POSSIBLE ACUTE COINFECTIONS IN THAI MALARIA PATIENTS

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Abstract. We conducted serodiagnostic testing for dengue virus infection, murine typhus, scrub typhus and leptospirosis in *Plasmodium falciparum*-infected individuals in Thailand. Sera from 194 malaria patients with a median age of 24 years were tested. No antibody titers diagnostic of dengue virus infection were demonstrated, but 29 (15%) of patients had serological evidence of scrub typhus, 45 (23.2 %) patients had evidence of murine typhus, and 15 (7.7%) sera tested positive for leptospirosis. Our serological results suggested that duel infections are not uncommon in malaria that is acquired in Thailand. However, our results must be confirmed by prospective studies aimed at describing the causative organisms. Mixed infections would have multiple implications for clinicians, including unexpected clinical findings and apparent poor responses to antimalarial treatment in patients thought only to have malaria.

INTRODUCTION

Simultaneous infections with more than one pathogen have received most attention in the context of human immunodeficiency virus type 1 infection (HIV-1), where both harmful opportunistic infections (Mussini *et al*, 2004; Kauffman and McMichael, 2005) and beneficial interactions (Watt *et al*, 2000; Xiang *et al*, 2004) have been described. Malaria is a prominent coinfection of HIV-infected individuals; millions of HIV-malaria duel infections are thought to occur annually in sub-Saharan Africa (Kublin *et al*, 2005). Co-infections in malaria-infected individuals other than HIV-1 have also been recognized. Algid malaria is a complication of severe malaria with secondary gram-negative bacteremia (Bradley and Warrell, 2003). *Ascaris lumbricoides* coinfection is apparently associated with protection from cerebral malaria (Nacher *et al*, 2000).

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Recently dual infections with leptospirosis and scrub typhus were described in Thai agricultural workers occupationally exposed to both diseases (Watt *et al*, 2003). Malaria in Thailand is often associated with activities in its mountainous border areas, such as logging and gem mining (Singanetra, 1993). These activities sometimes place individuals at increased risk of also acquiring acute infections other than malaria. Although there have been occasional reports of coinfections in malaria patients (Corne *et al*, 2004), systematic surveys have been lacking. We therefore examined the sera of patients who had acquired acute malaria on the Thai-Burmese border for evidence of scrub and murine typhus, leptospirosis, and dengue virus infection.

MATERIALS AND METHODS

Sera from adult patients with falciparum malaria, who were enrolled in antimalarial drug trials in Thailand, were tested for acute coinfections. Falciparum malaria was diagnosed if asexual forms of *P. falciparum* were detected in thick and thin Giemsa-stained blood films.

Acute coinfections were diagnosed using test kits provided by the Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand.

Dengue

Titers of IgG and IgM antibodies against dengue virus infection were determined by an enzyme-linked immunosorbent assay (Innis *et al*, 1989). The lower limit of positivity was designated as 40 units of antibody . The test kit used antigens from all four dengue serotypes as follows: DEN-1 (Hawaii), DEN-2 (TR1751), DEN-3 (S-87), and DEN-4 (S-241). The sensitivity of the test was stated to be 78% according to the package insert.

Scrub typhus

Orientia tsutsugamushi IgG and IgM antibody titers were determined by an indirect immunofluorescent assay (Chenchittikul *et al*, 1995). Test kits incorporated the Gilliam, Karp and Kato strains of *O. tsutsugamushi* as antigens. A titer of $\geq$ 1:400 on a single sera was considered to be positive. The sensitivity and specificity of this test kit were stated as 24.6% and 100%, respectively, according to the package insert.

Murine typhus

Murine typhus IgG and IgM antibody titers were measured by an indirect immunofluorescent assay (Chenchittikul and Saisongkorh, 1999). The test kit used the Wilmington antigen of *Rickettsia typhi* and a positive serodiagnosis was a titer of $\geq$ 1:400 on a single sera. The specificity of this test was described as 100%.

Leptospirosis

Leptospirosis IgG and IgM antibody titers were determined by an indirect immunofluorescent antibody assay using the *Leptospira interrogans* serotypes: sejroe, bratislava, icterohaemorrhagia and autumnalis as antigens (Biriraj *et al*, 1998). An IgM titer of $\geq$ 1:400 on a single specimen was considered positive. The sensitivity and specificity of this kit were described as 100% and 90%, respectively.

RESULTS

All patients acquired malaria on the Thai-Burmese border. Approximately 95% of the patients came from Mae Sot, Tak Province, while the remainders were from Kanchanaburi Province. The median age of the 194 patients was 24 years, and ranged from 15 to 87 years. Seventy percent were male. Seventy-nine individuals were Thai, six were Burmese, 34 were Karen, and 75 were Mon.

Dengue

The diagnosis of dengue virus infection requires IgG- and IgM-antibody titer determinations from acute and convalescent sera. Paired sera were available from only 29 patients. All 29 tested negative (< 40 units) for both IgM and IgG antibodies on both acute and later specimens.

A total of 194 admission sera were tested for scrub typhus, murine typhus and leptospirosis.

Scrub typhus

Twenty-nine of 194 sera (15.0 %) tested positive for scrub typhus using a cutoff titer of at least 1:400 for either IgG or IgM antibodies. In 25 patients (12.9%), the antibody titers were 1:800 or greater.

Murine typhus

Forty-five of 194 sera (23.2 %) tested positive for murine typhus using a cutoff titer of at least 1:400 for either IgG or IgM antibodies. Only 18 patients (9.3 %) had titers of 1:800 or greater.

Leptospirosis

Fifteen of 194 sera (7.7%) tested positive for leptospirosis at a cutoff titer of 1:400. However, titers of >1:400 were found in only six individuals (3.1%).

Serological findings suggested possible triple infections in 22 patients and quadruple infections in three individuals. Antibodies to both scrub and murine typhus were detected in 10 malaria patients, to scrub typhus and leptospirosis in five patients, and to murine typhus and leptospirosis in seven patients. In three malaria-infected individuals, antibodies to scrub and murine typhus as well as leptospirosis was demonstrated.

DISCUSSION

Our results suggest that additional acute infections, in patients who present with malaria

in Thailand, might be occurring more often than has previously been recognized. Not only are malaria and the other infections for which we tested common in Thailand, but individuals at risk for one are often at an increased risk for the others. For example, logging and land clearing have been associated with scrub typhus (Silpapojakul, 1997). These activities are performed in mountainous border regions of Thailand, and such activities would also place individuals at risk for acquiring malaria (Singanetra, 1993).

Our results strongly suggest that duel infections often complicate malaria. However, our data that indicate the proportions of multiple infections should be considered preliminary because infections other than malaria were only diagnosed serologically. Further investigations that demonstrate organisms would provide stronger evidence of mixed infections than investigations that only find antibodies. In addition to culture techniques, the causative organisms of leptospirosis, scrub typhus, and murine typhus can be demonstrated by PCR (Choi *et al*, 2005; Levett *et al*, 2005; Singhsilarak *et al*, 2005). Although the kits we used have been reported to be sensitive and specific, it is possible that background antibodies from previous exposures, rather than from an acute infection, were responsible for positive test results in some individuals. This risk was amplified because most of our testing was performed on a single, acute specimen rather than on paired samples. For example, the majority of patients who tested positive for murine typhus had antibody titers of only 1:400, which was considered to be at the lower limit of positivity. In addition, we cannot rule out with certainty that malaria does not induce antibodies that cross-react with scrub typhus, murine typhus, and leptospirosis. Similarly, the true number of mixed infections could have been higher than we measured if acute and convalescent sera had been available for every patient. Dengue virus infection was not demonstrated, but only 29 paired sera were available for testing.

Confirmation of dual infections of *P. falciparum* with other agents would have several implications for clinicians. Clinical features, not typically seen in malaria, might be encountered, and some malaria infections might not respond as expected to antimalarial drugs. Some compounds used in the treatment of malaria, such as doxycycline, also have activity against scrub typhus, murine typhus and leptospirosis. However, most antimalarials cannot be relied upon to cure these other infections.

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