Humoral Immune Response to Tetanus-Diphtheria Vaccine Given during Extended Use of Chloroquine or Primaquine Malaria Chemoprophylaxis


Immune suppression resulting from prolonged chemoprophylaxis and potential drug-vaccine interaction were investigated within the context of a randomized placebo-controlled trial that compared daily primaquine or weekly chloroquine administration for malaria prevention. After 11 months of prophylaxis, adult male subjects received a tetanus-diphtheria (Td) vaccination. Prophylaxis continued 4 weeks longer. Anti-tetanus and anti-diphtheria antibody levels were measured by ELISA at baseline and at 1, 3, 7, and 14 months after Td vaccination. All groups were comparable at baseline. Immunization triggered significant increases in anti-tetanus and anti-diphtheria IgG levels over each group’s pre-Td baseline levels and those of an unvaccinated control group. Geometric mean anti-tetanus titers (GMTs) in the primaquine group were significantly higher than those of the placebo group at 1, 3, and 14 months. Anti-tetanus GMTs in placebo and chloroquine groups declined over 14 months to levels comparable to those of unvaccinated controls, but levels in the primaquine group remained significantly higher than in controls.

Recent trials in Indonesia and Africa have generated new interest in primaquine for malaria chemoprophylaxis [1–3]. In Indonesian men, a daily regimen of 0.5 mg of primaquine base per kilogram of body weight was well-tolerated and yielded protective efficacies against Plasmodium falciparum and Plasmodium vivax of >90% [3]. In this year-long trial, primaquine was associated with enhanced lymphoproliferative responses to mitogens and antigens [4]. This unanticipated result prompted an in vivo immunization trial designed to further reveal the immunomodulating effects of the drug or the malaria protection it conferred.

A tetanus-diphtheria (Td) vaccination was administered to groups treated with primaquine, chloroquine, or placebo in the last month of the trial. Subjects’ in vitro lymphoproliferative responses to tetanus were measured at subsequent time points. Primaquine was associated with the highest initial post-Td responses of lymphocytes to tetanus, and no significant differences were observed between primaquine and placebo groups in the frequency, magnitude, or duration of these responses [5]. However, because protective immunity to tetanus is primarily if not wholly determined by levels of neutralizing antibody and because antimalarials can inhibit antibody response to rabies [6, 7], cholera, and typhoid vaccines [8], we were most interested in the drug’s effects on humoral responses to the Td vaccination. This study compared anti-tetanus and anti-diphtheria IgG responses evoked by the Td vaccine among groups receiving primaquine, chloroquine, and placebo.

Materials and Methods

Subjects and prophylaxis. The chemoprophylaxis and Td immunization (5 limits of flocculation units [LfU] of tetanus toxoid and 2 LfU of diphtheria toxoid/0.5 mL intramuscularly) components of this study have been described [2, 5]. Only 2 of 72 subjects recalled a previous immunization (>10 years prior). None of the subjects had been in military service where there might have been greater likelihood of immunization. Primaquine (n = 30), chloroquine (n = 21), placebo (n = 21), and control without prophylaxis (n = 20) groups were comparable in terms of age (~30 years), weight (~50 kg), ethnicity (Javanese/Sundanese), and socioeconomic status (new transmigrant farmers). Primaquine-treated subjects had been malaria-free during the 12 months of prophylaxis, but an estimated 0.8–1.0 cases of malaria/person-year had occurred in the chloroquine and placebo groups. The monthly incidence of primary postprophylaxis malaria over the 3- and 7-month post-Td vaccine sampling points was comparable in the 3 groups (0.15–0.3 infections/person-month). Subjects were malaria-free at vaccination and were screened for infection at each sampling point. Those with symptoms and parasitemia were treated, and their samples were omitted from analysis because of potential malaria-induced immune suppression.

Anti-tetanus and anti-diphtheria IgG assays. Standardized ELISAs were used to blindly measure anti-tetanus and anti-diph-
Diphtheria IgG levels in coded prophylaxis and control group sera that was collected at 5 time points: baseline (before Td vaccination) and 1, 3, 7, and 14 months after Td vaccination. Absorbance values for each sample were expressed as ELISA units (EU) and converted to international units (IU) by the formula: Sample EU/Reference EU × Reference Serum IU/mL. (The reference serum NCIV lot 1 contained 50 IU/mL anti-diphtheria IgG and 2.9 IU/mL anti-diphtheria IgG). In accordance with current conservative recommendations, IgG titers ≥0.1 IU/mL were considered protective [9].

Statistical analysis. Comparison between groups was based on log-transformation of each subject’s IU/milliliter values and analysis of variance (ANOVA) or Kruskal-Wallace nonparametric test. Student’s t test was used for paired comparisons when the ANOVA or Kruskal-Wallace test indicated significant differences among groups. Differences within or between groups in the proportion of subjects with protective IgG levels ≥1.0 IU/mL were compared by χ² or Fisher’s exact test. Two-tailed P values were calculated; the cutoff level for significance was P ≤ .05.

Results

Baseline IgG responses. Baseline anti-tetanus IgG responses in primaquine, chloroquine, and placebo groups were comparable, with individual titers ranging from 0 to 0.44 IU/mL. Protective anti-tetanus titers were found in 47%, 52%, and 29% of the respective groups (figure 1, P > .25). Anti-tetanus geometric mean titers (GMTs) were 0.09, 0.09, and 0.07 IU/mL, respectively (ANOVA, P = .66). Individual baseline anti-diphtheria IgG titers ranged from 0.02 to 5.28 IU/mL. Protective anti-diphtheria titers were found in 93%, 95%, and 100% of the primaquine, chloroquine, and placebo groups, respectively. Anti-diphtheria GMTs were 0.49, 0.49, and 0.40, respectively (Kruskal-Wallace, P = .66).

Post-Td anti-tetanus IgG responses (figures 1, 2A). All but 1 of the 72 prophylaxis group subjects had increased anti-tetanus IgG titers 1 month after Td vaccination. The proportion of subjects with protective titers increased significantly (P < .02) in each group of vaccinees. Conversion rates from unprotected to protected status in the primaquine (16/16) and placebo (15/15) groups were marginally greater (P = .05) than in the chloroquine group (7/10). Individual elevations in IgG ranged from 1 to >700 times those of baseline. There was no correlation between baseline levels and those attained 1 month after vaccination. Anti-tetanus IgG elevations 1 month after Td vaccination in primaquine, chloroquine, and placebo groups averaged 9.5, 5.6, and 5.2 times their respective baseline levels, and all group GMTs were significantly greater than at baseline (P < .01). Anti-tetanus GMTs 1 month after Td vaccination in the chloroquine and placebo groups were marginally higher than in the unvaccinated controls (P < .04). The anti-tetanus GMT in the primaquine group at this time was marginally above that of the vaccinated placebo group (P = .04) and significantly greater than that of the unvaccinated control group (P < .001).

In all vaccinated groups, the proportion of protective anti-tetanus titers and the GMTs fell from the peak levels attained 1 month post-Td. Relative to these peaks, end-point proportions with protective titers declined significantly in primaquine (100% vs. 82%, P = .03) and placebo (100% vs. 58%, P = .001) groups. End-point proportions of subjects with protective titers in the placebo (58%) and chloroquine (67%) groups fell

![Figure 1](image.png)
any postvaccination sample point ($P > .17$). Despite falling significantly from the peak attained 1 month after Td vaccination, the end-point GMT of the primaquine group remained significantly higher than its baseline ($P = .004$) or that of the unvaccinated control group ($P = .02$). End-point GMTs for vaccinated placebo and chloroquine groups fell to levels comparable with their baselines ($P > .10$) and that of the control ($P > .22$). There was no significant difference between anti-tetanus GMTs calculated for the unvaccinated control group at any of the 5 time points (ANOVA, $P = .42$).

Post-Td vaccination anti-diphtheria IgG responses (figure 2B). Anti-diphtheria IgG titers 1 month after Td vaccination ranged from 1.7- to 257-fold greater than at baseline. Highest anti-diphtheria GMTs, measured 1 month after vaccination, were 27.23, 24.38, and 19.95 IU/mL for the primaquine, chloroquine, and placebo groups, respectively (ANOVA, $P = .64$), and averaged 58-, 62-, and 46-fold higher than their respective baseline GMTs. Anti-diphtheria GMTs of the primaquine, chloroquine, and placebo groups were comparable to one another ($P > .26$) but were significantly greater than their respective baseline GMTs ($P < .0001$) and those of unvaccinated controls ($P < .0001$) at each post-Td vaccination sample point. Anti-diphtheria GMTs declined over time in each vaccinated group. A significant decline from the peak 1 month after Td vaccination was measured in the chloroquine group 7 months after vaccination ($P = .04$) and in the primaquine and placebo groups 14 months after vaccination ($P = .02$ and .03, respectively). In all 3 vaccinated groups, subjects that attained the highest anti-diphtheria titers 1 month after vaccination generally also registered the highest end-point titers ($r = .69$–.84). There was no correlation between anti-diphtheria and anti-tetanus antibody levels following immunization ($r = 0$ to $-0.01$). There was no significant difference between anti-diphtheria GMTs calculated for the unvaccinated control group at any of the 5 time points (ANOVA, $P = .53$).

Discussion
The results show that the attainment of protective anti-tetanus antibody titers and the magnitude of anti-tetanus GMTs were routinely highest in the vaccinated primaquine group. This finding complements the strong in vitro lymphocyte responses against tetanus that were measured in this treatment group [5]. Of interest, there was no correlation between cellular and humoral responses to tetanus: Men with the highest lymphoproliferative responses were equally likely to have either high or low anti-tetanus IgG titers. The heightened cellular and humoral responses observed in the primaquine group during the time of prophylaxis may relate more to the immunostimulatory properties of the drug than to its ability to prevent malaria-induced immune suppression. While it seems clear that long-term primaquine use was not immunosuppressive, the mechanisms underlying its effect on cellular and humoral immunity to levels comparable to their respective pre-Td vaccination baseline levels (placebo, $P = .11$; chloroquine, $P = .50$), but that of the primaquine group remained significantly above baseline (82% vs. 47%, $P = .03$). At end point, the proportion of primaquine subjects with protective titers was significantly greater than that of the unvaccinated control group (82% vs. 43%, $P = .03$), while proportions in placebo and chloroquine-treated groups were similar to that of the control ($P > .27$).

Anti-tetanus GMTs in the primaquine group exceeded those of the vaccinated placebo group at 3 ($P = .03$) and 14 months ($P = .002$) after Td vaccination. There was no statistical difference between GMTs of the placebo and chloroquine groups at
are unclear and may relate to drug-induced leukocytosis or hematopoiesis [10].

Since no association has been shown between asymptomatic malaria infections and reduced primary or secondary responses to tetanus immunization [11], it is unlikely that undetected or subpatent malaria infections could account for the large differences in cellular and humoral response that we measured. Unrecalled previous tetanus immunizations in our subjects and their chance clustering may have accounted for the significant differences observed; however, baseline pre-Td responses, both humoral and cellular, gave no indication of such bias. Furthermore, rapidly declining post-Td anti-tetanus titers in even the highest responders, asynchrony between the magnitude of individual anti-tetanus and anti-diphtheria titers, and the absence of a correlation between subject age and titer gave no supporting evidence of prior immunization.

On the basis of chloroquine’s established immunosuppressive quality [6–8, 12] and our observation of lymphoproliferative responses in the chloroquine group consistently below those of the primaquine and placebo groups [5], we had conjectured that anti-tetanus IgG levels would be similarly low. However, IgG titers in the chloroquine group exceeded or were comparable to those of the placebo group, and there was no difference at any sampling point in the proportion of subjects mounting protective titers. Nonintervention and possible enhancement of anti-toxin and anti-bacterial antibody responses have been previously reported for vaccinated African children maintained malaria-free by long-term chloroquine prophylaxis [11, 13].

The baseline and post-Td humoral antibody responses observed in our study subjects may be representative of the tetanus and diphtheria vaccination status of adult males living in rural Indonesia. Tetanus and diphtheria are important causes of death among infants and children in this nation [14], a situation that relates directly to the immunity and carrier status of adult Indonesians. The Indonesian National Immunization Program has targeted children and pregnant women since 1977 [15]. Many adults, particularly males, remain at risk of tetanus and may be reservoirs of diphtheria infection. The low frequencies of protective titers we observed at baseline, the magnitude of titers achieved by Td vaccination, and the rapid post-Td vaccination fall in titer are not indicative of a population that received full tetanus immunization as children. Paradoxically, however, virtually all subjects manifested protective anti-diphtheria titers at baseline and developed impressively high prolonged post-Td titers as a result of the single inoculation. On the basis of the low anti-tetanus responses seen and trends in coverage by the National Immunization Program, we do not believe that the high anti-diphtheria titers in these young adults resulted from prior vaccination. We suspect that toxigenic and nontoxigenic strains of Clostridium diphtheriae circulate naturally in the community, widely present in adults as inapparent, chronic, and immunizing infections.

In summary, long-term and concurrent malaria prophylaxis with primaquine or chloroquine did not inhibit the development and duration of humoral immune responses following Td vaccination. The initially high anti-tetanus and anti-diphtheria IgG titers observed in primaquine users complement their cellular responses against tetanus and may engender a more effective longer-lasting immunity. These results constitute an additional measure of safety assurance to support regulatory agency evaluation of primaquine for malaria prophylaxis.

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References