Kinetics of Antibody Responses in *Rickettsia africae* and *Rickettsia conorii* Infections

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African tick-bite fever, caused by *Rickettsia africae*, is the most common tick-borne rickettsiosis in sub-Saharan Africa. Mediterranean spotted fever due to *Rickettsia conorii* also occurs in the region but is more prevalent in Mediterranean countries. Using microimmunofluorescence, we compared the development of immunoglobulin G (IgG) and IgM titers in 48 patients with African tick-bite fever and 48 patients with Mediterranean spotted fever. Doxycycline treatment within 7 days from the onset of disease significantly prevented the development of antibodies to *R. africae*. In patients with African tick-bite fever, the median times to seroconversion with IgG and IgM were 28 and 25 days, respectively, after the onset of symptoms. These were significantly longer by a median of 6 days for IgG and 9 days for IgM than the times for seroconversion in patients with Mediterranean spotted fever (*P* < 10−3). We recommend that sera collected 4 weeks after the onset of signs of patients with suspected African tick-bite fever should be used for the definitive serological diagnosis of *R. africae* infections.

*Rickettsia africae* is the agent of African tick-bite fever, a tick-transmitted disease which was long mistaken for Mediterranean spotted fever caused by *Rickettsia conorii*. African tick-bite fever is a common disease in sub-Saharan Africa (7), where it occurs in people in rural areas when they come into contact with *Amblyomma* spp., ticks of cattle and game (4). Two cases of African tick-bite fever have also been reported from Guadeloupe (French West Indies) (6). In contrast to *Rhipicephalus* ticks, which transmit *R. conorii* and are very host specific, *Amblyomma* ticks feed readily on people who may frequently become infested with large numbers of these ticks. Consequently, African tick-bite fever often occurs in groups of people and is characterized by multiple inoculation eschars (2, 7). Mediterranean spotted fever, however, most often occurs as sporadic cases and patients present with a single eschar. *R. africae* also causes only mild clinical signs, and frequently there is no rash. When a rash is present, it is vesicular in half the patients and maculopapulous in the other half (7). Currently, the routine laboratory diagnosis of these diseases is based on serology, in particular microimmunofluorescence (MIF). The test is, however, limited by serological cross-reactivity between the spotted fever group rickettsiae and, at times, the test may not be able to identify the species causing an antibody response. Recently, we reported 119 cases of *R. africae* infection, the largest series to date, which enabled us to validate serological methods for the diagnosis of African tick-bite fever (7). We demonstrated that a specific diagnosis could be made when the immunoglobulin G (IgG) and IgM titers to *R. africae* were fourfold higher than against other species. Specific antibody responses could also be demonstrated by using Western blotting or cross-adsorption of sera.

We describe here the antibody responses to *R. africae* in 48 patients with African tick-bite fever and compare these to those against *R. conorii* in 48 patients with Mediterranean spotted fever. In addition, we estimated the influence of early doxycycline therapy on the development of anti-*R. africae* antibodies.

**MATERIALS AND METHODS**

**Case definition.** African tick-bite fever was diagnosed in patients if they met one of the following criteria: (i) evidence of direct evidence of *R. africae* infection by culture and/or PCR; (ii) both clinical symptoms consistent with African tick-bite fever, such as multiple inoculation eschars, a vesicular rash, and/or similar symptoms among other members of the same group of travelers coming back from an area where such infections are endemic (i.e., sub-Saharan Africa or the French West Indies), and positive serology against spotted fever group rickettsiae; and (iii) both clinical symptoms consistent with a spotted fever group rickettsiosis, such as fever, cutaneous rash, and/or eschar(s) after travel to sub-Saharan Africa or the French West Indies, and serology specific for a recent *R. africae* infection (with Western blot and/or cross-adsorption showing antibodies specific for *R. africae*). We did not include in our study patients in whom the diagnosis of *R. africae* infection was made based on the presence of levels of antibodies to *R. africae* that were greater than those to *R. conorii* by at least two dilutions since this criterion is also part of the question being investigated. A diagnosis of Mediterranean spotted fever was made in patients who had a diagnostic score of >25 as described elsewhere (8). Briefly, patients had such a score if they presented with a single IgG titer determined by MIF of ≥1:128 and/or an IgM titer of ≥1:64 and showed two of the following symptoms: fever, “tache noire,” or rash. Patients also had such a score if they only had one of the signs but also seroconverted or showed a fourfold increase in antibody titers after traveling in a Mediterranean area in summer.

**Patient selection.** For our study we selected patients from our laboratory databank. Patients with African tick-bite fever were selected if there were acute- and convalescent-phase sera available and if the attending physician had recorded the date of onset of signs and the treatment given. Patients with Mediterranean spotted fever were selected if both acute- and convalescent-phase sera were available.
Serology. R. conorii strain Seven (Malish, ATCC VR-613T) and R. africae strain ESF-5 (provided by G. Dasch) were grown in Vero cell monolayers of 150-cm² tissue culture flasks. Heavily infected cells (5 days postinoculation) were harvested with sterile glass beads and pelleted by centrifugation at 10,000 rpm for 15 min. For MIF, the pellets were resuspended in sterile distilled water so that each suspension had the same density of organisms as determined optically after Gimenez staining. These antigens were applied with a drawing pen at opposing poles of each well on 30-well microscope slides (Dynatech Laboratories, Ltd., Billingham, United Kingdom), air dried, and fixed in acetone for 10 min. Twofold serial dilutions of the sera from 1:8 to 1:4,096 were made in phosphate-buffered saline (PBS) with 3% nonfat powdered milk, applied to the antigens, and incubated in a moist chamber for 30 min at 37°C. After three 10-min washes in PBS, the slides were air-dried and the reactive antibodies detected with 1:300 dilutions of fluorescein isothiocyanate-conjugated goat anti-human IgG (Fluoline G; BioMerieux, Marcy l’Etoile, France) or anti-human IgM (Fluoline M; BioMerieux) by using incubation times and washing procedures as described above. The slides were mounted in buffered glycerol (Fluoprep; BioMerieux) by using incubation times and washing procedures as described above. The fluorescein was visualized with a Zeiss fluorescence microscope at ×400 magnification. Prior to IgM determination, rheumatoid factor absorbent (RF-Absorbent; Behring, Mannheim, Germany) was used to remove IgG.

The interval (in days) between the onset of clinical signs and the date of blood sampling was calculated for each patient. We regarded acute-phase sera as sera collected up to the 14th day from onset, when clinical signs were present, whereas convalescent-phase sera were sera collected after that point. Also for each patient, we calculated the median delay before IgG and IgM seroconversion to R. africae or R. conorii.

Influence of antibiotic therapy. To determine the effects of doxycycline on the development of antibodies to R. africae when administered to patients in the early stages (≤7 days of clinical signs) of African tick-bite fever, we compared treatments given to patients who did seroconvert and those who did not.

Statistical tests. Slopes of linear regression curves were compared as variances by using the Student’s t test. Medians were compared by using the Wilcoxon-Mann-Whitney test. Observed differences were considered significant when the P value was determined to be <0.05 by two-tailed tests.

RESULTS

Acute- and convalescent-phase sera were available for 65 patients with African tick-bite fever in our database who fulfilled the diagnostic criteria described in Materials and Methods. Seventeen patients had negative serology results but were PCR and/or culture positive for R. africae. Of these, 14 (82%) had received doxycycline before the seventh day of clinical signs: 2 on the first day, 2 on the second day, 4 on the third day, 2 on the fourth day, 2 on the fifth day, 3 on the sixth day, and 2 on the seventh day. By comparison, only 31.2% (15 of 48) of patients who seroconverted had received early doxycycline treatment (P < 10⁻²). Of these 15 patients, 1 on the first day of symptoms, 3 were treated on the second day, 2 were treated on the third day, 5 were treated on the fourth day, 1 was treated on the fifth day, and 3 were treated on the seventh day. We only included the 48 patients with positive serology in our comparative study. Seventeen patients were diagnosed as having African tick-bite fever by criterion i, 29 by criterion ii, and by criterion iii (see Materials and Methods). Cross-reactions between R. africae and R. conorii were observed with the sera of all patients with positive serology and all controls. In patients with African tick-bite fever, 21 of 48 (43.7%) had IgG and IgM titers to R. africae greater than those to R. conorii by at least two dilutions. Such differences in antibody titers were observed in the convalescent-phase sera from all 21 patients but only in the acute-phase sera from 3 patients. Similar findings were observed in patients with Mediterranean spotted fever, with 24 of 48 (50%) having IgG and IgM titers to R. conorii at least two dilutions greater than those to R. africae in convalescent-phase sera. Five of these patients also had four-fold-higher titers to R. conorii in their acute-phase sera.

The medians of the antibody titers each week after the onset of signs are shown in Fig. 1. In acute-phase sera, the IgG and IgM titers to R. africae in the 48 patients with African tick-bite fever and positive serology ranged from <1:8 to 1:16 and from <1:8 to 1:64, respectively. The IgG and IgM titers in the patients with African tick-bite fever against R. conorii were from <1:8 to 1:16 and from <1:8 to 1:32, respectively. In convalescent-phase sera, IgG and IgM titers to R. africac ranged from 1:16 to 1:1024 and from 1:32 to 1:512, respectively, whereas the IgG and IgM titers to R. conorii ranged from <1:8 to 1:1024 and from <1:8 to 1:512, respectively. In the acute-phase sera of the 48 patients with Mediterranean spotted fever, IgG and IgM titers to R. conorii ranged from <1:8 to 1:64 and from <1:8 to 1:128, respectively, whereas those to R. africac ranged from <1:8 to 1:64 and from <1:8 to 1:64, respectively. In the convalescent-phase sera the IgG and IgM titers to R. conorii ranged from 1:8 to 1:1024 and from 1:16 to 1:2048, respectively, and those to R. africac varied from <1:8 to 1,024 and from 1:16 to 1:1,024, respectively.

Seroconversion with IgM to R. africac in African tick-bite fever patients occurred between the second (12.5% of patients) and seventh (100% of patients) weeks after the onset of clinical signs (Fig. 2). Seroconversion with IgM to R. conorii in Mediterranean spotted fever patients occurred earlier, however, with 12.5% of patients developing antibodies in the first week after the onset of clinical signs and 100% of patients developing antibodies by the fifth week (P < 10⁻²). Seroconversion with IgG occurred between the third (14.5% of patients) and the seventh (100% of patients) weeks in patients with African tick-bite fever (Fig. 2), whereas in patients with Mediterranean spotted fever, it occurred between the first (6.2% of patients) and fifth (100% of patients) weeks (P < 10⁻²). When we compared the median delay before seroconversion, we found that in African tick-bite fever patients the median delay before IgM seroconversion (quartile 25% to quartile 75% was 25 (range, 10 to 34) days in African tick-bite fever versus 16 (range, 4 to 22) days in Mediterranean spotted fever patients (P < 10⁻²). The median delay before IgG seroconversion (quartile 25% to quartile 75%) was 28 (range, 16 to 34) days. This was significantly longer than the 22 (range, 10 to 28) days observed for Mediterranean spotted fever patients (P < 10⁻³). Among the 48 patients with African tick-bite fever, we observed similar antibody kinetics in the 15 patients who had received early doxycycline therapy and the 33 patients who had not.

DISCUSSION

Recently, we showed that in patients that were PCR or culture positive for R. africac, a combination of MIF analyses using titers to R. africac greater than those to R. conorii by two or more dilutions (titers to both antigens being read on the same spot), Western blot showing antibodies specific for R. africac, and cross-adsorption removing only antibodies to R. conorii had a sensitivity of 0.56, but each test had a positive predictive value of 1.0 (7). By using these serological criteria, we could diagnose an additional 80 patients as being infected with R. africac. However, among the 39 proven patients, MIF
alone had a sensitivity of 0.26 (10 of 39 patients), which allowed us to observe that antibody titers to \textit{R. africae} determined by MIF, the reference method for the serological diagnosis of rickettsioses, were lower during early stages of the disease than those used for Mediterranean spotted fever caused by \textit{R. conorii} (7). Therefore, in order to estimate the kinetics of antibodies to \textit{R. africae} during African tick-bite fever, we compared the MIF results of 48 patients infected with \textit{R. africae} and with those of 48 control patients infected with \textit{R. conorii}.

Although the kinetics of antibody responses in patients with Mediterranean spotted fever have been described (D. Raoult, S. Rousseau, B. Toga, H. Chaudet, J. Tamalet, and P. de Micco, Letter, Acta Virol. 29:516–518, 1985), there is little such data on patients with African tick-bite fever. In 17 of the 65 patients with African tick-bite fever that we studied, both

FIG. 1. (A) Comparative kinetics of IgM antibodies to \textit{R. conorii} (gray) and to \textit{R. africae} (gray cross-hatch) in Mediterranean spotted fever patients and to \textit{R. africae} (white) and to \textit{R. conorii} (black cross-hatch) in African tick-bite fever patients. The standard deviation for each median titer is shown. (B) Comparative kinetics of IgG antibodies to \textit{R. conorii} (gray) and to \textit{R. africae} (gray cross-hatch) in Mediterranean spotted fever patients and to \textit{R. africae} (white) and to \textit{R. conorii} (black cross-hatch) in African tick-bite fever patients. The standard deviation for each median titer is shown.
the acute-phase and the convalescent-phase sera were negative for antibodies reactive with *R. africae* by MIF. Fourteen of these seronegative patients had received doxycycline in the first week of clinical disease and, as for the other spotted fever group rickettsiae, *R. africae* is highly sensitive to the drug (9). We suspect, then, that the elimination of viable *R. africae* from the body early in the course of African tick-bite fever prevents the development of detectable titers of reactive antibodies.

The kinetics of IgG and IgM responses to *R. conorii* we observed in patients with Mediterranean spotted fever were similar to those described previously (1). When we compared these responses to those mounted against *R. africae*, we found that seroconversion was significantly delayed in patients with African tick-bite fever by a median of 6 days for IgG (range of 3 to 7 weeks versus 1 to 5 weeks) (*P* < 10\(^{-5}\)) and 9 days for IgM (range of 2 to 7 weeks versus 1 to 5 weeks) (*P* < 10\(^{-5}\)). This delay in immune response was not due to the administration of doxycycline in early stage of the disease. A limitation of the MIF test is that it is subjective, and thus different readers of slides may report different end titers. To avoid variations in

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**FIG. 2.** (A) Cumulative percentage of IgM seroconversion against *R. conorii* (gray curve) and *R. africae* (black curve) in patients with African tick-bite fever. (B) Cumulative percentage of IgG seroconversion against *R. conorii* (gray curve) and *R. africae* (black curve) in patients with African tick-bite fever.
the determination of antibody titers to *R. conorii* and *R. africae* in our study, both antigens were applied at the same density to opposite poles of each well of our MIF slides so that titers could be read simultaneously. Moreover, all antibody titers were determined by only one person. With these precautions we observed that, while graphs of the antibody responses in both diseases were very similar, the antibody responses in African tick-bite fever patients were delayed significantly compared to those in Mediterranean spotted fever patients. Additionally, serological cross-reactions are common among members of the spotted fever group rickettsiae (3), but in 46% of patients with African tick-bite fever and in 52% of patients with Mediterranean spotted fever we found fourfold-higher titers against the rickettsia causing the disease. This was especially apparent with sera collected later in the course of the disease. Usually, for the serological diagnosis of rickettsioses it is recommended that a serum sample be collected early in the disease and a second sample be obtained 2 weeks later (1). Our study shows, however, that in patients with African tick-bite fever this delay between the two specimens may be insufficient to enable rising antibody titers or seroconversion to be detected. Instead, we believe that the second sample should be collected after at least 4 weeks. However, a limitation of this late serology is that all patients may not be willing to provide a late-phase serum sample, especially because African tick-bite fever is a benign disease. Moreover, since Mediterranean spotted fever may be contracted in areas where African spotted fever is present, serology may not be able to distinguish between the two diseases, especially in patients with nonspecific symptoms. Determining the reasons for the delayed antibody response requires further investigation, but it may result from differences in virulence between the causative agents. Indeed, *R. africae* causes a far milder disease than that caused by *R. conorii* (7), and the considerable variation in the severity of rickettsioses has been linked to the relative virulence of the infecting species (5).

In conclusion, our study has shown that seroconversion with both IgG and IgM may not occur when doxycycline is given to patients with African tick-bite fever early in the course of the disease. Where seroconversion does occur, it is significantly later in African tick-bite fever patients than in Mediterranean spotted fever patients. Specific antibodies to *R. africae* develop in the sera of patients in the later stages of African tick-bite fever, and these may aid in the definitive diagnosis of *R. africae* infections.

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