Mefloquine Is Highly Efficacious against Chloroquine-Resistant *Plasmodium vivax* Malaria and *Plasmodium falciparum* Malaria in Papua, Indonesia

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(See the editorial commentary by Vinetz on pages 1073–4)

Background. During the period of 1996–1999, we prospectively monitored 243 Javanese adults and children after arriving in Papua, Indonesia, and microscopically documented each new case of malaria by active surveillance.

Methods. In a randomized, open-label, comparative malaria treatment trial, 72 adults and 50 children received chloroquine for each incident case of malaria, and 74 adults and 47 children received mefloquine.

Results. Among 975 primary treatment courses, the cumulative 28-day curative efficacies were 26% and 82% for chloroquine against *Plasmodium falciparum* malaria and *Plasmodium vivax* malaria, respectively. Mefloquine cure rates were far superior (96% against *P. falciparum* malaria and 99.6% against *P. vivax* malaria).

Conclusions. Mefloquine is a useful alternative treatment for *P. vivax* malaria and *P. falciparum* malaria in areas such as Papua, where chloroquine is still recommended as the first-line therapeutic agent.

The US Food and Drug Administration approves the use of mefloquine for treatment of mild-to-moderate acute malaria caused by mefloquine-susceptible strains of *Plasmodium falciparum* or by *Plasmodium vivax* [1]. However, published data supporting the use of mefloquine for *P. vivax* malaria is limited to treatment outcomes from 85 courses of therapy [2–6]. These data are also limited to settings where the comparator agent was chloroquine, for which clinical susceptibility has been confirmed. Assertions of mefloquine-resistant *P. vivax* have been described in only 3 case reports [7–9]. We evaluated the efficacy of mefloquine versus chloroquine, the approved first-line agent for treatment of malaria in Indonesia, against *P. falciparum* and *P. vivax* malaria in Indonesian Papua, where chloroquine resistance occurs and where historical treatment failure rates range from 54% to 80% for *P. falciparum* malaria and from 22% to 70% for *P. vivax* malaria [10–12].

SUBJECTS AND METHODS

We prospectively monitored a cohort of Javanese migrants starting immediately after their arrival to a malaria-endemic coastal settlement village 150 km west of the Papua provincial capital in eastern Indonesia. The study location and population are described in detail elsewhere [13]. In brief, this malaria-naive population arrived from Java between August and October 1996, during which period 243 migrants enrolled in a prospective study of the onset of naturally acquired immunity [14]. In this block-randomized, open-label comparative treatment trial, 72 adults (age, ⩾20 years) and 50 children (age, 6–12 years) were randomized to receive chloroquine for each incident primary case of uncomplicated malaria, and 74 adults and 47 children were randomized to receive mefloquine. Only individuals with normal glucose-6-phosphate dehydrogenase activity (as determined using the G-6-PDH Screening

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Table 1. Characteristics of 243 adults and children participating in a malaria treatment trial in Papua, Indonesia, 1996–1999.

	Treatment arm			
	Children		Adults	
Factor	Chloroquine	Mefloquine	Chloroquine	Mefloquine
No. of subjects	50	47	72	74
No. of male/female subjects	32/18	31/16	47/25	53/21
Age, mean years (range)	9.1 (6–12)	9.3 (6–12)	31.9 (20–58)	31.7 (20–50)
Weight, mean kg (range)	22.6 (15.4–38.2)	22.8 (13–34.7)	51.1 (35.5–64)	52.1 (37.8–83.3)

Test 203-A [Sigma Diagnostics]) participated in the study. From November 1996 to June 1999, native language–speaking study personnel visited subjects thrice weekly and obtained blood smear specimens during any visit in which a subject complained of having fever, rigors, nausea/vomiting, headache, or malaise significant enough to interfere with work or school activities. Technicians also prepared routine blood smears for all study subjects every 2 weeks, regardless of symptoms.

Each incident case of slide-proven uncomplicated malaria was treated according to the protocol for the group to which the subject had been assigned during randomization. Subjects in the chloroquine arm received chloroquine diphosphate (150-mg base; Resochin [P. T. Bayer Indonesia]) in 3 doses over 48 h: 10 mg base/kg on day 0, 10 mg base/kg on day 1, and 5 mg base/kg on day 2. Subjects assigned to the mefloquine arm received mefloquine hydrochloride (228-mg base; Lariam [Roche]) as a single 15-mg/kg dose. Subjects with *P. vivax* malaria also received primaquine phosphate (15 mg base daily for 14 days). Native language–speaking study personnel observed administration of all treatment doses and visited subjects on days 0, 1, 2, 4, 7, 14, 18, 21, and 28 after treatment was initiated, to assess symptoms and to obtain blood specimens for Giemsa smears. Unresolving or recurrent parasitemia during the follow-up period prompted the administration of rescue therapy with either sulfadoxine-pyrimethamine, quinine plus doxycycline, or quinine alone, depending on the primary treatment group and the clinical presentation. Any infection identified by routine screening after day 28 was considered to be a new infection and was treated in accordance with the primary treatment regimen assigned to that subject.

Treatment outcomes were assessed in accordance with the most recent recommendations of the World Heath Organization [15]. When outcomes could not be determined because rescue therapy was administered too early to assess response (when rescue treatment was provided on day 2 without a parasite density greater than that noted on day 0 of primary therapy or when rescue therapy with a second agent was provided on day 3 of follow-up without the parasite density exceeding 25% of that on day 0 of primary therapy), they were excluded from analysis. For actuarial (life table) estimates of the cumulative incidence of treatment failure, subjects who were lost to follow-up or who developed intercurrent infections with a new species only contributed person-time to the point of the last blood smear or the date of re-treatment, respectively.

All work was performed in accordance with code 32 of Federal Regulations Part 219 (Protection of Human Subjects) and US Department of Defense, US Navy (SECNAVINST 3900 .39C), and Indonesian Ministry of Health guidelines for the conduct of human use research. The protocols and informed consent processes were reviewed and approved by convening institutional review boards before research was initiated. Written informed consent was obtained from all study subjects.

RESULTS

The characteristics of subjects in the 2 treatment arms were similar (table 1). We treated a total of 1018 primary infections during the 32-month study period, of which we could evaluate 975 (96%) (table 2). Forty-three chloroquine treatment courses were excluded because rescue therapy was administered outside of the set parasitological parameters. Species-specific parasite densities among the 2 treatment groups were similar (table 2). We observed no treatment-related serious or severe adverse events in either cohort. Of the total number of infections diagnosed in the cohort during the study period, 9 were classified by the treating physician as severe and were treated with quinine. These treatment courses are therefore not included in this analysis but are reported elsewhere [13]. In all 9 cases, the patient recovered and resumed follow-up in the study, in accordance with the protocol. Intercurrent infections occurred more frequently during follow-up in individuals treated with chloroquine (relative risk [RR], 14; 95% CI, 4–64).

The overall 28-day mefloquine curative efficacy rates for *P. falciparum* and *P. vivax* malaria were 96.4% and 99.6%, respectively, compared with 26.2% and 81.6% among patients treated with chloroquine. These differences were significant. The RRs for treatment failure with chloroquine versus mefloquine were 20 (95% CI, 10–41) and 52 (95% CI, 7–376) for *P. falciparum* and *P. vivax,* respectively. The early treatment failure rates were relatively high in the chloroquine treatment

Table 2. Characteristics and outcomes for 1018 patients with malaria in Papua, Indonesia, 1996–1999.

	Plasmodium species, by treatment arm					
	Chloroquine			Melfoquine		
Variable	Pf	Pv	Pf/Pv	Pf	Pv	Pf/Pv
No. of treatment courses	161	232	28	232	295	27
No. of intercurrent infection with different species	11	12	0	1	1	0
No. of losses to follow-up before day 28	1	8	3	10	11	1
No. of 28-day cures	39	173	11	213	282	23
No. of recurrences	110	39	14	8	1	3
Geometric mean parasite density at day 0, parasites/μL (95% CI)	1757 (1362–2257)	799 (651–980)	…[a]	1526 (1217–1913)	677 (569–804)	…[a]

NOTE. A total of 464 treatments were administered in the chloroquine arm, and 554 were administered in the melfoquine arm. Forty-three and 0 patients were excluded from the chloroquine and melfoquine arms, respectively, because rescue therapy was administered too early to determine outcome, leaving 421 and 554 evaluable treatment courses. Pf, *Plasmodium falciparum;* Pf/Pv, mixed *P. falciparum* and *Plasmodium vivax;* Pv, *P. vivax.*

[a] Included in single species columns.

arm for *P. falciparum* and *P. vivax* malaria (39% and 20%, respectively). For mefloquine, only 1 early treatment failure for *P. falciparum* malaria occur, and the single case of recurrent *P. vivax* malaria after receipt of mefloquine treatment occurred on day 28 and was classified as late treatment failure. Among the treatment courses that we could evaluate, 56 (10%) of 554 and 117 (28%) of 421 of the total number of presumed new infections in the mefloquine and chloroquine groups, respectively, occurred 29–42 days after the initiation of therapy for the previous infection. Taking into account the time contributed by each subject, the periodic cumulative incidences of treatment failure are represented in figure 1.

Treatment outcomes in this cohort during the follow-up period were not affected by age or number of documented prior infections. The low number of treatment failures in the mefloquine group precluded analysis, but among subjects treated with chloroquine, age group did not affect the risk of treatment failure for *P. falciparum* malaria (RR, 1.15; 95% CI, 0.96–1.39) or *P. vivax* malaria (RR, 1.15; 95% CI, 0.75–1.93). The number of prior documented infections—a surrogate estimator of exposure-related immunity—had no clear relationship with chloroquine treatment outcome. Compared with first-infection treatment outcomes, the RRs of treatment failure (stratified by number of prior infections) were 1.31 (95% CI, 0.94–1.85) after 1 prior infection, 1.5 (95% CI, 1.08–2.09) after 2 prior infections, 1.39 (95% CI, 0.97–2) after 3 prior infections, and 1.1 (95% CI, 0.68–1.78) after 4 prior infections.

DISCUSSION

Chloroquine remains the first-line therapy for *P. falciparum* and *P. vivax* malaria in Indonesia, except in some Ministry of Health–designated areas where the combination of artesunate plus amodiaquine has been approved for special use to assess local efficacy, because combination therapies that contain artemisinin are emerging as the treatments of choice for *P. falciparum* malaria internationally. Because increasing antimalarial resistance impedes malaria-control efforts, new regimens are required. We demonstrated high efficacy of mefloquine for malaria in an area of eastern Indonesia where chloroquine is ineffective against *P. falciparum* and chloroquine-resistant *P. vivax* is well documented. In such areas where mefloquine-resistant *P. falciparum* has not been documented, mefloquine may be a suitable alternative treatment for either *P. vivax* or *P. falciparum* malaria. In an era of waning proficiency in microscopic diagnosis of malaria, use of a single agent that covers both *P. falciparum* and *P. vivax* would reduce the risk of morbidity and mortality associated with species misdiagnosis—specifically, mistaking *P. falciparum* for another species and treating the patient with an inferior regimen. The cost of mefloquine ($1.60–$4.85 [in US dollars] per treatment course) is comparable to that for combination regimens like artesunate-amodiaquine and artesunate-sulfadoxine/pyrimethamine [16], and its single-dose regimen increases the likelihood of compliance, compared with multiple-day regimens.

To our knowledge, the clinical efficacy of mefloquine against chloroquine-resistant *P. vivax* has not been previously described. The little data available on mefloquine therapy for *P. vivax* malaria relates to efficacy against chloroquine-susceptible *P. vivax*. Harinasuta et al. [3] compared the efficacy of a single 250 mg dose (4–6 mg/kg) of mefloquine with that of a single 450-mg dose of chloroquine in adult men with *P. vivax* malaria. Chloroquine treatment failures did not occur, and the authors attributed the 2 mefloquine treatment failures, occurring on days 12 and 28 after the initiation of therapy, to inadequate dosing. Both infections subsequently resolved after re-treatment with chloroquine. A study from Thailand [4] compared the efficacy of a single 1500-mg dose of mefloquine in 15 adults with *P. vivax* malaria with a 1500-mg dose of chloroquine, with or without primaquine, given over the course of 3 days to 25 adults with *P. vivax* malaria; it revealed 28-day cure rates of

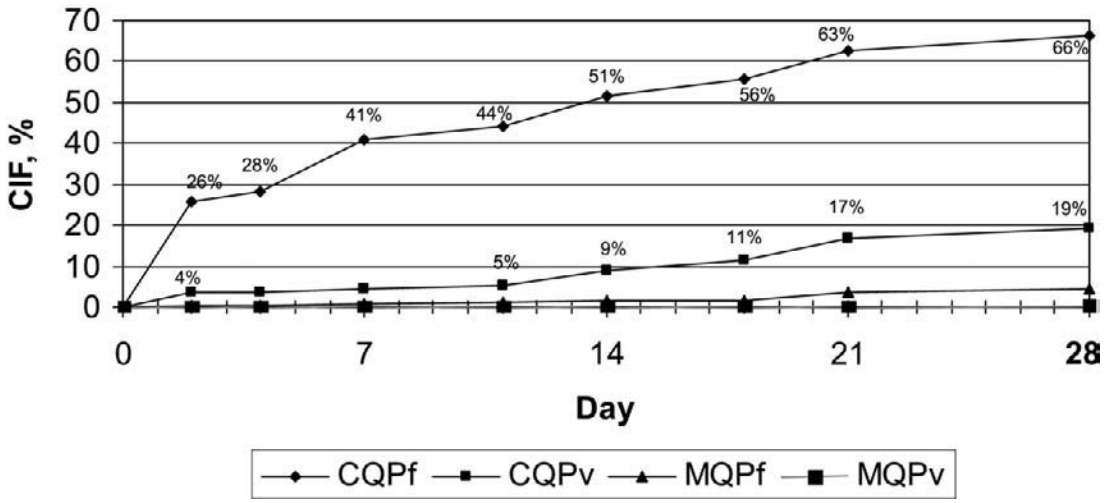

Figure 1. Cumulative incidence of therapeutic failure (CIF) among 975 treatment courses of chloroquine or mefloquine for uncomplicated *Plasmodium falciparum* or *Plasmodium vivax* malaria in Papua, Indonesia, 1996–1999. CQPf, chloroquine treatment for *P. falciparum* malaria; CQPv, chloroquine treatment for *P. vivax* malaria; MQPf, mefloquine treatment for *P. falciparum* malaria; MQPv, mefloquine treatment for *P. vivax* malaria.

100% in all groups. A more recent study from Thailand, where chloroquine remains effective against *P. vivax*, reported recurrent parasitemia in 2 of 17 mefloquine-treated patients (15 mg base/kg) and 1 of 21 chloroquine-treated patients (25 mg base/kg) cases only after day 28 [6].

Late, recurrent *P. vivax* parasitemia is generally attributed to either reinfection or, more commonly, relapse of Asian strains, as levels of slowly eliminated antimalarials like chloroquine and mefloquine decrease to less than the level required to suppress emergence of blood-stage parasites [17]. We observed an 18% rate of recurrent *P. vivax* parasitemia before day 28 among individuals who were treated with chloroquine plus primaquine, but there was only 1 instance of recurrent *P. vivax* parasitemia in the mefloquine plus primaquine group (on day 28). In this study, the 28-day rate of chloroquine treatment failure for *P. vivax* was much lower than was previously reported from this region (71%–78%) [12, 18]. We combined primaquine with chloroquine, a combination previously shown to improve 28-day cure rates for *P. vivax* malaria over chloroquine alone (85% vs. 22%) [18]—a finding that was not observed when therapeutic doses of primaquine are combined with chloroquine to treat chloroquine-resistant *P. falciparum* malaria, for which relapse does not occur [19]. These data suggest that previous methods for assessing treatment outcomes for blood-stage *P. vivax* infection, which did not control for relapse through administration of primaquine to cure hypnozoite stages, overestimate true recrudescence (i.e., blood-stage treatment failure) rates. Although we administered primaquine (15-mg base) to all subjects with *P. vivax* malaria, much higher doses are now recommended for strains from New Guinea, which appear to be tolerant of the standard 15-mg/kg dose that is generally recommended and that was used in this study [17]. Similarly, the higher degree of intercurrent infections with both *P. vivax* and *P. falciparum* observed in the chloroquine group, compared with the mefloquine group, illustrate the persistent blood-stage chemosuppressive effects of these agents, with mefloquine being far superior at suppressing recrudescence, relapse, and/or reinfection with *P. vivax* through day 28.

After careful review of the literature, we identified no clear evidence of the occurrence of mefloquine-resistant *P. vivax* [7–9]. Amor and Richards [7] reported the occurrence of *P. vivax* infection in an adult who was taking mefloquine prophylaxis while living in Papua, New Guinea. However, compliance with treatment was not confirmed, and blood concentrations of the drug were not measured to document adequacy of the level at the time of infection. Similarly, a reported case in India of mefloquine treatment failure on day 13 of treatment was based on the finding of 1 ring and 1 trophozoite on the thick smear that was not confirmed by thin smear, repeated thick smears, immunochromtography, or PCR [9]. Alecrim and colleagues report the most compelling evidence for mefloquine resistance in *P. vivax* in an adolescent female subject, who, after chloroquine therapy failed, was given mefloquine (20 mg/kg) on 2 occasions and who developed recurrent parasitemia [8]. Again, the possibilities of malabsorption and inadequate blood concentrations were not explored.

Individuals with *P. falciparum* malaria who received mefloquine had a 20-fold lower risk of recurrent parasitemia during the 28-day follow-up period, compared with those treated with chloroquine. Determining the appropriate follow-up period necessary to accurately estimate malaria treatment cure rates is often complicated by the risk of reinfection in locations where malaria is endemic. Many experts suggest that at least 42–63 days of follow-up is necessary to adequately assess therapeutic

responses to mefloquine [20]. Among patients treated with mefloquine and monitored in locations in Thailand where malaria is presumed to be nonendemic, 17%–50% of *P. falciparum* recrudescences occurred between days 28 and 42 after therapy [21]. Our study was conducted in an area of Papua where malaria is endemic and where malaria incidence rates were estimated as high as 3.2 cases per person-year [13]. Therefore, a risk of reinfection between days 28 and 42 of follow-up did exist. As observed in our study, 10% and 28% of presumed new *P. falciparum* infections in the mefloquine and chloroquine treatment groups, respectively, occurred during that window. Some of these infections are likely recrudescences. Although genotyping of the merozoite surface protein 2 gene has been established as a useful tool for distinguishing recrudescence from reinfection [22], and although the World Health Organization has recently advocated its use for this purpose [20], this technology was not available at our laboratory during the execution of this study. However, because we identified infections between days 28 and 42 after treatment of a prior infection as "new" in both groups and more commonly in the chloroquine group, we believe that this study still validates mefloquine's superiority over chloroquine, and we advocate use of this agent in the region as an efficacious alternative therapy for both *P. falciparum* and *P. vivax* malaria.

Finally, this study also provided the opportunity to examine the impact of immunity on clearance of parasitemia and clinical cure in the face of ineffective chloroquine therapy. In malaria, the role for immunity in clearing infection has been established [23]. Although age is frequently considered to be a surrogate marker of exposure-related immunity in locations where malaria is endemic, we observed no differences between adults and children in outcomes after chloroquine treatment for *P. falciparum* and *P. vivax*. However, in this cohort, both adults and children had no malaria exposure histories before initiation of the study. Still, despite evidence of clinical immunity (manifested by the lack of fever), by the fourth infection after arrival of this cohort in Papua [14], we observed no increase in chloroquine cure rates among individuals with increasing numbers of infections. However, very few individuals in this study had >5 infections during the course of the 3-year study, rendering statistically significant analysis of the impact of higher numbers of prior infections on chloroquine treatment outcome not possible.

This report constitutes the first published evidence, to our knowledge, from a large-scale clinical trial of the efficacy of a standard 15 mg/kg dose of mefloquine against chloroquine-resistant *P. vivax*. Although this study was completed 5 years ago, we do not anticipate emergence of mefloquine resistance during the intervening period, because mefloquine has not been available in Indonesia and, therefore, is not a factor in the development of selective resistance in Indonesian Papua. When used in malaria-endemic locations, it can provide the added benefit of short-term prophylaxis against chloroquine-resistant strains of *P. falciparum* and *P. vivax*. In areas where mefloquine-resistant *P. falciparum* does not occur, mefloquine provides an alternative for treating malaria, particularly when species determination at the local level may not be possible.

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References

1. In: Physicians' desk reference, 59th ed. Murray L, ed. Montvale NJ: Thompson PDR, **1995**:2903–6.
2. Trenholme GM, Williams RL, Desjardins RE, Frischer H, Carson PE, Rieckmann KH. Mefloquine (WR 142,490) in the treatment of human malaria. Science **1975**; 190:792–4.
3. Harinasuta T, Lasserre T, Bunnag D, Leimer R, Vinijanont S. Trials of mefloquine in vivax and of mefloquine plus 'Fansidar' in falciparum malaria. Lancet **1985**; 1(8434):885–8.
4. Dixon KE, Pitaktong U, Phintuyothin P. A clinical trial of mefloquine in the treatment of *Plasmodium vivax* malaria. Am J Trop Med Hyg **1985**; 34:435–7.
5. Alcantara AK, Uylangco CV, Sangalang RP, Cross JH. A comparative clinical study of mefloquine and chloroquine in the treatment of vivax malaria. Southeast Asian J Trop Med Public Health **1985**; 16:534–8.
6. Pukrittayakamee S, Chantra A, Simpson JA, et al. Therapeutic responses to different antimalarial drugs in vivax malaria. Antimicrob Agents Chemother **2000**; 44:1680–5.
7. Amor D, Richards M. Mefloquine resistant *P. vivax* malaria in PNG. Med J Aust **1992**; 156:883.
8. Alecrim M das G, Alecrim W, Macedo V. *Plasmodium vivax* resistance to chloroquine (r2) and mefloquine (r3) in Brazilian Amazon region. Rev Soc Bras Med Trop **1999**; 32:67–8.
9. Kshirsagar NA, Gogtay NJ, Rajgor D, Dalvi SS, Wakde M. An unusual case of multidrug-resistant *Plasmodium vivax* malaria in Mumbai (Bombay), India. Ann Trop Med Parasitol **2000**; 94:189–90.
10. Baird JK, Basri H, Jones TR, Purnomo, Bangs MJ, Ritonga A. Resistance to antimalarials by *Plasmodium falciparum* in Arso PIR, Irian Jaya, Indonesia. Am J Trop Med Hyg **1991**; 44:640–4.
11. Murphy GS, Basri H, Purnomo, et al. Vivax malaria resistant to treatment and prophylaxis with chloroquine. Lancet **1993**; 341(8837): 96–100.
12. Taylor WR, Widjaja H, Richie TL, et al. Chloroquine/doxycycline combination versus chloroquine alone, and doxycycline alone for the treatment of *Plasmodium falciparum* and *Plasmodium vivax* malaria in northeastern Irian Jaya, Indonesia. Am J Trop Med Hyg **2001**; 64:223–8.
13. Krisin, Basri H, Fryauff DJ, et al. Malaria in a cohort of Javanese migrants to Indonesian Papua. Ann Trop Med Parasitol **2003**; 97: 543–56.
14. Baird JK, Krisin, Barcus MJ, et al. Onset of clinical immunity to *Plasmodium falciparum* among Javanese migrants to Indonesian Papua. Ann Trop Med Parasitol **2003**; 97:557–64.
15. Bloland P, Ringwald P, Snow RW. Assessment and monitoring of antimalarial drug efficacy for the treatment of uncomplicated falciparum malaria. Geneva: World Health Organization, **2003**.
16. International Drug Price Indicator Guide. McFadyen JE, ed. Cambridge, ME: Management Sciences for Health. **2004**:112–3.

17. Pukrittayakamee S, Imwong M, Looareesuwan S, White NJ. Therapeutic response to antimalarial and antibacterial drugs in vivax malaria. Acta Tropica **2004**; 89:351–6.
18. Baird JK, Basri H, Subianto B, et al. Treatment of chloroquine-resistant *Plasmodium vivax* with chloroquine and primaquine or halofantrine. J Infect Dis **1995**; 171:1678–82.
19. Baird JK, Wiady I, Sutanihardja A, et al. Short report: therapeutic efficacy of chloroquine combined with primaquine against *Plasmodium falciparum* in northeastern Papua, Indonesia. Am J Trop Med Hyg **2002**; 66:659–60.
20. World Health Organization (WHO). Monitoring antimalarial drug resistance, report of a WHO consultation Geneva, Switzerland, 3–5 December 2001. Available at: http://www.who.int/emc. Accessed 5 December 2005.
21. Smithuis FM, Van Woensel JBM, Nordlander E, Vantha WS, Ter Kuile FO. Comparison of two mefloquine regimens for treatment of *Plasmodium falciparum* malaria on the northeastern Thai-Cambodian border. Antimicrob Agents Chemother **1993**; 37:1977–81.
22. Ohrt C, Mirabelli-Primdahl L, Karnasuta C, Chantakulkij S, Kain KC. Distinguishing *Plasmodium falciparum* treatment failures from reinfections by restriction fragment length polymorphism and polymerase chain reaction genotyping. Am J Trop Med Hyg **1997**; 57:430–7.
23. Cohen S, McGregor IA, Carrington S. Gamma-globulin and acquired immunity to human malaria. Nature **1961**; 192:733–7.