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CAUSES OF FEVER IN ADULTS ON THE THAI-MYANMAR BORDER

RUTH D. ELLIS,* MARK M. FUKUDA, PHILIP MCDANIEL, KATHERINE WELCH, ANANDA NISALAK, CLINTON K. MURRAY, MICHAEL R. GRAY, NICHAPAT UTHAIMONGKOL, NILLAWAN BUATHONG, SABAITHIP SRIWICHAI, RUNGNAPHA PHASUK, KRITSANAI YINGYUEN, CHAIYAWAT MATHAVARAT, AND ROBERT S. MILLER

Armed Forces Research Institute for Medical Sciences (AFRIMS), Bangkok, Thailand; Kwai River Christian Hospital, Sangkhlaburi, Thailand; Brooke Army Medical Center and VETCOM Leptospirosis Reference Laboratory, Fort Sam Houston, Texas

Abstract. A hospital-based study was conducted along the Thai-Myanmar border to provide greater knowledge of the causes of febrile illness and to determine what zoonotic and vector-borne emerging infectious diseases might be present. A total of 613 adults were enrolled from June 1999 to March 2002. Cases were classified based on clinical findings and laboratory results. An etiologic diagnosis was made for 48% of subjects. Malaria was the most common diagnosis, accounting for 25% of subjects, with two-thirds *Plasmodium falciparum*. Serologic evidence for leptospirosis was found in 17% of subjects. Other etiologic diagnoses included rickettsial infections, dengue fever, and typhoid. The most frequent clinical diagnoses were nonspecific febrile illness, respiratory infections, and gastroenteritis. Clinical associations were generally not predictive of etiologic diagnosis. Apparent dual diagnoses were common, particularly for malaria and leptospirosis. Findings have been used to modify treatment of unspecified febrile illness in the area.

INTRODUCTION

For health care providers and public health officials, knowledge of local patterns of disease is critical for making informed treatment and prevention decisions. In many developing nations, prevalence and incidence of infectious disease is largely unknown, with estimates based on scanty or unreliable data.[1] Diagnostic tools for infectious disease in particular require an investment in training, infrastructure, and technology that is beyond the reach of many developing nations.[2] Presumptive treatment, even for a relatively easily diagnosed cause of fever such as malaria, remains the standard of care in many locales.[3]

One approach to improving knowledge of local patterns of infectious disease is to identify a sentinel site (typically a hospital) and to intensively study patients presenting with febrile illness at that site. Some of these hospital-based cohort studies have focused on bacterial infections, and others have included serologic testing as well as bacterial cultures.[4–10] Results from these studies have been used to improve local empirical treatment decisions and to enhance the appropriate use of resources and to improve quality of diagnostic services.[2] Three reports of studies of febrile illness in Thailand have been published: of bacterial infections in adults in Bangkok; of causes of fever in children during flooding in a provincial hospital; and of etiologies of acute pyrexia of unknown origin in children and adults at 10 community hospitals.[5,10,11]

The primary objectives of this study were, first, to provide greater knowledge of the causes of febrile illness among patients presenting for care at this community hospital, and second, to determine what zoonotic and vector-borne emerging infectious diseases might be prevalent at this sentinel site.

MATERIALS AND METHODS

Setting. Sangkhlaburi District is located in Kanchanaburi Province in western Thailand, on the border with Myanmar (Burma). Several ethnic groups are represented in the district, including Thai, Karen, Mon, and Burmese. The district is rural and mountainous and is a heavily traveled border crossing, with large numbers of people entering and leaving for trade and occasionally because of unrest inside Myanmar. Malaria is common, as is nonspecific febrile illness. Kwai River Christian Hospital (KRCH) is a 60-bed hospital and has served local residents as well as those who travel to the hospital seeking medical care since 1961. The Armed Forces Research Institute for Medical Science (AFRIMS) is a joint U.S.-Royal Thai Army tropical medicine research institute based in Bangkok. The AFRIMS/KRCH Clinical Center (AKCC) was established in 2000 and is a center for malaria and other infectious disease research at the hospital.

Subjects. Adult inpatients and outpatients presenting with temperature $\geq 38°C$ or history of fever over the previous 48 hours were admitted to the study and evaluated for cause of fever. Patients with fever longer than 48 hours were also eligible for enrollment, as long as the cause of fever was not yet known. Patients presenting for continuation of treatment of known cause of fever were excluded, as were those who were unable or unwilling to provide blood samples 2–4 weeks after enrollment. Clinical information and blood samples were obtained at enrollment and after approximately 3 weeks. Informed consent was obtained from each study subject before enrollment. Patients were enrolled under Walter Reed Army Institute of Research (WRAIR) protocol no. 745. The protocol was approved by the WRAIR Human Use Review Committee and by the Thai Ministry of Public Health's Ethical Review Committee for Research in Human Subjects.

Laboratory and diagnostic techniques. Routine laboratory tests included complete blood count (CBC), and a standard biochemistry panel—alanine transferase (ALT), gamma glutamyl transferase (GGT), blood urea nitrogen (BUN), and creatinine. Other diagnostic tests, for example, chest x-ray, sputum studies, or rapid human immunodeficiency virus (HIV) testing, were done at the discretion of the attending physician. Cultures were not done. All reference diagnostic testing was performed at AFRIMS, with the exception of malaria blood smears, microscopic agglutination test (MAT), and immunofluorescent antibody (IFA) as discussed below.

Malaria. All subjects were screened for malaria at enrollment by thick and thin blood smear Giemsa microscopy performed by expert microscopists located at KRCH. If asexual

* Address correspondence to Ruth D. Ellis, 2530 N. 24th Street, Arlington, VA 22207. E-mail: schwartzellis@hotmail.com

parasites were seen on the smear, the patient was considered to have malaria.

Leptospirosis. All subjects were screened by a commercially available IgM enzyme-linked immunosorbent assay (ELISA; PanBio, Inc., Baltimore, MD). All cases with a level of ≥ 9 PanBio units on acute or convalescent samples were sent for confirmatory MAT, performed at the Veterinary Command (Fort Sam Houston, TX). Cases with an MAT titer of ≥ 800 on a single sample or a ≥ fourfold change between acute and convalescent titers were considered to be positive for leptospirosis. A panel of 24 serovars was used for MAT.

Dengue fever and Japanese encephalitis. All cases with paired samples available were tested for IgG and IgM antibodies for dengue and Japanese encephalitis by an in-house ELISA.[12] Acute cases were defined as > 40 IgM antibody units with a rise in titer between the acute and convalescent specimens. The ratio of antibody to dengue versus Japanese encephalitis was used to differentiate the two infections, and the ratio of IgM/IgG was used to determine whether the infection was primary or secondary dengue infection. For specimens positive by ELISA, detection of dengue viral RNA was performed by reverse-transcription polymerase chain reaction using a modification of the primers used in the Lanciotti procedure.[13]

Rickettsioses. Forty-six (46) cases selected by clinical criteria (rash or eschar, history of arthropod bites or jungle exposure) were evaluated for evidence of rickettsial infection (scrub typhus, murine typhus, and spotted fever group rickettsiosis) at Unité des Rickettsies (Marseille, France). IFA techniques used for diagnosis have previously been described.[14] IgG titers ≥ 64 and/or IgM titers ≥ 32 on acute or convalescent specimens were considered to be positive for acute infection.

Typhoid. Real-time PCR for *Salmonella enterica* serovar *typhi (S. typhi)* was done on a selected subset of 31 patients based on clinical criteria including nonlocalized rash or admitting or reviewing physician's presumptive diagnosis of typhoid. DNA was extracted from reconstituted whole blood and sequences (DNA fragments) from the Vi antigen (ViaB gene) specific for *S. typhi* were amplified using the TaqMan PCR assay, with a lower detection limit of 1,000 cfu/mL of extract (Phasuk R, Sethabutr O, unpublished data) Patients with a clinical illness consistent with typhoid and with ilial perforation found on laparotomy were also counted as confirmed typhoid cases.

Pulmonary TB and HIV/AIDS. To be classified as pulmonary tuberculosis (PTB), subjects must have had a cavitary lesion on chest x-ray and/or acid-fast bacilli seen on sputum smear. Subjects counted as HIV/AIDS had a positive rapid diagnostic test for HIV and met the World Health Organization case definition for AIDS surveillance and did not have a pathogen-specific diagnosis otherwise identified.[15]

Melioidosis (Burkholderia pseudomallei) and Q fever (Coxiella burnetii). Commercially available IgG ELISA kits (PanBio, Inc.) were used to screen 44 selected subjects for melioidosis and 133 selected subjects for Q fever. The manufacturer's recommended cutoff of ≥ 11 PanBio units was considered positive. These results were not used for diagnostic classification, as discussed below.

Clinical (nonetiologic) diagnoses were based on investigator review of charts after all information was compiled. For descriptive purposes, diagnoses were grouped. For example, all pharyngitis, sinusitis, and tonsillitis diagnoses were included in upper respiratory infection; all influenza-like illnesses, viral syndromes, "rule out" or presumptive diagnoses without confirmatory laboratory findings were counted as fever not specified. Cases counted as lower respiratory infection had abnormal chest x-ray or were classified as pneumonia or bronchitis by the admitting or reviewing physician. Gastroenteritis included subjects who presented with nausea and vomiting or diarrhea.

Analysis. Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 12.0 for Windows (Chicago, IL). Clinical predictors of specific etiologic diagnoses were analyzed using Pearson χ^2 coefficients for dichotomous variables, or Fisher's exact test. A nonparametric Mann-Whitney rank sum test was used for continuous variables. Multivariate logistic regression was performed for variables associated with diagnoses at a significance level of $P < 0.1$. Variables significant at a level of 0.05 after regression were reported as independent predictors of etiologic diagnosis.

RESULTS

Six hundred thirteen (613) subjects were enrolled into the study from June 1999 to March 2002. Demographic data are shown in Table 1. Follow-up specimens were obtained from 530 (86%) of subjects at a median of 21 days after enrollment. Nineteen percent of subjects were inpatients.

Etiologic diagnoses. Table 2 shows combined etiologic and clinical diagnoses ($N = 613$). A specific etiologic diagnosis was made for 294 (48%) of subjects. Malaria was the most frequent etiology, with 25.3% of subjects found to be positive for malaria parasites on enrollment. *Plasmodium falciparum* accounted for 61% of malaria cases.

Leptospirosis was the next most frequent etiologic diagnosis, followed by rickettsial infection. All cases of dengue were diagnosed during an outbreak in 2001. Most of the dengue cases (8 of 9) were secondary infections. Seven of the nine cases were positive for dengue viral RNA by nested PCR, including one case also seropositive for leptospirosis. One case of Japanese encephalitis was diagnosed (included in "other" in Table 2). A diagnosis of typhoid fever was made in five cases, three by PCR and two on the basis of surgical

TABLE 1
Demographic data ($N = 613$)

Characteristic	Number (%)
Age (years), range	Median 38, 20–87
Male	325 (53)
Inpatient	118 (19)
Deaths	8 (1)
Ethnicity	
Karen	320 (52)
Mon	120 (20)
Thai	112 (18)
Burmese	41 (7)
Other/not specified	12 (2)
Occupation	
Farmer/forestry	224 (37)
Housewife/housekeeper	77 (13)
Hired worker not specified	79 (13)
Shop owner	34 (6)
Other	199 (33)

TABLE 2
Diagnoses overall ($N = 613$)

Diagnosis	Number of cases	Percent* (%)
Malaria total	155	25.3
P. falciparum	(95)	(15.5)
P. vivax	(48)	(7.8)
Mixed or other	(12)	(2.0)
Fever not specified	153	25.0
Leptospirosis	107	17.5
Lower respiratory infection	64	10.4
Upper respiratory infection	57	9.3
Rickettsiosis total	36	5.9
Spotted fever group (SFG) rickettsiosis	(20)	(3.3)
Murine typhus (*R. typhi*)	(9)	(1.5)
Scrub typhus (*O. tsutsugamushi*)	(7)	(1.1)
Gastroenteritis	16	2.6
Pyelonephritis/urinary tract infection	13	2.1
Dengue fever†	9	1.5
Japanese encephalitis	1	0.2
AIDS	7	1.1
Typhoid	5	0.8
Pulmonary tuberculosis (PTB)	7	1.1
Other	17	2.8

* Total is > 100% due to dual diagnoses.
† All Dengue cases occurred between March and August 2001.

findings of ilial perforation. One of these cases was also seropositive for SFG rickettsiosis.

Of the 44 cases screened for serologic evidence of melioidosis by IgG ELISA, 7 had a rise in titer from negative (< 9 PanBio units) to positive (> 11 PanBio units). Six (6) cases were serofast positive. Of the 133 cases screened for serologic evidence of Q fever by IgM ELISA, 14 were positive (> 11 PanBio units) on either an acute or convalescent specimen or both. One case showed a rise in titer from negative to positive, although 90 of these cases had convalescent titers only. These results were not felt to be convincing evidence of acute infection and were not included in the etiologic diagnoses.

Clinical diagnoses. For clinical diagnoses, fever not specified was most common, followed by respiratory infections, gastroenteritis, and urinary tract infections. Of the gastroenteritis cases, the most frequent presentation was diarrhea (12 of 16).

Of the eight deaths in the cohort, two were positive for typhoid by PCR, one was seropositive for leptospirosis, two had clinical end-stage acquired immune deficiency syndrome (AIDS), two were fever not specified, and one had clinical hepatitis with no etiology identified.

Dual etiologic diagnoses. Table 3 shows the numbers of cases with apparent dual diagnoses. Twenty-six (26) of 155 (16.8%) smear-positive malaria cases had laboratory evidence of a second infection, most commonly leptospirosis. Twenty-eight (28) of 107 (26%) leptospirosis cases had evidence of a second infection, most commonly malaria. Evidence of dual infection was also seen for rickettsial infections (4 of 36, 11%) and for dengue (2 of 9, 22%).

TABLE 3
Apparent dual diagnoses

Diagnoses	Number
Malaria and leptospirosis	22
Malaria and rickettsiosis	2
Malaria and other (dengue, PTB)	2
Leptospirosis and rickettsiosis	4
Leptospirosis and other (dengue, PTB)	2
Rickettsiosis and other (typhoid, PTB)	2
Total	34

PTB, pulmonary tuberculosis.

Clinical correlations for etiologic diagnoses. Cases of malaria (grouped and *P. falciparum* only), leptospirosis, rickettsiosis (grouped), and dengue fever were compared with the remainder of the cohort to look for differences in age, sex, outpatient versus inpatient status, symptoms at presentation (documented fever, presence of rash, complaints of headache, myalgia, cough, and abdominal pain), and presence of leukopenia (white blood cells $< 4.0 \times 10^3/\mu L$), anemia (hematocrit < 35%) thrombocytopenia (platelets $< 150{,}000 \times 10^3/\mu L$), elevated BUN (> 20 mg/dL), elevated creatinine ($\geq$ 1.6 mg/dL), elevated ALT (> 45 U/L), and elevated GGT (> 76 U/L). Associations were examined including and excluding dual diagnosis cases. Results for all associations and for those significant after logistic regression are shown in Table 4.

For malaria overall, associations were seen for several hematologic and clinical variables. After regression, results remained significant for outpatient status, documented fever, and thrombocytopenia. Results were similar when *P. falciparum*–only cases were examined, but with fewer variables reaching statistical significance.

The only significant association for leptospirosis with the above variables was elevated ALT, which lost significance after regression was performed. For rickettsial diagnoses, significance was seen for age, with cases being slightly older than noncases, for the presence of rash, and for elevated GGT. However, the increased likelihood of rash is likely due to confounding as rash was one of the diagnostic criteria by which cases were chosen for serologic testing. No associations were seen for dengue fever, although the small number of cases ($N = 9$) makes statistical significance unlikely. For all diagnoses, results were similar when dual diagnosis cases were excluded (not shown).

DISCUSSION

As expected in this malaria-prevalent area, *Plasmodium* infection was the most common diagnosis among subjects, with *P. falciparum* being predominate. Malaria diagnosis was based on expert microscopy, and all patients enrolled were screened. Thus, the number of malaria diagnoses is likely to accurately reflect the true number of cases.

Leptospirosis was the next most frequent etiologic agent, accounting for 17.5% of cases overall. As with malaria, all subjects enrolled were screened, although subjects who did not follow up were less likely to be diagnosed due to lack of a convalescent specimen. Diagnosis of leptospirosis relied on serology. The MAT cutoffs used to define positive cases are the more stringent levels typically used in endemic regions (i.e., a titer of 800 rather than 200 for single sera). However, MAT titers can sometimes remain elevated for long periods after infection, so some proportion of the cases identified may be false positives. Conversely, treatment with antibiotics can blunt the immune response in leptospirosis, reducing the number of cases detectable by serology.[16] As results of this study became available, clinicians at the hospital were more likely to treat for suspected leptospirosis, which had previ-

TABLE 4
Associations between variables seen at presentation and etiologic diagnosis

Diagnosis	Variable*	Cases vs. rest of cohort (%)	Independent predictor *P* value	Odds ratio (95% CI)
Malaria all ($N = 155$)	Outpatient	86 vs. 79	< 0.001	3.2 (1.7–6.0)
	Age	36 vs. 39		
	Male sex	61 vs. 50		
	Temp ≥ 38°C	64 vs. 44	0.001	2.3 (1.4–3.8)
	WBC < 4.0	18 vs. 6		
	Platelets < 150,000	81 vs. 21	< 0.001	17.2 (10.4–28.5)
	Creatinine > 1.6	2 vs. 7		
	Cough	45 vs. 55		
	Headache	95 vs. 86		
Malaria pf only ($N = 96$)	Temp ≥ 38°C	66 vs. 46	0.006	2.1 (1.2–3.5)
	Platelets < 150,000	80 vs. 29	< 0.001	8.8 (5.1–15.5)
	Age	35 vs. 39		
	WBC < 4.0	22 vs. 7		
	HCT ≤ 35	35 vs. 24		
Leptospirosis ($N = 107$)	ALT > 45	40 vs. 29		
Rickettsioses all ($N = 36$)	Age	42 vs. 38		
	Rash	14 vs. 3		
	GGT > 76	36 vs. 19		

pf, *Plasmodium falciparum;* Temp, temperature; WBC, white blood cell count; HCT, hematocrit; ALT, alanine transferase; GGT, gamma glutamyl transferase.
* Variables listed were significant at the 0.05 level before regression. Variables with confidence intervals shown were also significant after regression; *P* values are above.

ously not been known to be common in the area. Leptospirosis is an increasingly frequently recognized cause of unspecified febrile illness in Southeast Asia.[17] During the late 1990s, Thailand experienced a 30-fold increase in reported leptospirosis cases, with the majority of cases reported in the northeast region.[18,19] In a recent report of an investigation of a dengue outbreak in Bangladesh, leptospirosis was found to be present in 18% of dengue-negative cases and to have a higher mortality rate than dengue.[20] Other studies of febrile illness have also found unexpectedly high rates of leptospirosis in Thailand and Nepal.[9,11]

Dengue fever was episodic and did not account for many cases. Both Japanese encephalitis and dengue fever are more frequent in childhood, with adults often immune. Consistent with this, most dengue cases that were seen were secondary rather than primary infections. Diagnosis required paired specimens, so some cases of dengue may have been missed. Serologic diagnosis was confirmed by isolation of viral RNA in 7 of 9 cases, including one also diagnosed with leptospirosis by MAT.

Rickettsioses, particularly scrub typhus, were thought to be common in the area prior to the study and were diagnosed in 36 patients overall. Data on 15 of these cases is reported separately.[14] However, only 46 patients were screened for rickettsial illness, and this number is likely to underestimate the true number of infections. SFG rickettsiosis, previously undescribed in the area, was more than twice as common as scrub or murine typhus among the cases tested. The agent for scrub typhus, *Orientia tsutsugamushi*, is well described in Southeast Asia.[21] However, several other rickettsial agents, including tick-borne spotted fever group (SFG) rickettsiae and related organisms, may also exist or are yet to be discovered.[14,22]

The number of typhoid cases is likely to be underestimated, as blood cultures were not done and diagnosis relied on surgical findings of ilial perforation or molecular diagnosis done on selected cases. A reliable serologic test for typhoid is not currently available.[23] The true numbers of tuberculosis and HIV/AIDS cases are also likely to be higher than shown, as these diagnoses rely on tests ordered by the attending physician on a case by case basis, and not all subjects enrolled were screened for these diseases. The fact that 4 of 8 deaths reported were due to typhoid and HIV/AIDS suggests that these infections were more common than was documented. In addition, extrapulmonary TB would be missed by our strict case definition.

Results of serologic testing for melioidosis and Q fever were not used for diagnostic classification. Accurate diagnosis of melioidosis requires isolation of the pathogen *(Burkholderia pseudomallei)*, as serology is not reliable.[24,25] However, it seems likely that melioidosis is a cause of illness in this population, with a high incidence of IgG seropositivity among the cases tested and with a rise in titer shown in several cases. As this part of Thailand has not been regarded as a melioidosis-prevalent area, confirmation of the presence of the disease is critical, particularly as specific therapy with ceftazidime is required for effective therapy, particularly for diabetic or immunocompromised hosts.[26] The serologic evidence for the presence of Q fever is not as strong, as only one case was shown to have a rise in titer. In general, interpretation of serologic tests in a population where the prevalence of infection is unknown is problematic. Antibodies from previous infection may persist, and cross-reactivity is common, particularly for poorly characterized emerging infections.[27,28]

Another important finding of the study is the high apparent frequency of coinfection, particularly for malaria and leptospirosis, both with each other and with other infectious agents. Despite the use of reference laboratory standards and strict criteria for diagnoses, 34 cases met criteria for two infections. Details on seven likely malaria-leptospirosis coinfections from this study are reported elsewhere.[29] However, infection with malaria parasites often causes a polyclonal amplification of the immune response and may confound results of serologic testing.[30,31] Isolation of leptospires or rickettsial agents in patients with a positive malaria smear would be useful to confirm these findings. Possible coinfection between leptospirosis and scrub typhus has also been reported in Thailand.[32] A study of febrile illness in Nepal showed 5 out of 36

likely leptospirosis cases had positive blood cultures for *S. typhi* or *S. paratyphi.*[9] Coinfection may be common, and the clinical assumption that only one etiologic agent is responsible for a given illness may not hold true in these settings. In an environment where exposure to multiple pathogens is common, patients not responding to treatment of a particular infection or those in whom the presentation is atypical or severe should be suspected of harboring a second infectious agent.

After logistic regression, significant clinical associations were seen for malaria only, partly because malaria was the most common diagnosis and numbers are large, reducing Type II error. Yet the number of leptospirosis cases is also large, and if clear clinical patterns were present, they should have been statistically significant. These results demonstrate the difficulty of making a clinical diagnosis of leptospirosis, which presents with nonspecific findings such as headache, cough, and myalgia. Clinical presentations of all of the infectious agents identified in this population overlap considerably.

Intensive surveillance to determine etiologic causes of febrile illness in underdeveloped regions with unique environments provides both public health and local community benefits. Study findings have been presented to the Sangkhlaburi medical community and have been used to modify treatment practices in the area. For patients with nonspecific febrile illness who are malaria smear negative, doxycycline is now the antibiotic of choice for patients ill enough to require treatment, as it effectively treats leptospirosis, scrub and murine typhus, and spotted fever group rickettsiosis, as well as most community-acquired pneumonia. The drug is inexpensive and readily available. In addition, clinicians now have a heightened suspicion of the presence of dual infection in patients not responding to treatment.

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Authors' addresses: Ruth D. Ellis, 2530 N. 24th Street, Arlington, VA 22207, Telephone: 703-350-1936, E-mail: schwartzellis@hotmail.com. Mark M. Fukuda, Nichapat Uthaimongkol, Nillawan Buathong, Sabaithip Sriwichai, Kritsanai Yingyuen, Chaiyawat Mathavarat, and Robert S. Miller, Department of Immunology and Medicine, Armed Forces Research Unit of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Bangkok 10400, Thailand. Ananda Nisalak, Department of Virology, AFRIMS, 315/6 Rajvithi Road, Bangkok 10400, Thailand. Rungnapha Phasuk, Department of Enterics, AFRIMS, 315/6 Rajvithi Road, Bangkok 10400, Thailand. Clinton K. Murray, Department of Medicine (MCHE-MDI), Brooke Army Medical Center, 3851 Roger Brooke Drive, Fort Sam Houston, TX 78216. Michael R. Gray, VETCOM Leptospirosis Reference Laboratory, Fort Sam Houston, TX 78216. Philip McDaniel and Katherine Welch, 2140 College Ave SE, Grand Rapids, MI 49507.

Reprint requests: Mark M. Fukuda, Department of Immunology and Medicine, Armed Forces Research Unit of Medical Sciences (AFRIMS), 315/6 Rajvithi Road, Bangkok 10400, Thailand, Telephone: 66-2-644-5775, Fax: 66-2-644-5775, E-mail: mark.fukuda@afrims.org.

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