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Increased endothelial and macrophage markers are associated with disease severity and mortality in scrub typhus

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KEYWORDS
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Summary *Objectives:* Scrub typhus is endemic in the Asia-Pacific region. Mortality is high even with treatment, and further knowledge of the immune response during this infection is needed. This study was aimed at comparing plasma levels of monocyte/macrophage and

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endothelial related inflammatory markers in patients and controls in South India and to explore a possible correlation to disease severity and clinical outcome.
Methods: Plasma levels of ALCAM, VCAM-1, sCD163, sCD14, YKL-40 and MIF were measured in scrub typhus patients ($n = 129$), healthy controls ($n = 31$) and in infectious disease controls ($n = 31$), both in the acute phase and after recovery, by enzyme immunoassays.
Results: Patients had markedly elevated levels of all mediators in the acute phase, differing from both healthy and infectious disease controls. During follow-up levels of ALCAM, VCAM-1, sCD14 and YKL-40 remained elevated compared to levels in healthy controls. High plasma ALCAM, VCAM-1, sCD163, sCD14, and MIF, and in particular YKL-40 were all associated with disease severity and ALCAM, sCD163, MIF and especially YKL-40, were associated with mortality.
Conclusions: Our findings show that scrub typhus is characterized by elevated levels of monocyte/macrophage and endothelial related markers. These inflammatory markers, and in particular YKL-40, may contribute to disease severity and clinical outcome.

Introduction

Scrub typhus is a multi-system infection caused by the obligate intracellular gram-negative, vector-borne bacteria *Orientia tsutsugamushi* (*O. tsutsugamushi*). This tropical infectious disease is endemic to the Asia-Pacific region and is responsible for one million new infections each year. If untreated, mortality can be as high as 30—50%,[1] and even with treatment, significant fatality rates of 10—20% has been reported in studies from India.[2–4] Scrub typhus is manifested by fever and multiple organ involvement with an eschar in variable proportion of patients, and in severe forms, multiorgan failure and death may develop.[5] *O. tsutsugamushi* is considered to be an intracellular pathogen that primarily infects endothelial cells both *in vitro*[6] and *in vivo*.[7] Subsequent to infection of endothelial cells, perivascular infiltration of T cells and monocytes/macrophages may occur, resulting in vasculitis.[8,9]

In mice, *O. tsutsugamushi* infects and multiplies in peritoneal macrophages[10,11] leading to death of infected animals, and human autopsy tissues from scrub typhus patients revealed *O. tsutsugamushi* in macrophages from the liver and spleen.[7] It also appears that the interaction of *O. tsutsugamushi* with myeloid cells, progenitors for monocytes/macrophages among others, plays a pivotal role in *O. tsutsugamushi* infection.[12] Recently, Tantibhedhyangkul et al. showed that *O. tsutsugamushi* replicates in human monocytes and macrophages isolated from healthy donors,[13,14] findings strengthened by earlier published data on tropism of *O. tsutsugamushi* for monocytes and dendritic cells within eschars.[15] Tantibhedhyangkul et al. also showed that *O. tsutsugamushi* alters a large number of host genes in human monocytes isolated from healthy donors, including genes encoding inflammatory cytokines and chemokines.[13] However, data on monocyte/macrophage activation *in vivo* in patients with scrub typhus are scarce.

We have recently reported up-regulation of a number of inflammatory cytokines in scrub typhus patients from South India.[16] Several studies have confirmed monocyte/macrophage and endothelial cell activation in scrub typhus patients.[13,14,17] Additionally, recent *in vitro* observations have shown that *O. tsutsugamushi* infect and multiply inside monocytes and macrophages.[13,18] Based on these findings, we wanted to examine inflammatory markers of monocyte/macrophage and endothelial cell activation in patients with scrub typhus. We selected established (i.e., vascular cell adhesion molecule 1 [VCAM-1]) and novel (i.e., activated leukocyte cell adhesion molecule [ALCAM)] markers of endothelial cell activation as well as established (i.e., soluble [s]CD14 and sCD163) and novel (i.e., macrophage migration inhibitory factor [MIF] and Chitinase-3-like protein 1 [YKL-40]) markers of monocyte/macrophage activation, and the latter two have also been involved in monocyte/endothelial cell interaction.

Materials and methods

Ethics statement

Blood samples from patients and controls were collected after obtaining informed and written consent from each participant. The study was approved by the local ethic committees and conducted according to the ethical guidelines from the Helsinki declaration.

Patients and controls

Patients >15 years of age admitted to Christian Medical College, Vellore, Tamil Nadu, India between November 2009 and February 2011 with suspected scrub typhus were considered for inclusion to the study. All patients with confirmed diagnosis of scrub typhus based on a positive IgM ELISA test were included as cases.[19,20]

The scrub typhus patients were further divided into subgroups according to disease severity. Those with no organ dysfunction were considered to have mild disease, those with one organ dysfunction moderate, while two or more organ dysfunctions was defined as severe disease. Organ dysfunction was defined as follows: Renal dysfunction, creatinine ≥2.5 mg/dl; hepatic dysfunction, bilirubin (total) ≥2.5 mg/dl; pulmonary dysfunction, bilateral pulmonary shadows on chest X-rays with moderate or severe hypoxia (PaO_2/FiO_2 <300 mmHg/PaO2 <60 mmHg/SpO_2 <90%); cardiovascular dysfunction, systolic blood pressure <80 mmHg despite fluid resuscitation and central nervous