$ (T suppressor and cytotoxic cells), CD3/$CD16^+$ CD56 (natural killer cells), CD3/anti-HLA-DR (activated T cells) cells and the $CD4^+/CD8^+$ ratios. Lymphocyte stimulation indices were statistically comparable among the placebo, primaquine, and chloroquine groups at both time points, although the primaquine group was distinguished by having repeatedly greater proportions of subjects with high (>3.0) stimulation indices. The lymphocyte subset profiles of these groups at both time points were also similar and undistorted relative to those of healthy reference populations matched for age, sex, and ethnicity. The results provide quantitative support for the safety of daily primaquine prophylaxis.

The 14-day regimen of primaquine for the radical cure and terminal treatment of relapsing malaria has been in use for nearly half a century, but owing to real or perceived issues of toxicity, this drug has only recently been evaluated for use as a malaria prophylactic (1, 12, 23). During a 12-month chemoprophylaxis trial in Irian Jaya, Indonesia, daily primaquine provided better than 90% protection against falciparum and vivax malaria, and no difference in the occurrence of physical complaints or nonmalaria illness was seen between the primaquine, chloroquine, and placebo groups. With the exception of asymptomatic methemoglobinemia, which was expected, no evidence of primaquine toxicity was apparent in endpoint measures of renal and hepatic function (12). Nevertheless, immune suppression and derangement of lymphocyte populations were considered to be potentially insidious side effects that might occur during extended chemoprophylaxis (4, 17, 19). Therapeutic levels of both chloroquine and primaquine in vitro have been shown to depress mitogen-driven lymphocyte proliferation (14, 20). Chloroquine is concentrated in vivo by lymphocytes, where it is known to inhibit antigen processing (15, 18). The in vivo effects of primaquine on lymphocyte function are not known with certainty, but in vitro biotransformation of this drug to its principal metabolite, carboxyprimaquine [or 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline], has been demonstrated by hematopoietic cells (11), and high doses have induced leukocytosis, neutropenia, and granulocytopenia in humans (5, 6, 8, 9). Evaluations of the short- and long-term effects of primaquine prophylaxis on lymphocyte function and subset profiles were therefore important additional safety issues. This report compares lymphocyte function and subset profiles in primaquine, chloroquine, and placebo study groups after moderate (10-week) and long-term (48-week) periods of supervised chemoprophylaxis.

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MATERIALS AND METHODS

Study site and subjects. Details of the chemoprophylaxis study have been published elsewhere (12). The study took place in Arso XI, northeastern Irian Jaya, during the period between July 1993 and August 1994. This work was conducted in accordance with U.S. Navy and Republic of Indonesia regulations governing the protection of human subjects in medical research. American and Indonesian committees for the protection of human subjects reviewed and approved the procedures followed in this research. After providing informed, written consent, 129 adult male Javanese volunteers were tested to confirm that they had normal glucose-6-phosphate dehydrogenase activity, curatively treated with quinine-doxycycline-primaquine for any malaria infection, and randomized to receive either daily primaquine (0.5 mg of base per kg of body weight daily; 30 mg daily for men weighing >55 kg), weekly chloroquine (300 mg of base one time weekly), or a daily placebo over a 12-month period. Testing for lymphocyte function and subset composition was delayed until week 10 of prophylaxis in order to (i) allow the subjects to recover from preexisting malaria infections, (ii) avoid the drug effects induced by the 14-day radical cure, and (iii) permit the subjects to normalize to their prophylactic regimen. Laboratory testing was performed by technicians blinded to the drug groups of the subjects. Subjects reported no physical complaints or illness at the time of testing, and blood films were negative for malaria parasites.

Lymphocyte function. A random cross-sectional sample of volunteers (approximately 30%) was drawn from the study population to yield approximately equal numbers of subjects receiving primaquine, chloroquine, or placebo for lymphocyte proliferation assays. Venous blood (20 ml) was collected during fasting into heparinized tubes at the 10- and 48-week points of prophylaxis. After centrifugation, the plasma was removed and the buffy coat and top layer of erythrocytes were transferred to an equal volume of RPMI wash medium (RPMI 1640 medium supplemented with 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], 2% $NaHCO_3$, 100 μg of gentamicin per ml, 2 mM glutamine). Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (Ficoll-Hypaque; Sigma Chemical Co., St. Louis, Mo.). Washed PBMCs were counted with a hemocytometer, and the volume was adjusted with cRPMI (RPMI medium supplemented with 10% pooled human type AB serum) to achieve a final concentration of 10^6 PBMCs per ml. The PBMCs (2×10^5 cells in 200 μl) were plated into each well of a round-bottom microtiter plate (Costar, Cambridge, Mass.), and 10 μl of mitogen or antigen in cRPMI was added to the well. The mitogen or antigen concentrations in the wells at week 10 were as follows: pokeweed mitogen (PWM; Sigma), 2.5, 0.83, and 0.28 μg/ml; phytohemagglutinin A (PHA; Sigma), 10.0, 3.3, and 1.1 μg/ml; concanavalin A (ConA; Sigma), 12.5, 1.25, and 0.125 μg/ml; and purified protein derivative of *Mycobacterium tuberculosis* (PPD; Connaught Laboratories Ltd., Willowdale, Ontario, Canada), 10.0, 3.3, and 1.1 μg/ml. The mitogen or antigen concentrations in the wells at week 48 were as follows: PHA, 5.0 and 2.5 μg/ml; PPD, 10.0 and 5.0 μg/ml; and *Clostridium tetani* toxoid (TET; Connaught), 10.0, 1.0, and 0.1 μg/ml. The PBMCs of the subjects were tested in quadruplicate against each concentration of mitogen and antigen. cRPMI alone was added to four to eight control wells containing PBMCs from each subject. The plates were covered and were maintained at 37°C in humidified air containing 5% CO_2. On day 6 each well was pulsed with 0.5 μCi of tritiated thymidine (Amersham International plc, Buckinghamshire, United Kingdom) in cRPMI, and after an additional 24 h of incubation the cells were harvested and washed and tritium uptake was counted with a liquid scintillation counter (Top Count; Packard Instrument Co., Meridien, Conn.). A stimulation index (SI) was determined, by subject, for each mitogen and antigen concentration on the basis of the ratio of the mean counts per minute of the stimulated wells to the mean counts per minute of the unstimulated control wells. A significant proliferative response is conventionally an SI of >2.0, but we arbitrarily applied the more stringent level of an SI of ≥3.0 to define responders in this study.

Lymphocyte subset profiles. Differential counts of total leukocytes, percent lymphocytes plus monocytes, percent granulocytes, and platelets were measured by the quantitative buffy coat-complete blood count (QBC-CBC) centrifugal method (Becton Dickinson Centrifugal Hematology System) from venous blood at the baseline and at weeks 10 and 48 of prophylaxis. A venous blood sample of 3 ml was taken from the same subjects in the prophylaxis group subsets at the 10- and 48-week points of prophylaxis and placed in EDTA-containing tubes (Becton Dickinson) for the enumeration of lymphocyte subsets by flow cytometry. All reagents, procedures, equipment, and software used for flow cytometry were from Becton Dickinson Immunocytometry Systems, San Jose, Calif. Respective aliquots of blood in EDTA-containing tubes were incubated in the dark at room temperature for 30 min with (i) a Simultest LeucoGATE (CD45/CD14 [anti-HLe-1/Leu-M3]) negative control to set an exclusive lymphocyte gate free of debris, monocytes, and granulocytes; (ii) Simultest Control γ_1/γ_{2a} (immunoglobulin G1 [IgG_1], fluorescein isothiocyanate [FITC], and IgG_{2a} phycoerythrin [PE]) to set the fluorescence intensity and to measure nonspecific staining of the sample; (iii) Simultest T- and B-cell test reagent (CD3/CD19 [anti-Leu-4 FITC/anti-Leu-12 PE) for the percentages of total T cells and total B cells; (iv) Simultest CD4/CD8 (Leu-3a/2a) for the percentage of $CD4^+$ T-helper and inducer cells, the percentage of $CD8^+$ T-suppressor and cytotoxic cells, and the $CD4^+/CD8^+$ ratio; (v) Simultest CD3/Anti-HLA-DR (Leu-4/anti-HLA-DR) for the percentage of activated T cells; and (vi) Simultest $CD3/CD16^+$ CD56 (Leu-4/11c+19) for the percentage of natural killer cells. Staining was followed by lysing of erythrocytes with fluorescence-activated cell sorter lysing solution, washing to remove unbound antibody, and fixation in phosphate-buffered saline–1% formaldehyde. Negative control and sample aliquots for each subset of interest were analyzed with a FACScan two-color flow cytometer equipped with SimulSET software and interfaced with a desktop computer. Instrument calibration and quality control were performed daily by using CaliBRITE beads and AutoCOMP software in accordance with the manufacturer's recommendations. Data for each sample and its negative controls were visually and automatically inspected to determine quality. Samples with insufficient leukocytes or those which did not meet all gating criteria were excluded from the analysis. Data for samples which met all quality criteria proceeded through the software option for percent lymphocyte conversion and were reported as appropriate estimates of the true subset values.

Statistical analyses. SIs for the mitogen and antigen concentrations for the subjects in the prophylaxis and placebo groups were log transformed and were statistically compared by analysis of variance (ANOVA) if they were normally distributed or by the Kruskal-Wallis nonparametric test if Bartlett's test for homogeneity of variance showed that the data were not normally distributed (2). Statistical analyses between groups for each mitogen or antigen concentration in the panel compared SIs for (i) the combined responder plus nonresponder cohorts or (ii) the responder cohort only. A geometric mean SI (GMSI) and standard deviation were calculated for each group and for each mitogen or antigen concentration. The proportion of responder subjects in each group was compared by the chi-square or Fisher's exact test. Differential counts of leukocytes at the baseline and at 10 and 48 weeks were compared by one-way ANOVA, and selected groups were compared by the *t* test. The lymphocyte subsets for each study subject were expressed as the percentage of the subject's total lymphocyte count. One-way ANOVA was applied to compare the counts between the prophylaxis and placebo groups and against a larger reference population ($n = 68$) of healthy Indonesian men matched for age and Javanese-Sundanese ethnicity.

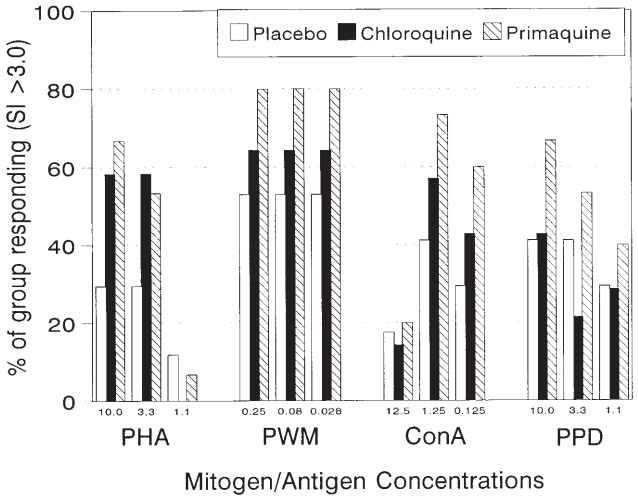

FIG. 1. Proportion of groups responding to mitogen or antigen after 10 weeks of malaria chemoprophylaxis. Responder subjects had an SI (mean counts per minute for stimulated PBMCs/mean counts per minute for unstimulated PBMCs) of ≥3. The mitogens or antigens were as follows: PWM tested at 2.5, 0.83, and 0.28 μg/ml; PHA tested at 10.0, 3.3, and 1.1 μg/ml; ConA tested at 12.5, 1.25, and 0.125 μg/ml; and PPD tested at 10.0, 3.3, and 1.1 μg/ml.

RESULTS

Lymphocyte proliferative response. The frequency or proportion of responder subjects with SIs of >3.0 was repeatedly

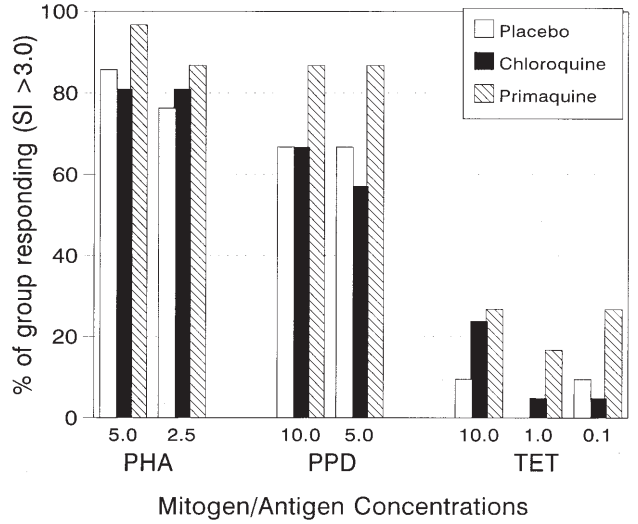

FIG. 2. Proportions of groups responding to mitogen or antigen after 48 weeks of malaria chemoprophylaxis. Responder subjects had an SI (mean counts per minute for stimulated PBMCs/mean counts per minute for unstimulated PBMCs) of ≥3. The mitogens or antigens were as follows: PHA tested at 5.0 and 2.5 μg/ml; PPD tested at 10.0 5.0 μg/ml; and TET tested at 10.0, 1.0, and 0.1 μg/ml.

TABLE 1. GMSIs of mitogen- or antigen-driven lymphocyte proliferation by combined prophylaxis group and responder cohort after 10 weeks of malaria chemoprophylaxis[a]

Stimulant and concn	GMSI (SD) for the following prophylaxis group:					
	Placebo		Primaquine		Chloroquine	
	Combined	Responder	Combined	Responder	Combined	Responder
PWM						
1:400	5.2 (7.5) ($n = 17$)	28.8 (2.5) ($n = 9$)	13.9 (4.2) ($n = 15$)	25.5 (2.2) ($n = 12$)	7.5 (10.1) ($n = 14$)	37.9 (3.4) ($n = 9$)
1:1,200	6.7 (6.5) ($n = 17$)	28.8 (2.5) ($n = 9$)	14.9 (3.8) ($n = 15$)	26.6 (2.0) ($n = 12$)	7.6 (9.8) ($n = 14$)	39.7 (3.0) ($n = 9$)
1:3,600	7.0 (6.6) ($n = 17$)	28.8 (2.5) ($n = 9$)	16.9 (3.6) ($n = 15$)	29.1 (1.9) ($n = 12$)	7.5 (8.8) ($n = 14$)	36.0 (2.8) ($n = 9$)
PHA						
10.0 μg/ml	1.5 (4.6) ($n = 15$)	10.5 (1.8) ($n = 5$)	4.7 (2.7) ($n = 15$)	8.3 (1.7) ($n = 10$)	2.4 (3.4) ($n = 12$)	7.3 (1.4) ($n = 7$)
3.3 μg/ml	1.6 (4.0) ($n = 16$)	10.4 (1.7) ($n = 5$)	3.1 (2.6) ($n = 15$)	6.6 (1.7) ($n = 8$)	2.2 (3.1) ($n = 12$)	6.7 (1.7) ($n = 7$)
1.1 μg/ml	0.7 (2.8) ($n = 15$)	5.9 (1.0) ($n = 2$)	1.0 (2.2) ($n = 15$)	4.8 ($n = 1$)	0.9 (2.1) ($n = 12$)	0
ConA						
12.5 μg/ml	1.2 (3.2) ($n = 17$)	7.9 (3.4) ($n = 3$)	1.3 (2.4) ($n = 15$)	5.0 (1.3) ($n = 3$)	0.9 (2.6) ($n = 14$)	4.1 (1.2) ($n = 2$)
1.25 μg/ml	3.3 (5.6) ($n = 17$)	20.7 (2.6) ($n = 7$)	7.6 (3.6) ($n = 15$)	13.9 (2.3) ($n = 11$)	3.8 (4.8) ($n = 14$)	15.5 (1.9) ($n = 8$)
0.125 μg/ml	2.1 (5.2) ($n = 17$)	21.3 (2.0) ($n = 5$)	4.3 (4.2) ($n = 15$)	12.0 (2.0) ($n = 9$)	1.8 (3.5) ($n = 14$)	8.7 (1.4) ($n = 6$)
PPD						
10.0 μg/ml	3.2 (5.8) ($n = 16$)	19.3 (2.0) ($n = 7$)	4.6 (3.3) ($n = 15$)	9.0 (2.0) ($n = 10$)	2.9 (5.0) ($n = 14$)	17.6 (2.8) ($n = 6$)
3.3 μg/ml	2.7 (4.5) ($n = 17$)	12.8 (2.7) ($n = 7$)	3.6 (3.2) ($n = 15$)	8.7 (1.8) ($n = 8$)	1.6 (2.8) ($n = 14$)	14.8 (1.6) ($n = 3$)
1.1 μg/ml	2.1 (3.8) ($n = 17$)	13.0 (2.2) ($n = 5$)	2.2 (2.4) ($n = 15$)	5.1 (1.6) ($n = 6$)	1.5 (2.7) ($n = 14$)	8.6 (2.0) ($n = 4$)

[a] Combined refers to responders plus nonresponders, with responders referring to subjects with SIs of >3.0. The *n* values refer to the number of subjects.

more pronounced in the primaquine group during weeks 10 (Fig. 1) and 48 (Fig. 2) of prophylaxis. Individually, for each mitogen or antigen concentration, these higher proportions were not significantly different from those for the placebo or chloroquine groups at either time point. Collectively, however, there was statistical significance over the placebo group in the occurrence of high responder frequencies in the primaquine group for all three concentrations of a particular mitogen or antigen ($P = 0.037$) and in the occurrence of higher responder frequencies for all four mitogens and antigens tested ($P = 0.012$). The random probabilities of primaquine group responder frequencies exceeding those of the placebo group for 11 of 12 concentrations of mitogen or antigen tested during week 10 ($P = 0.333^{11}$) and all 7 test concentrations during week 48 ($P = 0.333^{7}$) were exceedingly small and therefore indicative of an in vivo drug effect. GMSIs based on the responder and nonresponder subjects combined were, accordingly, also consistently high for the primaquine group, but with one exception (TET, 0.1 μg/ml; $P = 0.04$) they were not significantly greater than those for the placebo or chloroquine group for test concentrations of mitogen or antigen at either sampling time (Tables 1 and 2). Statistical analyses which compared only the high responder (SI > 3.0) cohorts in the three groups revealed that the GMSIs for the primaquine cohort were not significantly different ($P > 0.10$) from those for the placebo or chloroquine cohort at either the 10-week (Table 1) or the 48-week (Table 2) time points. Analyses between responder cohorts at a less stringent cutoff (SI ≥ 2.0) but with a greater power to detect differences also identified no significant differences ($P > 0.07$) between the groups (data not shown). In contrast to the GMSIs for the primaquine group derived from the values for the responders and nonresponders combined, the GMSIs for the primaquine responder cohort alone, calculated at low (SI > 2.0) and high (SI > 3.0; Table 1)

TABLE 2. GMSIs of mitogen- or antigen-driven lymphocyte proliferation by combined prophylaxis group and responder cohort after 48 weeks of malaria chemoprophylaxis[a]

Stimulant and concn (μg/ml)	GMSI (SD) for the following prophylaxis group:					
	Placebo		Primaquine		Chloroquine	
	Combined	Responder	Combined	Responder	Combined	Responder
PHA						
5.0	32.1 (2.3) ($n = 21$)	36.9 (1.8) ($n = 18$)	29.0 (2.9) ($n = 30$)	31.4 (2.7) ($n = 29$)	18.0 (6.5) ($n = 21$)	38.0 (2.6) ($n = 17$)
2.5	9.2 (7.2) ($n = 21$)	19.9 (1.6) ($n = 16$)	14.3 (3.7) ($n = 30$)	21.0 (1.9) ($n = 26$)	13.6 (2.9) ($n = 21$)	18.5 (2.2) ($n = 17$)
PPD						
10.0	6.6 (3.0) ($n = 21$)	10.9 (2.1) ($n = 14$)	7.3 (2.6) ($n = 30$)	9.4 (1.9) ($n = 26$)	5.5 (3.5) ($n = 21$)	11.4 (2.3) ($n = 14$)
5.0	4.9 (5.3) ($n = 21$)	10.9 (2.0) ($n = 14$)	7.7 (2.2) ($n = 30$)	8.9 (2.0) ($n = 26$)	6.5 (2.7) ($n = 21$)	10.9 (2.5) ($n = 12$)
TET						
10.0	1.3 (1.9) ($n = 21$)	3.8 (1.1) ($n = 2$)	2.2 (2.6) ($n = 30$)	8.4 (1.9) ($n = 8$)	1.9 (2.4) ($n = 21$)	7.1 (1.9) ($n = 5$)
1.0	0.9 (1.7) ($n = 21$)	0	1.4 (2.4) ($n = 30$)	7.4 (1.4) ($n = 5$)	0.9 (2.5) ($n = 21$)	15.9 ($n = 1$)
0.1	1.3 (1.7) ($n = 21$)	3.9 (1.4) ($n = 2$)	1.8 (2.5) ($n = 30$)	5.8 (2.0) ($n = 8$)	1.0 (2.3) ($n = 21$)	17.5 ($n = 1$)

[a] See footnote *a* of Table 1.

TABLE 3. Comparison of hematologic parameters in prophylaxis groups at baseline versus week 48 of chemoprophylaxis[a]

Treatment group and time period[b]	Leukocyte count (10^9/liter)	Percent		Platelet count (10^9/liter)
		Granulocytes	Lymphocytes plus monocytes	
Primaquine				
Pre (n = 30)	7.2 ± 2.1	61.2 ± 10.3	40.2 ± 13.9	283.5 ± 76.7
Post (n = 23)	7.3 ± 2.2	52.2 ± 11.2[c]	47.8 ± 11.8[c]	281.5 ± 100.4
Chloroquine				
Pre (n = 29)	7.7 ± 2.0	62.6 ± 8.1	37.4 ± 8.1	261.2 ± 97.5
Post (n = 17)	7.3 ± 2.2	56.1 ± 10.2[c]	44.5 ± 9.1[c]	274.8 ± 87.9
Normal range	4.3–10.0	42–72	40–50	140–400

[a] Values are means ± 1 standard deviation.
[b] Pre, baseline; Post, week 48.
[c] Significantly different ($P < 0.05$; unpaired comparison by t test).

response cutoff levels, were consistently below those for the placebo cohort during week 10 of prophylaxis. Responder cohort GMSIs for all PHA and PPD concentrations during week 48 were comparable for the three groups (Table 2). There were insufficient subjects with SIs of >3.0 for statistical comparison of the responses of the cohorts to tetanus antigen during week 48, but at the less stringent level of an SI of >2.0, GMSIs for the primaquine cohort were not significantly different from those for the placebo or the chloroquine cohort ($P > 0.15$; data not shown). No subject recollected having received a tetanus immunization, but the disproportionately high SIs against low tetanus concentrations registered by one subject in the chloroquine group may reflect a recent vaccination (Table 2). The uptake of tritiated thymidine by unstimulated control cells for the primaquine group (271 cpm [GM]; 95% confidence interval, 192 to 382 cpm) was significantly greater ($P = 0.01$) than that for placebo controls (129 cpm [GM]; 95% confidence interval, 110 to 153 cpm) at week 10, but the uptakes for the groups were comparable ($P = 0.86$) at week 48.

Lymphocyte subset composition. Comparison between differential counts for both the primaquine and chloroquine groups at the baseline and at week 48 identified significant increases in the percentage of lymphocytes and monocytes at week 48 over that at the baseline and significant reductions in the percentage of granulocytes compared with that at the baseline. These changes did not fall outside the normal range, and the baseline values may have been abnormally skewed by malaria infections at the time of enrollment (Table 3). The mean leukocyte counts at week 48 were highest in the primaquine group, but they were not significantly different ($P = 0.23$; data not shown) from those in the other groups. More-definitive measures of the effect of prophylaxis on lymphocyte populations are profiled in Tables 4 and 5. The data in Tables 4 and 5 indicate that the mean percentages of total T cells, total B cells, $CD4^+$ T cells, $CD8^+$ T cells, natural killer cells, and activated T cells and the mean $CD4^+/CD8^+$ ratios were comparable among the placebo, primaquine, and chloroquine groups at both time points. No significant differences were identified between the groups within a time point or between times within a prophylaxis group. The values obtained for the prophylaxis groups all fell within the normal ranges derived by profiling a larger reference sample of subjects matched to the study subjects for age, sex, and ethnicity.

DISCUSSION

Lymphoproliferative responses to a panel of mitogens and antigens were comparable among placebo, primaquine, and chloroquine groups during weeks 10 and 48 of a malaria chemoprophylaxis trial and yielded no evidence of drug-induced immune suppression. The lymphocyte subset profiles for these groups at both time points were also similar to one another and were undistorted relative to the profiles for a healthy reference population matched for age, sex, and ethnicity.

The absence of evidence linking chloroquine prophylaxis with lymphocyte disturbance was not surprising. Previous studies have documented only a reduced in vitro response to mitogenic stimulation by lymphocytes from patients undergoing prolonged high-dose daily chloroquine treatment (250 mg/day) for rheumatoid arthritis or by normal lymphocytes preincubated with high concentrations of the unmetabolized drug (14, 18).

The negative results for primaquine are similarly unspectacular but are ideal from the standpoint of its potential toxicity. Even though primaquine has long been used for the treatment of relapsing malaria, its pharmacology, metabolism, and mode of action remain poorly understood (7, 21). It has been shown that at least 11 metabolites can be formed in mammalian

TABLE 4. Lymphocyte subset composition among test groups after 10 weeks of chemoprophylaxis

Treatment group	% of total lymphocyte count (mean ± 1 SD)						$CD4^+/CD8^+$ ratio
	CD3 (total T cells)	CD19 (total B cells)	$CD4^+$ T cells	$CD8^+$ T cells	Natural killer cells	CD3/anti-HLA-DR (activated T cells)	
Placebo (n = 17)	65.1 ± 9.0	13.9 ± 4.3	34.5 ± 6.1	38.9 ± 5.2	21.2 ± 8.7	16.2 ± 8.7	0.91 ± 0.22
Primaquine (n = 15)	71.1 ± 7.6	12.3 ± 3.7	33.4 ± 5.2	37.3 ± 6.1	17.3 ± 8.9	14.9 ± 7.6	0.94 ± 0.23
Chloroquine (n = 14)	69.4 ± 11.5	13.3 ± 4.2	33.2 ± 7.2	39.3 ± 8.8	23.7 ± 15.4	21.4 ± 10.2	0.88 ± 0.24
Reference (n = 68)[a]	61.0 ± 10.3	13.2 ± 5.0	30.2 ± 7.4	43.0 ± 8.0	26.6 ± 11.2	21.0 ± 6.4	0.75 ± 0.33

[a] Healthy Indonesian men of Javanese-Sundanese ethnicity.

TABLE 5. Lymphocyte subset composition among test groups after 48 weeks of chemoprophylaxis

Treatment group	% of total lymphocyte count (mean ± 1 SD)						$CD4^+/CD8^+$ ratio
	CD3 (total T cells)	CD19 (total B cells)	$CD4^+$ T cells	$CD8^+$ T cells	Natural killer cells	CD3/anti-HLA-DR (activated T cells)	
Placebo (n = 15)	70.2 ± 8.1	14.7 ± 5.8	36.2 ± 8.7	38.9 ± 8.8	22.2 ± 11.3	20.5 ± 12.7	1.04 ± 0.51
Primaquine (n = 27)	69.8 ± 9.1	12.8 ± 4.0	36.6 ± 6.0	39.8 ± 7.9	26.3 ± 14.1	22.6 ± 7.4	0.99 ± 0.33
Chloroquine (n = 9)	68.6 ± 7.2	13.4 ± 5.6	35.4 ± 4.8	41.8 ± 5.3	23.6 ± 14.8	19.0 ± 4.6	0.91 ± 0.22
Reference (n = 68)[a]	61.0 ± 10.3	13.2 ± 5.0	30.2 ± 7.4	43.0 ± 8.0	26.6 ± 11.2	21.0 ± 6.4	0.75 ± 0.33

[a] Healthy Indonesian men of Javanese-Sundanese ethnicity.

systems and that some have potent antimalaria activity and a capacity to induce hemolysis and methemoglobinemia (21). Carboxyprimaquine, the principal metabolite formed in humans, differs markedly from the parent compound in that it attains higher concentrations in plasma (16) and is eliminated more slowly (22). By virtue of these kinetics, progressive metabolite accumulation in tissue or plasma occurs when the drug is used in a daily or alternate-day prophylactic regimen. The general uncertainty that surrounds primaquine metabolite function and toxicity provided the rationale for our efforts to monitor subjects for subtle evidence of chronic immune suppression and lymphocyte imbalance.

There were repeatedly higher proportions of responder subjects and greater combined (responder plus nonresponder) indices of lymphocyte proliferation in the primaquine group during both time points. However, the proportions and indices attained were not significantly greater than those for the placebo or chloroquine group. SIs derived from just the responder cohorts of each group during week 10 of prophylaxis were also not significantly different, but the indices for the primaquine group responders were consistently below those for the placebo group. These outcomes appear to conflict and challenge a simple interpretation of the in vivo effect of primaquine on the basis of limited in vitro results. Relative to the placebo group, the reverse trend from consistently high GMSIs for the entire primaquine group to low GMSIs for its responder cohorts may have arisen partially and artificially from the effects of unequal sample size reductions (reductions of 47 to 70% for the placebo and chloroquine groups versus reductions of 20 to 40% for the primaquine group) on the GMs calculated for the responder cohorts. An alternative or contributory explanation may be that the actual drug effect in the primaquine group manifested as a broadened but moderated enhancement of mitogen- and antigen-stimulated lymphocyte proliferation.

Our results are in contrast to an earlier published finding obtained with normal human lymphocytes preincubated in a therapeutic concentration of primaquine (20). The in vitro immunosuppressive effect that had been observed in that study has been a widely cited aspect of primaquine toxicity (3, 6, 7, 13) and one that has possibly contributed to a long-standing reluctance to consider the prophylactic value of this drug. Our current methodology, which used lymphocytes chronically exposed in vivo to primaquine and its accumulated metabolites, was intended to reflect more accurately the effect of prolonged daily drug use upon lymphocyte function. Despite the improvement, our assays measured the responses of cells that had been removed from exposure and placed into an entirely different and metabolite-free environment. To gain a more realistic picture of the effect of primaquine, we have begun studying the mitogen-driven proliferative responses of unexposed and chronically exposed PBMCs incubated in autologous serum containing the naturally produced metabolites.

Normal therapeutic regimens with primaquine are reported to induce mild disturbances in leukocyte populations (5, 6, 8, 9), and comparison within the primaquine group at the 52-week endpoint of prophylaxis did measure a significant increase in the proportion of lymphocytes and monocytes and a decrease in the proportion of granulocytes compared with the proportions at the baseline. Notably, however, the same changes occurred in the chloroquine group, and endpoint means for both groups fell within the normal ranges. Comparative profiling of lymphocyte subsets in the chloroquine and primaquine prophylaxis groups against those of either their companion placebo group or a larger ethnically similar reference population did not yield evidence of a disturbance. The mean percentages of total T cells, $CD4^+$ T cells, and $CD8^+$ T cells and the $CD4^+/CD8^+$ ratios at both time points for each of the three test groups were also comparable to the reference values derived from a population of healthy adult Caucasians (10). However, drug-induced changes in $CD4^+$ and/or $CD8^+$ cells, not reflected in absolute counts or subset percentages, may have occurred and may have subsequently altered the regulation of the proliferative response.

In summary, repeated testing for lymphocyte function and subset composition during a year-long chemoprophylaxis trial produced no evidence of suppression or disturbance induced by moderate or long-term daily primaquine or weekly chloroquine regimens. While these results appear to provide a measure of safety assurance for the use of primaquine, they are more appropriately intended to serve as a stimulus to further study this drug.

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