at an earlier age than that of our study population.

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References


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Babesia microti and Borrelia burgdorferi Coinfection Associated with Increased Severity of Arthritis

To the Editor—In the 1 November 2005 issue of the Journal of Infectious Diseases, Coleman et al. [1] reported that simultaneous infection (coinfection) with the etiological agent of Lyme disease (Borrelia burgdorferi) and the etiological agent of human babesiosis (Babesia microti) did not result in increased severity of either disease in mice. However, we would like to point out the following observations, which may have influenced their final analysis.

Two factors can have an important influence on the outcome of this type of study: (1) the mouse model used and (2) the time interval selected for arthritis analysis. The C3H mouse is a well-defined model for the study of arthritis caused by B. burgdorferi, and mice of this strain are considered to be among the most likely to develop severe arthritis [2]. Peak arthritis, determined by histopathological changes, is observed from 14 to 30 days after initial infection, especially in mice inoculated before age 8 weeks. The increased susceptibility of C3H mice to Lyme arthritis is most likely caused by genetic and immunoinflammatory factors [3, 4]. If simultaneous infection with B. microti has an effect on arthritis induced by B. burgdorferi infection, this probably would not have been detected with the single time point selected (22 days) by Coleman et al. In other words, C3H and Babesia–infected mice already have a high level of arthritis by day 22 that may not be statistically different from coinfected mice, as was demonstrated by Moro et al. [5]. Furthermore, Coleman et al. did not perform a histopathological analysis of joints in BALB/c mice—a strain of mice that are significantly more resistant to Lyme arthritis than are C3H mice [2] and in which coinfection of B. microti and B. burgdorferi has resulted in increased Lyme arthritis at day 30 after infection [5]. We hypothesize that B. microti induces a Th1-mediated proinflammatory response [5, 6] that contributes to the pathogenesis of arthritis caused by B. burgdorferi infection. Because C3H mice tend to have strong Th1 (inflammatory) responses when challenged with B. burgdorferi, the contribution of B. microti to this response may not be detected in this mouse strain, especially during the first few weeks after infection. This may also explain why Coleman et al. did not detect more severe arthritis in spleenectomized C3H mice, which are more susceptible to B. microti infection.

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References


No pfmdr1 Amplifications in Samples from Guinea- Bissau and Liberia Collected between 1981 and 2004

To the Editor—Uhlemann et al. [1] recently reported a $5% prevalence of pfmdr1 amplifications in Gabon in 1995 and described an association between these amplifications and low-level mefloquine (MQ) resistance. Surprisingly, no amplifications were seen 7 years later. Previously, pfmdr1 amplifications have been associated with Plasmodium falciparum resistance to MQ in vivo and in vitro [2]. In addition, it was recently suggested that pfmdr1 amplifications are associated with lumefantrine resistance in vitro [3]. Am-
plifications have also been linked to decreased *P. falciparum* susceptibility to halofantrine, quinine (QN), and artemisinine derivatives in vitro [2, 4].

Guinea-Bissau recently changed its first-line treatment for uncomplicated *P. falciparum* infection to artemether-lumefantrine [5]. Because of this change and the detection of amplifications in Gabon, we decided to determine the prevalence of multiple copies of *pfmdr1* in Guinea-Bissau. Our secondary aims were to determine whether *pfmdr1*-amplification prevalence decreased over time and after treatment with chloroquine (CQ) or amodiaquine.

We assessed *pfmdr1* copy number using a reverse transcriptase–polymerase chain reaction (RT-PCR) method described elsewhere [3]. All samples were run in triplicate. Each run contained 3 reference samples of the *P. falciparum* 3D7 clone (1 *pfmdr1* copy) and 1 control sample of Dd2 (2–3 *pfmdr1* copies). We used the same cutoff criteria as previous studies [1, 2]; that is, assays were repeated if any of the following results were obtained: ΔΔCt (cycle threshold) spread >1.5, Ct values >35, or copy number equal to 1.3–1.6. Values obtained were then rounded to the closest integer.

Blood samples came from 3 previous studies. One was a clinical trial conducted between 2001 and 2004 [6] in which 729 children with uncomplicated *P. falciparum* infection were randomly assigned treatment with 25 mg/kg CQ, 50 mg/kg CQ, 15 mg/kg AQ, or 30 mg/kg AQ. Blood for DNA analysis was not collected at the beginning of the trial. Therefore, 560 samples were available for RT-PCR; of these samples, 523 (93%) were successfully amplified.

Samples also came from routine in vitro monitoring of CQ resistance conducted at Laboratório Nacional Saúde Pública, Guinea-Bissau (authors’ data). In summary, surveys of children conducted in 1992, 1993, and 1995 identified asymptomatic carriers of *P. falciparum*. Before treatment, blood was obtained by fingerprick and used for in vitro CQ-susceptibility testing; in the present study, it was used for DNA analysis. RT-PCR analysis was successful in 73 (90%) of 81 samples.

A third set of archival patient samples came from a survey conducted in northern Liberia in 1981 [7], a time when very little CQ was used. Blood was obtained before therapy with CQ and then frozen. We randomly selected 40 samples for inclusion in this study and successfully amplified 34 (85%) samples. All the studies had the required ethics approval from the Karolinska Institute ethics committee and local ethics committees.

Despite the large number of samples and the geographical and chronological spread, we found no *pfmdr1* amplifications (table 1). This indicates that the genetic basis of MQ resistance, and potentially that of lumefantrine resistance, associated with multiple copies is very rare, if it even exists in Guinea-Bissau. Similarly, artemisinin and QN tolerance associated with *pfmdr1* amplifications should be very rare. Because QN has been and still is used in Guinea-Bissau, this result casts some doubt on the importance of *pfmdr1* amplifications as a marker of QN resistance in vivo.

The lack of any amplification in the clinical trial could be due to the use of CQ in the decades before the trial. However, the fact that no *pfmdr1* amplifications were found in infections as far back as the early 1980s suggests that amplifications are and have been rare in West Africa, but probably not as a result of CQ use. Rather, amplifications are probably rare because they do not provide sufficient benefit in an environment that does not include specific stressors such as MQ.

It has been suggested that *pfmdr1* amplifications arise only in *P. falciparum* with the *pfmdr1* 86N genotype. Because this genotype has a prevalence of ≥60% in the analyzed samples (authors’ data), lack of it is not an explanation for not detecting amplifications.

Finally, finding no *pfmdr1* amplifications is very encouraging in light of Guinea-Bissau’s recent switch to artemether-lumefantrine as first-line therapy for uncomplicated *P. falciparum* infection. However, amplifications were previously detected in Gabon, and genotypes associated with drug resistance can spread rapidly through Africa. Therefore, it will be important to monitor *pfmdr1*-amplification prevalence in the future, because an increase might be the first sign of resistance to artemether-lumefantrine developing or spreading.

**References**

It is reassuring that these data support the idea of no preexisting increase in pfmdr1 copy number in parasites from these 2 West African countries. It would have been useful to have an estimate of the degree of polyclonality of infections, because multiple clones can lead to underestimation of the frequency of increased pfmdr1 copy number [2]. Nonetheless, the relatively large sample size provides a useful baseline against which molecular surveillance can be implemented to detect emerging resistance to drugs such as mefloquine (MQ) and lumefantrine.

These findings are from an area with no MQ pressure and strengthen the hypothesis that MQ is the principal agent for selection of pfmdr1 amplification in field isolates (making quinine a less likely selective agent). Quinine has been implicated as a possible cause of cross-resistance to MQ [3]. Of note, our study from Gabon was performed at a time when low-dose MQ studies were being performed in that area [3].

Previous studies show that pfmdr1 amplification and the N86Y point mutation in this gene are inversely related [4], for reasons that remain incompletely understood. In African isolates, the pfmdr1 86Y allele may occur infrequently by itself or more frequently in association with the pfcrt K76T allele, depending on the endemic setting [5–7]. This combined genotype (N86Y and K76T) has been strongly associated, in some but not all studies, with resistance to CQ. It has been proposed that N86Y is selected for because of CQ pressure, either to augment resistance or to compensate for altered functional properties of mutant pfcrt harboring K76T. The N86Y mutation may in turn have rendered the parasites hypersensitive to MQ [2]. If so, then in areas with MQ pressure, wild-type 86N could be favored and predispose to pfmdr1 amplification. Multicopy pfmdr1 is highly associated with MQ resistance and may also increase CQ susceptibility in some parasite genetic backgrounds.

Ursing et al. report that ≥60% of their samples carry the pfmdr1 86N allele, suggesting that pfmdr1 amplification is theoretically possible [1]. Although present at lower levels, CQ resistance is not as widespread in Guinea-Bissau as it is in Gabon [7, 8]. Perhaps the lack of high-level CQ resistance provides a protective role for the development of MQ resistance by pfmdr1 amplification. Monitoring for these amplifications will become increasingly important as African countries move away from using CQ and instead use MQ and other agents in artemisinin-based combination therapy.

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