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Clinical outcome of experimental human malaria induced by *Plasmodium falciparum*-infected mosquitoes

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ABSTRACT

Background: Human experimental malaria infections have been safely carried out previously. The objective of this study was to evaluate infection rates and clinical safety of different protocols for human experimental malaria induced by *Plasmodium falciparum*-infected mosquitoes.
Methods: Thirty nonimmune volunteers were infected by bites of 1-2 or 4-7 *Anopheles stephensi* mosquitoes infected with the NF54 strain of *P. falciparum*.
Results: A 100 or 50% infection rate was obtained after bites of 4-7 and 1-2 infected mosquitoes, respectively. Median prepatent period was 8.8 days. The most common symptoms after a median incubation time of eight days were headache, malaise/fatigue and fever. There was no significant difference in clinical and parasitological presentation between groups infected by 4-7 or 1-2 mosquitoes. Delay of treatment by maximally 48 hours after the first positive thick smear was generally well tolerated but fever was higher and more frequently observed. The most prominent laboratory abnormality was uncomplicated thrombocytopenia. Two volunteers with parasitaemia developed psychiatric side effects after chloroquine treatment.
Conclusion: With stringent inclusion criteria, close monitoring and immediate administration of treatment upon detection of parasitaemia, experimental human malaria challenges can be considered safe and generally well tolerated.

INTRODUCTION

Malaria is one of the most important infectious diseases worldwide. The number of infected people is increasing due to human migration, climate changes, failure of programmes for malaria control and the global spread of drug resistance. Today, malaria is found throughout the tropical and subtropical regions of the world and causes more than 300 million acute illnesses and at least one million deaths annually.[1] The potential effect of vaccines on the devastating malaria situation worldwide justifies the highest priority for its development. Pre-erythrocytic vaccines are partly developed to prevent disease in people travelling to malaria-endemic countries and for children in endemic countries. Asexual vaccines, which contain blood stage antigens, are developed to reduce the severity and lethality of malaria in endemic countries.[2] Preclinical studies have proven to be useful to test malaria vaccine candidates, but the ultimate validation of efficacy depends on human studies.[3] Due to limited resources, only the most promising vaccines can be tested in elaborate field trials in endemic areas. In addition, human challenges have shown to be safe, reliable and ethically acceptable for testing the efficacy of potential malaria vaccines.[4] Hundreds of volunteers have been experimentally infected by bites of generally five infected mosquitoes.[4-8]
The objective of this study was to evaluate infection rates and clinical safety of different protocols for human experimental malaria induced by *Plasmodium falciparum*-infected mosquitoes.

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MATERIALS AND METHODS

Production of infected mosquitoes

Culture of *P. falciparum* parasites and the infection of *Anopheles stephensi* mosquitoes has been a routine procedure for the past ten years.[9] The chloroquine-sensitive NF 54 strain was used in all challenge studies. Batches with more than 90% infected *Anopheles stephensi* mosquitoes were used with a mean of at least 10,000 sporozoites per paired salivary gland. A small cage containing the desired number of mosquitoes was placed between the forearms and the mosquitoes were allowed to feed for ten minutes. Blood engorged mosquitoes were dissected to confirm the presence of sporozoites in the salivary glands. If this was not the case, another feeding session followed (maximum of three) until the desired number of infected mosquitoes had fed.

Recruitment

Thirty healthy volunteers (18 to 45 years) were included. Exclusion criteria were 1) previous history of malaria or travel to malaria endemic areas, 2) previous history of dermatological, central nervous system, renal, cardiac, pulmonary, hepatic, and splenetic disease, splenectomy, pregnancy and lactation, 3) need for medication, and 4) known allergy to antimalarial agents. Volunteers were recruited through general advertisements in public places and local journals. All volunteers had to live in the vicinity of our hospital. Screening included a physical examination, complete blood count, liver and renal function tests, urinalysis for glucosuria, protenuria and pregnancy test, and serological testing for antimalarial antibodies, HIV, and hepatitis B and C. The protocol was adapted to more stringent criteria of <10% for risk of coronary heart disease. Risk was calculated according to the Framingham Heart Study Coronary Heart Disease Risk Prediction Chart.[10] The volunteers were informed about the expected adverse events and risks before inclusion. An informed consent form was signed by all subjects. An independent specialist in internal medicine could be consulted by the subjects to obtain information on the studies. The subjects' general practitioners were asked to mention any conditions known to them that could increase the risk of an adverse outcome. The studies were approved by the institutional ethical board (CWOM numbers 0004-0090, 0011-0262, 2001/203, and 2002/170).

Experimental set-up

The studies were conducted from 1999 to 2003 at the Centre for Clinical Malaria Studies in the Radboud University Medical Centre, Nijmegen, the Netherlands. In Group A, 15 (three groups of five) volunteers were challenged by bites of 4-7 infected mosquitoes. Thick smears were taken following the World Health Organisation's standard procedure. Smears were screened for parasites in 200 fields at high-power magnification. Standard chloroquine (base 100 mg, salt 136.3 mg, Aventis) treatment, 10 mg/kg initially followed by 5 mg/kg after 6, 24 and 48 hours, was started immediately after detection of parasitaemia by thick smear.

In group B (5 volunteers), curative treatment was delayed for maximally 48 hours after the first microscopic detection of parasitaemia, to monitor parasite multiplication. To ensure maximal safety, we admitted the volunteers to the hospital as soon as the thick smear was positive. They were closely monitored with review by a physician unrelated to the study. Treatment was immediately initiated when parasitaemia was >500/μl, in case of severe laboratory abnormalities, or on development of clinical symptoms that required prompt treatment according to either the investigator, the independent physician, or the volunteer. In Group C, ten (two groups of five) volunteers were challenged by bites of 1-2 infected mosquitoes.

Follow-up

Follow-up of volunteers in group A and C was on an outpatient basis with close monitoring. Ear temperature was measured, and all symptoms were recorded on a case report form at every visit. Volunteers were requested to measure their temperature twice daily, and note their symptoms in a booklet. At the end of the study the subjects were asked to complete a questionnaire on their perception on inconveniences and severity of disease during the study. Thick smears were done twice daily from day 6 after infection until they were positive and treatment had been initiated. Chloroquine treatment was provided to all volunteers including the ones whose thick smears remained negative to the end of the study. Standard blood and urine laboratory tests were done once daily in the three days post-treatment including haemoglobin, platelet count, white blood cell count with differentiation, creatinine, blood urea nitrogen, sodium, potassium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase, lactic dehydrogenase, gamma glutamyl transpeptidase and blood glucose. Urine analysis included protein and glucose measurement. Thick smears were carried out daily until two negatives had been obtained for *P. falciparum*. At the end of the study (ten days after first detection of parasitaemia) blood was drawn for standard clinical laboratory tests and a control thick smear.

Data analysis

All available data were analysed in SPSS 10.0 for Windows. Data of three volunteers were excluded from clinical and laboratory analysis, because of the development of concurrent illnesses (influenza A, flu-like syndrome, and myocardial infarction). Prepatent period was defined as the period of time between the challenge and microscopic detection

of parasites; incubation period was defined as time from the challenge until the first fever episode (>38°C). If fever did not occur, incubation time was regarded as missing. Duration of symptoms was defined as the number of days with malaria or chloroquine-related symptoms. Parasite densities were compared after transformation (ln). Clinical differences between group B, group C and reference group A were tested using the nonparametric Wilcoxon's rank-sum test for continuous variables and the Fisher's exact test for dichotomous variables. Thrombocytopenia was defined as a platelet count below 120 x 10^9/l. Changes in blood cell counts were tested by the nonparametric Friedman's rank test.

RESULTS

Infection by 4-7 mosquitoes

Group A of 15 subjects consisted of five males, 13 Caucasians, one Asian and one Black. All volunteers developed parasitaemia after bites of 4-7 infected mosquitoes and were immediately treated with chloroquine. Results of two volunteers were excluded from the analysis because of concurrent illnesses (influenza A and flu-like syndrome). The median prepatent period was 8.8 days (*table 1*). Geometric mean of maximum parasite density was 39.9 parasites/µl. The median incubation period was eight days while median duration of symptoms was six days (from 4 to 122 days after infection). Of the volunteers, 46% (6/13) developed fever, of which 67% in the prepatent period. Altogether, 93% (12/13) of the volunteers showed signs and symptoms one to four days before detection of parasitaemia.

All volunteers in group A developed a mild, uncomplicated episode of clinical malaria. The most common symptoms were headache, malaise and/or fatigue, and myalgia and/or arthralgia (*table 2*).

White blood cell (WBC) count was decreased on the day the thick smear became positive (day 0, *figure 1A*) with a nadir at day 2 and recovery to baseline levels by day 10. Thrombocytopenia (<120 x 10^9/l) occurred in three of the 13 (23%) volunteers. Platelet counts (*figure 1B*) also showed a pattern of significant decline and recovery with a nadir at days 1 to 3. Lymphocyte counts (*figure 1C*) also decreased, but recovered somewhat sooner. Absolute neutrophil counts did not change significantly (data not shown, Friedman rank test: $\chi^2 = 11.2$; df = 6; p=0.08).

Chloroquine treatment was generally uneventful but two volunteers developed side effects. One female became depressed but recovered completely within five days. A second female suffered from paranoia, depersonalisation, nightmares, and concentration problems. There was no medical or family history of neuro-psychiatric disease. Symptoms started on the second day after the start of chloroquine treatment (a total dose of 1.5 g, 27.3 mg/kg). Most symptoms subsided within five days, but the concentration problems took 122 days to resolve.

Infection by 4-7 mosquitoes with delay of treatment

All five volunteers developed signs and symptoms of mild malaria (*table 1*). Clinical presentation of group B was similar to group A, but there was a tendency towards higher fever frequencies with higher maximum temperatures (*tables 1* and *2*). All volunteers developed thrombocytopenia. One volunteer had to be treated with chloroquine after 41.5 hours because of a platelet count of 15 x 10^9/l, without symptoms of bleeding. This was a single observation in a series of measurements showing a gradual decline from 248 to 127 x 10^9/l in four days, followed by a sudden drop to 15 x 10^9/l and a recovery to 120 x 10^9/l

Table 1
Clinical responses to experimentally induced P. falciparum malaria

	A	B	C
NUMBER OF MOSQUITOES	4-7	4-7	1-2
NUMBER OF VOLUNTEERS	13	5	5
ONSET OF TREATMENT	AFTER FIRST POSITIVE THICK SMEAR	DELAYED 48 HOURS	AFTER FIRST POSITIVE THICK SMEAR
Prepatent period (days)	8.8 (7.3-10.3)		9.0 (8.0-13.0)
Incubation period (days)	8 (7-11)[$]		8 (4-11)
Duration of parasitaemia (days)	2 (1-2)	3 (3-5)[*]	2 (1-3)
Duration of symptoms (days)	6 (2-122)[#]	5 (3-6)	5 (1-7)
Highest parasite density (GM[#], per/µl)	39.9 (23-55)	9.6 (32-124)	38.8 (32-55)
Highest temperature (°C)	37.8 (37.0-39.9)	39.4 (38.0-40.2)[**]	38.0 (37.3-39.8)

All values are median (range), except [#] = geometric mean (range); [$]6/13 volunteers did not develop fever, see table 2; [#]due to chloroquine-induced psychiatric side effects; [*]difference between group A and B, Wilcoxon's rank sum p=0.001; [**]difference between group A and B, Wilcoxon's rank sum p=0.05 (borderline significance).

Table 2

Frequency of signs and symptoms in experimentally induced P. falciparum malaria

	A	B	C
NUMBER OF MOSQUITOES	4-7	4-7	1-2
NUMBER OF VOLUNTEERS	13	5	5
ONSET OF TREATMENT	AFTER FIRST POSITIVE THICK SMEAR	DELAYED 48 HOURS	AFTER FIRST POSITIVE THICK SMEAR
Fever	6 (46.2)*	5 (100)	4 (80)
Headache	12 (92.3)	5 (100)	5 (100)
Malaise and/or fatigue	12 (92.3)	4 (80)	5 (100)
Myalgia and/or arthralgia	9 (69.2)	2 (40)	2 (40)
Nausea with/without vomiting	5 (38.5)	2 (40)	3 (60)
Chills	3 (23.1)	1 (20)	3 (60)
Diarrhoea	2 (15.4)	1 (20)	0
Abdominal pain	2 (15.4)	0	1 (20)
Psychiatric symptoms after onset of chloroquine treatment	2 (15.4)	0	0
Thrombocytopenia#	3 (23.1)	5 (100)§	0

Number of volunteers (%); #<120 x 10^9/l; §thrombocytopenia occurred in the period of treatment delay.

Figure 1

Haematological changes after infection with P. falciparum malaria

The influence of infection on white blood cell (1A), platelet (1B) and absolute lymphocyte count (1C) were visualised by plotting the median of all infected volunteers (n=16-21), after subtracting the values on the day of inclusion (=0 on the y-axis).

Day 0 on the x-axis indicates the first thick smear positive day.

Differences were tested using Friedman's rank test. The error bars indicate the interquartile range (IQR).

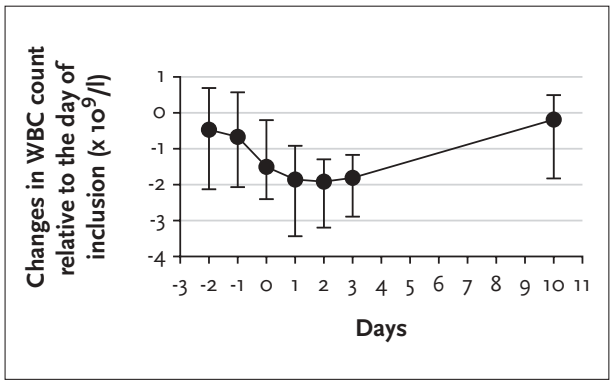

Figure 1A

White blood cell count (WBC)

(Friedman rank: χ^2 = 24.2, df = 6, p<0.001)

Range on the day of inclusion: 4.0- 9.6 x 10^9/l.

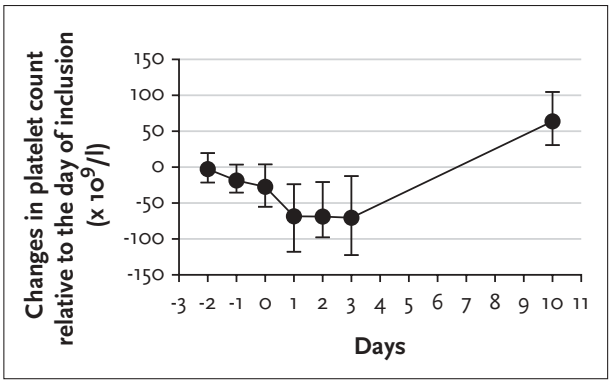

Figure 1B

Platelet count

(Friedman's rank: χ^2 = 67.4; df = 6; p<0.001).

Range on the day of inclusion: 187- 443 x 10^9/l.

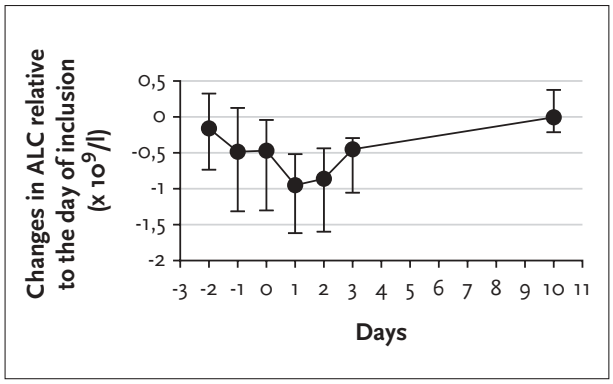

Figure 1C

Absolute lymphocyte count (ALC)

(Friedman's rank: χ^2 = 63.6; df = 6; p<0.001)

Range on the day of inclusion: 1.2- 3.7 x 10^9/l.

within nine hours. An inaccurate reading is most likely in this case. Severity of the thrombocytopenia correlated with parasite density (Pearson's correlation coefficient -0.51, p=0.02). As expected, duration of and maximum parasitaemias were higher than group A (Wilcoxon's rank sum, p=0.001).

Infection by 1-2 mosquitoes

A total of ten volunteers (group C) were exposed to bites of 1-2 infected mosquitoes; five of these ten subjects developed parasitaemia. Parasitaemia and symptoms were similar to volunteers infected with 4-7 mosquitoes (*tables 1* and *2*). None of the volunteers developed thrombocytopenia. No significant differences in laboratory results were observed (data not shown). Volunteers recovered uneventfully from the malaria episode after chloroquine treatment. The five volunteers who remained negative were excluded from data analysis. One of the negative volunteers had an unexpected event during the study. One day after chloroquine treatment, he developed signs of a myocardial infarction. An infero-posterior infarction with a significant stenosis in the circumflex coronary artery was diagnosed. He was transferred to the Cardiology Department and recovered.

Volunteer perception

On the last follow-up visit, we retrospectively evaluated the volunteers' own perceptions on inconveniences and severity of disease through questionnaires. In group A, burden of disease was considered to be severe by 54%, and mild by 46% of the volunteers. Of the volunteers of group B 20% experienced the disease episode as severe, and 80% as mild. Disease perception was comparable in group C: 20% experienced severe disease and 80% mild. Median duration of perceived illness was longer in group A and B, compared with group C (3.0 *vs* 2.0 days). Volunteers perceived headache (37%), malaise/fatigue (19%), and fever/flu-like feeling (15%) as the most unpleasant symptoms. Of the volunteers, 35% considered disease severity higher than anticipated, but 78% of volunteers would participate again.

DISCUSSION

A 100 and 50% infection rate was obtained in 20 and 10 volunteers, respectively, who were experimentally infected by 4-7 and 1-2 *Anopheles stephensi* mosquitoes carrying *P. falciparum* parasites. It has been reported that at least five infected mosquitoes should be used to ensure 100% infection rate, because lower numbers of mosquitoes result in inconsistent infection rates.[6-8] Exposure to 1-2 infected mosquitoes induced parasitaemia in only five out of ten volunteers, which corroborates previous findings.[11]
There are ethical aspects to an infection-inducing challenge experiment, which should be evaluated. Such experiments should not pose risks of irreversible harm if they are confined to self-limiting and completely curable diseases. The expected risks and discomforts for volunteers should be taken into account before the assessment of the study's scientific rationale.[12] In a prospective study, ambulatory management of imported malaria is safe.[13] Follow-up of our volunteers was in principle on an outpatient basis with intensive monitoring, which proved to be satisfactory. Volunteers with an uncomplicated course were only admitted for observation if chloroquine treatment was delayed for 48 hours (group B). The risk of complications was considered to be minimal because of close monitoring and a low threshold for intervention at this low density of parasitaemia. Volunteers participating in other studies had been allowed to develop parasitaemias >10^5 parasites/μl before treatment was initiated.[5,14]

Our protocol with delayed treatment was used for a more precise measure of parasite multiplication. A statistical model was developed that can provide detailed estimates of parasite growth rates and may substantially improve the capacity to evaluate asexual vaccines.[15] In addition, treatment delay provides a possibility to collect data on the initial immune responses during a malaria episode with possibilities to study immune correlates of protection and susceptibility to malaria. All five volunteers in group B developed uncomplicated malaria with a mild increase in severity of symptoms compared with the group that was immediately treated when the thick smear was positive.

Thrombocytopenia was present in all volunteers of group B, but one single platelet count of 15 x 10^9/l was obtained in one individual. This measurement, however, is likely to be incorrect because values directly before and after were similar within a nine-hour period of time. In group A (immediate treatment) 23% (3/13) of the volunteers developed thrombocytopenia (<120 x 10^9/l), while 100% (5/5) developed a low platelet count in group B (delayed treatment). Church *et al.* found thrombocytopenia (<100 x 10^9/l) in ten of 83 (12%) volunteers compared with four of 27 (15%) in our entire study group.[4] A correlation between severity of thrombocytopenia and parasite density has been reported in 89 cases of acute and imported malaria.[15] Nonetheless, this event stresses the need to stay alert and perform frequent tests. The significant decrease in WBC, in particular lymphocytes, is consistent with previous studies.[4,16] It has been speculated that redistribution of lymphocytes into body compartments and apoptosis of T cells occur in parallel during malaria attacks.[17-19]

The clinical response to all challenges, i.e. duration of parasitaemia, and geometric mean parasitaemia corroborates previous studies.[4-8,14] However, our studies show shorter

prepatent periods, which may be due to differences in parasite strain (as has been shown before) or different protocols of laboratory diagnosis.[4,11] The number of sporozoites released from the mosquitoes may vary, or a higher efficiency of liver stage development may be obtained with some strains. A weak inverse relationship was found between prepatent period and number of mosquitoes (data not shown, Pearson's correlation coefficient: -0.397, p=0.04). Previous studies have shown inverse correlations between the estimated inoculum dose and prepatent period.[8]
Our study shows that incubation time is often shorter than the prepatent period, which is in contrast to other challenge studies.[6-8,11] Incubation period has been previously reported from six to 32 days. It is, however, difficult to compare results from various studies, due to different monitoring of volunteers and definitions of incubation time.

Clinical symptoms are comparable with previous studies, but headache was more frequently reported by our volunteers.[4] A relation between parasite inoculum and severity of disease has been suggested.[20] Challenging with 1-2 mosquitoes does not decrease the symptoms, although disease perception is less severe.

Unexpected side effects occurred in three volunteers after the onset of chloroquine treatment. Two volunteers developed reversible psychiatric symptoms following treatment (Telgt, *et al.* in press). Psychiatric side effects following therapeutic doses of chloroquine have been reported but are relatively rare.[21,22] Symptoms usually occur when 2 to 6 g of chloroquine is administered, but both our volunteers received a chloroquine dose below 2 g. For future studies, chloroquine will be replaced by another antimalarial agent, such as co-arthemeter.
One male volunteer who did not develop parasitaemia had a myocardial infarction two days after chloroquine administration. Cardiac complications during and after adequate treatment of malaria are extremely rare (0.6%).[23,24] Retrospectively, this volunteer appeared to have a moderate risk of a coronary event within ten years. Volunteers with a risk of a coronary event greater than 10% will be excluded in future challenge studies.

In conclusion, *P. falciparum* (NF54) experimental human malaria infections with *Anopheles stephensi* mosquitoes induced a 100% infection rate after bites of 4-7 infected mosquitoes and 50% after 1-2 mosquitoes. Using stringent criteria, including risks for cardiac events, and close monitoring, with immediate administration of antimalarial treatment on first detection of parasitaemia, experimental human malaria challenges can be considered to be safe and generally well tolerated. In this way, phase IIa challenge trials can be a powerful tool in the difficult decision-making process of malaria vaccine development and testing.

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REFERENCES

1. WHO. http://www.rbm.who.int/cmc_upload/0/000/015/372/RBMInfosheet_1.htm.
2. Dubovsky FAAP. Creating a vaccine against malaria. 2001. http://www.malariavaccine.org/files/Creating_a_Vaccine_against_Malaria.pdf.
3. Herrera S, Perlaza BL, Bonelo A, Arevalo-Herrera M. Aotus monkeys: their great value for anti-malaria vaccines and drug testing. Int J Parasitol 2002;32(13):1625-35.
4. Church LW, Le TP, Bryan JP, et al. Clinical manifestations of Plasmodium falciparum malaria experimentally induced by mosquito challenge. J Infect Dis 1997;175(4):915-20.
5. Boyd MF, Kitchen SF. Observations on induced falciparum malaria. Am J Trop Med Hyg 1937;17:213-35.
6. Chulay JD, Schneider I, Cosgriff TM, et al. Malaria transmitted to humans by mosquitoes infected from cultured Plasmodium falciparum. Am J Trop Med Hyg 1986;35(1):66-8.
7. Herrington DA, Clyde DF, Murphy JR, et al. A model for Plasmodium falciparum sporozoite challenge and very early therapy of parasitaemia for efficacy studies of sporozoite vaccines. Trop Geogr Med 1988;40(2):124-7.
8. Powell RD, McNamara JV. Infection with chloroquine-resistant Plasmodium falciparum in man: prepatent periods, incubation periods, and relationships between parasitemia and the onset of fever in non-immune persons. Ann N Y Acad Sci 1970;174(2):1027-41.
9. Ponnudurai T, Lensen AH, van Gemert GJ, Bensink MP, Bolmer M, Meuwissen JH. Infectivity of cultured Plasmodium falciparum gametocytes to mosquitoes. Parasitology 1989;98(Pt 2):165-73.
10. Anderson KM, Wilson PWF, Odell PM, Kannel WB. An updated coronary risk profile: a statement for health professionals. Circulation 1991;83:356-62.
11. Rickman LS, Jones TR, Long GW, et al. Plasmodium falciparum-infected Anopheles stephensi inconsistently transmit malaria to humans. Am J Trop Med Hyg 1990;43(5):441-5.
12. FG, Grady C. The ethical challenge of infection-inducing challenge experiments. Clin Infect Dis 2001;33(7):1028-33.
13. D'Acremont V, Landry P, Darioli R, Stuerchler D, Pecoud A, Genton B. Treatment of imported malaria in an ambulatory setting: prospective study. BMJ 2002;324(7342):875-87.
14. Jeffery GM, Young MD, Burgess RW. Early activity in sporozoite induced Plasmodium falciparum infections. Ann Trop Med Parasitol 1959;53:51-8.
15. Hermsen CC, de Vlas SJ, van Gemert GJA, Telgt DSC, Verhege DF, Sauerwein RW. Testing vaccines in human experimental malaria: statistical analysis of parasitemia measured by a quantitative real-time polymerase

chain reaction. Am J Trop Hyg 2004;71(2):196-201.
16. Richards MW, Behrens RH, Doherty JF. Short report: hematologic changes in acute, imported Plasmodium falciparum malaria. Am J Trop Med Hyg 1998;59(6):859.
17. Hviid L, Kurtzhals JA, Goka BQ, Oliver-Commey JO, Nkrumah FK, Theander TG. Rapid reemergence of T cells into peripheral circulation following treatment of severe and uncomplicated Plasmodium falciparum malaria. Infect Immun 1997;65(10):4090-3.
18. Hviid L, Kemp K. What is the cause of lymphopenia in malaria? Infect Immun 2000;68(10):6087-9.
19. Kern P, Dietrich M, Hemmer C, Wellinghausen N. Increased levels of soluble Fas ligand in serum in Plasmodium falciparum malaria. Infect Immun 2000;68(5):3061-3.
20. Glynn JR. Infecting dose and severity of malaria: a literature review of induced malaria. J Trop Med Hyg 1994;97(5):300-16.
21. Good MI, Shader RI. Lethality and behavioral side effects of chloroquine. J Clin Psychopharmacol 1982;2(1):40-7.
22. Mohan D, Mohandas E, Rajat R. Chloroquine psychosis: a chemical psychosis? J Natl Med Assoc 1981;73(11):1073-6.
23. Franzen D, Curtius JM, Heitz W, Hopp HW, Diehl V, Hilger HH. Cardiac involvement during and after malaria. Clin Invest 1992;70(8):670-3.
24. Sprague HB. The effects of malaria on the heart. Am Heart J 1946;31:426-30.

Verhage, et al. Experimental human malaria induced by *P. falciparum*-infected mosquitoes.