Systemic Inflammatory Responses in African Tick-Bite Fever

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Information regarding the inflammatory response in African tick-bite fever (ATBF), an emerging spotted-fever-group rickettsiosis, in international travelers to sub-Saharan Africa, is scarce. Plasma/serum levels of von Willebrand factor (vWF), soluble (s) E-selectin, tumor necrosis factor-α, interleukin (IL)–6, interferon-γ, IL-10, IL-13, IL-8, RANTES, macrophage inflammatory protein–1α, and C-reactive protein were studied, at both first presentation and follow-up, in 15 patients with travel-associated ATBF and in 14 healthy travelers who served as control subjects. Our main and novel findings are the following: (1) patients with ATBF had increased levels of vWF and sE-selectin, with a subsequent decrease at follow-up; (2) with the exception of IFN-γ, levels of cytokines and chemokines were also increased in these patients at the first presentation; and (3) IL-10 and IL-13 tended to increase during follow-up, whereas most of the inflammatory cytokines decreased. The induction of these mediators and the balance between them may be critical both for the regulation of inflammation and for protective immunity in ATBF.

African tick-bite fever (ATBF) is an acute flulike illness frequently accompanied by multiple inoculation eschars, regional lymphadenitis, aphthous stomatitis, and vesicular cutaneous rash. ATBF is caused by Rickettsia africae, a newly identified spotted-fever-group (SFG) rickettsia, and is transmitted by ticks of cattle and wild game, of the Amblyomma genus, in rural sub-Saharan Africa [1]. Paralleling the rapid expansion of international safari tourism, notably to southern Africa, the incidence of travel-associated ATBF has recently increased significantly in Europe and elsewhere. Characteristically, travel-associated ATBF frequently occurs in clusters that may affect up to 100% of exposed subjects [2].

The pathophysiological hallmark of SFG rickettsioses comprises infection of endothelial cells and subsequent perivascular infiltration of T cells and macrophages, resulting in vasculitis [3]. Although complex alterations in the cytokine profile, accompanied by T cell activation, have been described during the 2 potentially most severe forms of SFG rickettsioses—Mediterranean spotted fever (MSF), caused by Rickettsia conorii, and Rocky Mountain spotted fever (RMSF), caused by Rickettsia rickettsii [4]—little is known about the inflammatory response in ATBF. However, the milder clinical course and the frequent lack of skin rash suggest that underlying processes of ATBF may be different from those of MSF and of RMSF.

To identify systemic inflammatory responses that possibly have clinical and pathological importance in ATBF, we examined circulating levels of von Willebrand factor (vWF) and soluble (s) E-selectin (both of which are parameters of endothelial-cell activation), as well as circulating levels of various inflammatory and anti-inflammatory cytokines and inflammatory chemokines. The balance between inflammatory and anti-inflammatory cytokines seems to be important in the pathogenesis of several infectious disorders, such as human immunodeficiency virus infection and malaria. Moreover, chemokines play a major role in the direction of leukocytes into inflamed tissue and in their activation there, and, accordingly, these mediators could play a pathogenic role in infectious disorders characterized by endothelial-cell activation, such as rickettsiosis.

Subjects, materials, and methods. Fifteen consecutive patients—3 women and 12 men, 20–57 (mean 38.7) years old—diagnosed with serologically verified ATBF at an outpatient clinic (Aker University Hospital, Oslo) were included in the study. The 2 main purposes of travel by patients were game hunting (n = 11) and leisure safari (n = 4). Thirteen of the cases occurred in clusters. All patients presented with flulike symptoms that manifested within the first 10 days after they had left rural areas in South Africa or Botswana; 11 patients had inoculation eschars, 12 had regional lymphadenitis, 3 had vesicular cutaneous rash, and 5 had aphthous stomatitis.
complicated course was recorded in 1 patient, a 44-year-old woman who developed reactive arthritis. Antirickettsial therapy with doxycycline was instituted in 12 case subjects. Fourteen healthy travelers >18 years old who had returned to Norway from sub-Saharan Africa during the preceding 7 days served as control subjects.

Blood was collected from patients with ATBF, both at first presentation, 1–7 (mean 3.8) days after symptom onset, and at follow-up, 11–21 (mean 17.4) days after symptom onset. Peripheral venous blood was drawn into pyrogen-free blood-collection tubes, either without any additives (in the case of serum) or with EDTA as anticoagulant (in the case of plasma). The tubes were immediately immersed in melting ice and were centrifuged, either within 30 min (in the case of plasma) or 1 h (in the case of serum), for 10 min at 3000 g. Plasma and serum were stored at −80°C.

vWF antigen was determined in plasma samples, by an IL ACL Futura system (IL Test vWF; Viale Monza). Plasma levels of interleukin (IL)-6, IL-8, IL-10, interferon-γ (IFN-γ), and macrophage inflammatory protein–1α (MIP-1α), and serum levels of RANTES and sE-selectin were measured by enzyme immunoassays (EIAs) (R&D Systems). Plasma levels of tumor necrosis factor–α (TNF-α) and of IL-13 were measured by EIAs from BioSource Europe and from Bender Medsystems, respectively. Serum C-reactive protein (CRP) was measured by a nephelometer. To minimize run-to-run variability, all samples from a given patient were analyzed in the same microtiter plate.

Microimmunofluorescence assay (MIF), the serological reference method, was performed as reported elsewhere [2], by use of both R. conorii strain Seven (Malish, ATCC VR-613T) and R. africae strain ESF-5 (provided by Dr. G. Dasch) as antigens. Titer ≥1/64, for IgG, and/or ≥1/32, for IgM, were considered evidence of recent infection by a rickettsia species. Western-blotting procedures and cross-adsorption for serological testing were performed as described elsewhere [5, 6]. We considered the following as definite serological evidence of R. africae infection: IgG plus IgM MIF in which titers of antibodies to R. africae were ≥2 dilutions higher than to those to R. conorii; a Western-blot profile that revealed only R. africae–specific antibodies; or cross-adsorption studies that demonstrated that the homologous antibodies were directed against R. africae [2].

Differences between groups were compared by the Mann-Whitney U rank-sum test. Within groups, differences were analyzed by the Wilcoxon rank-sum test. Relationships between variables were tested by Spearman’s rank-correlation test. The level of significance was set at P < .05.

Results. Vascular endothelial cells seem to be a primary target of SFG rickettsial infection. We therefore first analyzed levels of vWF and sE-selectin (both of which are markers of endothelial-cell activation [7]) in patients with ATBF and in control subjects. As shown in figure 1, the patients had vWF levels that, compared with those of the healthy control subjects, were significantly increased at first presentation and that were significantly decreased at follow-up. A similar pattern was seen for sE-selectin (figure 1).

In addition to endothelial-cell infection, a subsequent infiltration of leukocytes, resulting in vasculitis, seems to be a pathogenic hallmark of SFG rickettsioses. This migration and activation of cells within the vessel wall could involve chemokines and inflammatory cytokines. We therefore next analyzed plasma/serum levels of 3 inflammatory cytokines (TNF-α, IL-6, and IFN-γ), 1 anti-inflammatory cytokine (IL-10), 1 CXC-chemokine (IL-8), 2 CC-chemokines (RANTES and MIP-1α), and CRP (as an unspecific marker of inflammation), in patients with ATBF and in control subjects. As shown in figure 1, patients with ATBF had, at first presentation, significantly increased levels of TNF-α, IL-6, IL-10, IL-8, MIP-1α, RANTES, and CRP (with particularly high concentrations of TNF-α, IL-6, and CRP) compared with control subjects. At follow-up, several of these mediators—TNF-α, IL-6, RANTES, MIP-1α, and CRP—were significantly decreased, with a particularly marked decrease in TNF-α, IL-6, and CRP, which fell to levels comparable to those in control subjects. At follow-up, in contrast, levels of the anti-inflammatory cytokine IL-10 increased in 5 of 15 patients, but this trend was not statistically significant. A similar pattern, with an increase rather than a decrease, at follow-up, was also seen for the Th2-related cytokine IL-13. Notably, the highest level of plasma IL-10 (24.5 pg/mL, on day 11 after symptom onset) was observed in the patient with a complicated course (figure 1). IFN-γ was not detectable in either patients or control subjects (data not shown).

During follow-up, there was a tendency of significant correlation between changes in levels of vWF and changes in levels of IL-6 (r = 0.58; P < .08).

Discussion. Details of the inflammatory response during SFG rickettsial infection, which is believed to involve complex interactions between T cells, macrophages, natural killer cells, B lymphocytes, antibodies, cytokines, and chemokines, are not fully understood. Human in vivo studies are few, and data on circulating levels of inflammatory mediators are available for only a handful of diseases [4]. Herein we have reported data on the inflammatory response during ATBF, data that show, as the major findings, marked endothelial-cell involvement (as assessed by enhanced levels of vWF and sE-selectin) accompanied by a marked increase in inflammatory cytokines and chemokines.

Infection of the endothelium, with secondary endothelial-cell activation and inflammation, seems to play an important pathogenic role in SFG rickettsioses. In the present study, we have reported levels of vWF and sE-selectin, in patients with ATBF, that are increased during the acute phase and that are decreased at follow-up. An increase in plasma levels of vWF,
which has also been reported in patients with MSF [8], is thought to reflect persistent endothelial-cell activation, with increased release and synthesis of vWF triggered by microorganisms [7]. Although vWF may also be released from platelets upon activation, we and others have shown that platelets are not major contributors (<15%) to plasma levels of vWF [9]. Serum levels of sE-selectin, which has endothelial cells as its only cellular source, show a pattern similar to that of vWF, a finding that strongly suggests the presence of endothelial-cell activation during ATBF. Moreover, since vWF may foster platelet adhesion to damaged endothelium, the increased concentration of vWF may not only be a marker of endothelial-cell activation but also contribute to the development of thrombosis and vascular injury.
activation but may also augment the cascade of events leading to endothelial damage [7].

Increased plasma levels of inflammatory cytokines have been reported in some rickettsioses [10–12]. In the present study, we have shown that ATBF also is accompanied by a systemic increase of several inflammatory cytokines, with particularly high levels of TNF-α and IL-6. Even more important, at follow-up there tended to be a significant correlation between changes in vWF and changes in IL-6 levels, which is further support for the involvement of this cytokine in ATBF. However, although increased TNF-α levels could represent a protective immune response against intracellular pathogens [4], an inappropriate and persistent elevation of inflammatory cytokines could contribute to prolonged endothelial-cell activation and inflammation, which could possibly lead to prolonged clinical disease. The exact role that TNF-α and IL-6 play in ATBF and in other SFG rickettsioses will have to be further clarified. It has been suggested that IFN-γ plays an essential role in clearing rickettsial infection, a suggestion that may seem in conflict with our data. However, the lack of detectable IFN-γ levels in plasma of our patients does not exclude a role for this cytokine in ATBF.

Previous studies have reported that IL-8 expression in endothelial cells during in vitro infection with R. rickettsii and R. conorii is enhanced and also that CXCL9 and CXCL10 are expressed in livers of mice infected with R. conorii and in vascular lesions in the brains of case subjects with fatal RMSF [4, 13]. However, with the exception of a report on levels of circulating IL-8 in MSF [10], the literature is virtually devoid of in vivo data on chemokines in SFG rickettsioses in humans. In the present study, we have found that ATBF is associated with increased plasma levels of both CC-chemokines (RANTES and MIP-1α) and CXC-chemokines (IL-8), and, for the CC-chemokines, there was a significant decrease at follow-up. Interestingly, in R. conorii infection in vitro, RANTES activates the bactericidal function of human endothelium and of hepatocytes [14], and our findings may further support a role for chemokines in SFG rickettsioses.

Plasma levels of IL-10, a potent inhibitor of TNF-α and IL-6 secretion, were significantly increased in our patients with ATBF. However, in contrast to the decrease in inflammatory cytokines, IL-10 levels tended to be increased at follow-up. Previous studies of SFG rickettsioses suggest a peak activation of IL-10 at around day 12 after the onset of symptoms (when infection is considered well under control [4]), which possibly prevents an inappropriate inflammatory response. In contrast with the possible preventive effect of increased levels of IL-10, overproduction is associated with the development of poorly controlled rickettsial infection [15]. It is noteworthy that our only patient with complicated course had by far the highest plasma levels of IL-10 at follow-up. However, this finding must be interpreted with caution, and more patients must be examined before any firm conclusion can be drawn regarding the role that this anti-inflammatory cytokine plays in ATBF.

Thus, the present study has reported, for the first time, that ATBF inflammatory response comprises endothelial-cell activation accompanied by an up-regulation of several inflammatory cytokines and chemokines, and that there is a tendency toward a more delayed increase in the anti-inflammatory cytokine IL-10. The induction of these mediators and the balance between them may be critical both for the regulation of inflammation and for protective immunity in ATBF, and they may potentially explain some clinical and pathological features of this emerging tick-borne disease.

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References

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