Controlled Human Malaria Infection with Graded Numbers of *Plasmodium falciparum* NF135.C10- or NF166.C8-Infected Mosquitoes

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**Abstract.** Controlled human malaria infections (CHMIs) with *Plasmodium falciparum* (Pf) parasites are well-established. Exposure to five Pf (NF54)-infected *Anopheles* mosquitoes results in 100% infection rates in malaria-naive volunteers. Recently Pf clones NF135.C10 and NF166.C8 were generated for application in CHMIs. Here, we tested the clinical infection rates of these clones, using graded numbers of Pf-infected mosquitoes. In a double-blind randomized trial, we exposed 24 malaria-naive volunteers to bites from one, two, or five mosquitoes infected with NF135.C10 or NF166.C8. The primary endpoint was parasitemia by quantitative polymerase chain reaction. For both strains, bites by five infected mosquitoes resulted in parasitemia in 4/4 volunteers; 3/4 volunteers developed parasitemia after exposure to one or two infected mosquitoes infected with either clone. The prepatent period was 7.25 ± 4.0 days (median ± range). There were no serious adverse events and comparable clinical symptoms between all groups. These data confirm the eligibility of NF135.C10 and NF166.C8 for use in CHMI studies.

**INTRODUCTION**

Controlled human malaria infections (CHMIs) are a well-accepted tool used since the 1980s for the exploration of immunology and pathophysiology of malaria infections and for evaluation of candidate vaccines and drugs. The majority of studies have been conducted with 3D7 and its parental strain NF54. Most volunteers have been infected using laboratory-reared *Plasmodium falciparum* (Pf)-infected *Anopheles* mosquitoes, which reproducibly result in optimal infection rates in malaria-naive volunteers. Reducing the number of NF54 or 3D7 Pf-infected mosquitoes to one or two reduces the infection rate.

Given the diversity of Pf isolates in the field, the value of CHMI trials can be increased by expanding the portfolio with Pf clonal isolates from different geographical origins. The Pf isolate NF54, most likely originates from Africa. The more recently generated clones are NF135.C10 and NF166.C8, originating from Cambodia and Guinea, respectively. Initial studies with NF135.C10 and NF166.C8 show that these clones are safe and give high infection rates with five infectious mosquitoes (80% [8/10] and 100% [5/5], respectively). However, these clones show more effective hepatocyte invasion and liver merozoite development as compared with NF54, which results in higher first peak of parasitemia and shorter prepatent period. In order to establish an NF135.C10- or NF166.C8 CHMI model with comparable parasite dynamics as obtained in NF54-infected volunteers, the objective of the current study was to compare infection rates and dynamics of parasitemia as well as clinical manifestations using one, two or five NF135.C10- or NF166.C8- infected mosquitoes.

**MATERIALS AND METHODS**

The study is a single-center, double-blind, randomized trial. The flowchart, screening procedures, and inclusion and exclusion criteria were described previously. Briefly, 24 healthy male and female volunteers, aged 18–35 years, were randomly assigned to six groups and exposed to bites from five mosquitoes of which five, two, or one were infected with either NF135.C10 or NF166.C8. Uninfected mosquitoes were added to preserve blinding of all trial personnel except the staff members preparing and aliquotting infected mosquitoes. *Anopheles stephensi* mosquitoes were reared and infected according to standardized protocols. The average number of sporozoites per mosquito used in this study was 69,000 for NF135.C10 and 51,000 for NF166.C8. Mosquito feeding was performed for 10 minutes and infectivity of mosquitoes was assessed after feeding by dissecting salivary glands for the presence of sporozoites. Feeding was repeated until the predefined number of infected bites was reached. Eight volunteers needed two sessions, one needed three sessions (all in the five- or two-mosquito bite groups).

After CHMI, volunteers were followed-up twice daily from day 5 post-infection until two consecutive blood samples were positive by quantitative polymerase chain reaction (qPCR) (≥500 parasites/mL), or, when they remained negative, until day 13. At this point, volunteers were treated with atovaquone/proguanil and followed on days 1, 2, 3, and 7 after treatment and on day 35 post-infection. Follow-up was prolonged if volunteers remained qPCR-positive or symptomatic. Throughout the study, high-sensitive troponin T, lactate dehydrogenase (LDH), and platelet counts were measured as safety parameters. The first peak of parasitemia was calculated as the geometric mean of *Pf* parasites per milliliter between day 6.4 and 8.4.

The trial was registered at www.clinicaltrials.gov, identifier NCT02149550. Ethical approval from the Central Committee on Research Involving Human Subjects (NL48704.000.14) was obtained.

All statistical analyses were performed with IBM SPSS statistics for Windows (Version 23.0; IBM Corp., Armonk, NY). Mean prepatent period, first peak of parasitemia, and the frequency of adverse events (AEs) between the groups were assessed by using unpaired t test, one-way analysis of
variance (ANOVA), or their nonparametric variants. Adverse
events were reported as mild (grade 1), moderate (grade 2), or
severe (grade 3).

RESULTS

In total, 20 of 24 volunteers developed parasitemia; all vol-
unteers exposed to five infected mosquitoes and 3/4 volun-
tees exposed to one or two infected mosquitoes with either
strain (Table 1).

The prepatent period in the NF135.C10 groups ranged from
7 to 9 days and did not differ significantly between the dosage
groups (median [range] five mosquitoes: 7.5 [7.0–9.0] versus
two mosquitoes: 9.0 [7.5–9.0] versus one mosquito: 7.0
[7.0–7.0]; P = 0.06, Table 1). There was no difference in first
peak of parasitemia in parasites/mL (geometric mean (GM)
[95% confidence interval (CI)] five mosquitoes: 2.9 [1.8–4.0],
versus two mosquitoes: 2.4 [1.0–3.8], versus one mosquito:
3.0 [2.3–3.7]; P = 0.32) (Figure 1A). All parasiticemic volunteers
experienced one or more AEs. When comparing subjects chal-
enged with either five, two, or one NF135.C10-infected mosqui-
toes, there were no differences in the total number of
grade 1, 2, or 3 AEs (P = 0.81, P = 0.60, and P = 0.87, re-
spectively) (Figure 1B), or the number of solicited AEs (head-
ache, nausea, malaise, chills and myalgia, data not shown). A
few unsolicited AEs were reported, two or three per group, all
grade 1.

For the NF166.C8 clone, no significant difference was
found in the prepatent period between the three dosage
groups either (median [range] five mosquitoes: 7.0 [7.0–7.0]
versus two mosquitoes: 7 [7.0–11.0] versus one mosquito:
9.0 [7.5–9.0]; P = 0.09, Table 1). In contrast to NF135.C10, the
first peak of parasitemia was significantly higher in volunteers
bitten by higher numbers of NF166.C8-infected mosquitoes
(GM [95% CI] five mosquitoes: 3.8 [3.3–4.2], versus two
mosquitoes: 2.4 [0.9 to 5.6], versus one mosquito: 2.2
[1.7–2.7]; P = 0.04, Figure 2A). There were no differences in the
number of grade 1, 2, or 3 AEs (P = 0.11, P = 0.33, and P =
0.90, respectively) (Figure 2B), nor in the number of solicited
AEs between the volunteers challenged with either five, two,
or one NF166.C8-infected mosquitoes (data not shown). A
few unsolicited AEs were reported, four or six per group, all
grade 1.

When comparing all subjects who developed parasitemia
with either strain, the prepatent period was similar for NF135.
C10 and NF166.C8 (median [range] 7.5 [7.0–9.0] versus 7.0
[7.0–11.0]; P = 0.65 see Table 1) as was the height of the first
peak of parasitemia (GM [95% CI] NF135.C10: 2.8 [2.4–3.2]
parasites/mL versus NF166.C8: 2.9 [2.1–3.6]; P = 0.48). In
addition, there were no significant differences in the number of

| Table 1 |
|———|———|———|———|———|———|
|      | No. of infectious bites | Five, two, or one | Five | Two | One | P value |
| Strain | Subjects PCR positive (n/total) | 10/12 | 4/4 | 3/4 | 3/4 | – |
|        | Prepatent period (days (median [range])) | 7.5 [7.0–9.0] | 7.5 [7.0–9.0] | 9.0 [7.5–9.0] | 7.0 [7.0–7.0] | 0.06 |
| NF166.C8 | Subjects PCR positive (n/total) | 10/12 | 4/4 | 3/4 | 3/4 | – |
|        | Prepatent period (days (median [range])) | 7.0 [7.0–11.0] | 7.0 [7.0–7.0] | 7.0 [7.0–11.0] | 9.0 [7.5–9.0] | 0.09 |

Differences between groups were tested using the Kruskal-Wallis method.
DISCUSSION

This study shows that CHMI with five bites of NF135.C10- or NF166.C8-infected mosquitoes results in 100% patent parasitemia, which is comparable with the outcomes of previous CHMIs with NF54, 3D7, or 7G8.3,14,15 Lowering the number of infectious bites of both test clones to one or two bites reduced the patency to 75% (3/4 individuals infected), which is similar to previous trials with 3D7 or NF54-infected mosquitoes showing parasitemia in 40–60% of the cases.4,5

The kinetics of emerging NF135.C10 parasitemia remained relatively similar irrespective of the number of infected mosquitoes used. Theoretically, a relation with the number of infectious mosquito bites could be expected, as seen in NF166. C8. However, the variance in first peak of parasitemia in NF135.C10 and NF166.C8 of either clone consistently gives 80–100% patent parasitemia in the three studies performed with these heterologous clones. In future CHMI studies using clones NF135.C10 and NF166.C8 and lower numbers of mosquito bites may suffice to achieve blood-stage dynamics which are more similar to NF54, albeit reaching lower infection rates.

In conclusion, this study with limited numbers of volunteers per group shows that bites of five mosquitoes of NF135.C10 and NF166.C8 of either clone consistently gives 80–100% patent parasitemia in the three studies performed with these heterologous clones. In future CHMI studies using clones NF135.C10 and NF166.C8 and lower numbers of mosquito bites may suffice to achieve blood-stage dynamics which are more similar to NF54, albeit reaching lower infection rates.

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