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Correlation between the concentrations of tumor necrosis factor-α and the severity of disease in patients infected with *Orientia tsutsugamushi*

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ABSTRACT

Background: Patients with tsutsugamushi disease sometimes die if they do not receive appropriate chemotherapy. This study measured the concentration of several cytokines both before and after the administration of tetracyclines, and evaluated the changes in cytokine levels in patient serum to investigate the relationship between serum levels of cytokines and disease severity.
Methods: A total of nine patients were infected with *Orientia tsutsugamushi*. The diagnosis of tsutsugamushi disease was made using an indirect immunoperoxidase antibody test. The serum concentrations of cytokines were measured using enzyme-linked immunosorbent assays.
Results: The levels of interleukin (IL)-10 (mean 71.7 pg/ml) and IL-12p40 (mean 588 pg/ml) were elevated in all patients in the acute phase, above the normal upper limits. Tumor necrosis factor-α (TNF-α) levels (mean 9.20 pg/ml) were elevated in 89% and interferon-γ (IFN-γ) levels (mean 41.0 pg/ml) in 44% of patients. The down-regulation of these overproduced cytokines was observed after chemotherapy. There was a significant correlation between the concentrations of TNF-α in the acute phase and the severity of disease ($r = 0.918$).
Conclusion: The concentration of TNF-α may predict the severity of tsutsugamushi disease in the acute infectious phase.

1. Introduction

Infection with *Orientia tsutsugamushi* occurs frequently in Southeast Asia; this infection is called mite-borne typhus or scrub typhus and also tsutsugamushi disease. The etiologic agent of tsutsugamushi disease was previously known as *Rickettsia tsutsugamushi*.[1,2] Because of phenotypic and genotypic differences between this pathogen and other species belonging to the genus *Rickettsia*, the genus *Orientia* was proposed for this pathogen.[3] Serum antigenic serotypes of *O. tsutsugamushi* have been reported to differ in their virulence in experimental animals.[4] Gilliam, Karp, and Kato are highly virulent strains, and they can spread to become severe and potentially life-threatening.[5,6] However, other antigenic serotypes, such as Shimokoshi, Kawasaki, Kuroki, and others, have also recently been described.[7–10] The vector of this infectious disease is the trombiculid mite, named tsutsugamushi, which is distributed in mountainous areas and around rivers throughout Southeast Asia. These environments where mites are prevalent coincide with areas that are routinely used by human beings such as farmers, woodcutters, and hikers.

Recently, almost 500 patients per year have been reported with tsutsugamushi disease in Japan. Tsutsugamushi disease is characterized by fever, rash, and eschar, and is sometimes complicated with pneumonia, meningitis, disseminated intravascular coagulation (DIC), and systemic inflammatory response syndrome (SIRS),[11] leading to severe multiorgan failure if patients do not receive appropriate treatment. It has been recognized that SIRS is associated with an exacerbated production of cytokines,[12] and fulminant cases of tsutsugamushi disease have also been found to show hypercytokinemia.[2,13]

It is well known among physicians that tetracyclines are very effective against tsutsugamushi disease, and a majority of patients show dramatic defervescence within 24 h after starting antirickettsial chemotherapy using tetracyclines, although the mechanism of this dramatic effect is still unknown.[1] Therapeutic approaches directed against various components of the inflammatory processes such as down-regulation of the immune response by inflammatory cell apoptosis, inhibition of matrix

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metalloproteinase (MMP) expression,[14] and blockade of proinflammatory cytokines, could offer more effective treatments. Therefore, tetracyclines may not only have an antimicrobial function but also anti-inflammatory properties in various disorders.[15–17] This study measured the concentration of several cytokines both before and after the administration of tetracyclines, and evaluated changes in cytokine levels in patient serum.

2. Materials and methods

2.1. Patients

The present study investigated nine Japanese patients with confirmed *O. tsutsugamushi* infection between 1998 and 2005, to determine changes in serum cytokine levels. All patients lived in Yamagata Prefecture, in the north of Honshu, Japan. Each of these patients required intensive treatment in hospital to control symptoms and complications. Every patient responded well to treatment with tetracyclines for 2 weeks (one patient was administered doxycycline 200 mg/day and the others were administered minocycline 200 mg/day). A diagnosis of tsutsugamushi disease was made upon determination of a four-fold rise in IgG antibody titer to strains of *O. tsutsugamushi* (Gilliam or Karp serotype) in serum, as detected by an indirect immunoperoxidase antibody test[18] performed on paired serum samples collected during the acute and convalescent phases. No patient had an autoimmune disease or was in an immunocompromised state before this infectious episode happened.

2.2. Clinical severity scoring system

To evaluate the severity of tsutsugamushi disease, severity scores were used as reported previously.[13] In brief, the score was calculated as the sum of different point values assigned to specific criteria: central nervous system involvement, severe myalgia, radiographically documented pulmonary involvement, renal or liver dysfunction, hepatosplenomegaly, and DIC (Table 1).

2.3. Cytokine measurement

Serum levels of tumor necrosis factor-α (TNF-α) and interleukin (IL)-12p40 as proinflammatory cytokines, IL-10 as an anti-inflammatory cytokine, interferon-γ (IFN-γ) as a Th1 cytokine, and IL-4 as a Th2 cytokine, were measured using an enzyme-linked immunosorbent assay kit (Cytoscreen, Biosource, Camarillo, CA, USA). The amount of cytokine in each sample was determined by comparison with a standard curve. Data are reported as the concentrations of each cytokine in the acute and convalescent phases. Sera were frozen within 8 h of being received and were stored at − 80 °C until analysis. In our experience, freezing had no influence on the results on retesting. Serum cytokine levels in the nine patients were determined routinely on admission in the acute phase; moreover they were also measured 2 weeks after the initiation of anti-rickettsial chemotherapy, i.e., in the convalescent phase. We adopted the values that were determined in normal subjects from eight through 23 samples in the protocols for each cytokine measurement as normal values.

Table 1
Clinical severity scoring system

Central nervous system involvement	1
Severe myalgia	1
Radiographically documented pulmonary involvement	1
Elevated serum creatinine level:	
1.5–3.5 mg/dl	1
>3.5 mg/dl	2
Elevated serum ALT level:	
41–100 IU/l	1
>100 IU/l	2
Hepatosplenomegaly	1
Thrombocytopenia with platelet count:	
$100–149 \times 10^9$/l	1
$<100 \times 10^9$/l	2
Elevated level of FDP	1

ALT, alanine aminotransferase; FDP, fibrin/fibrinogen degradation products.
The score was calculated as the sum of different point values assigned to specific criteria. These scores, with possible total points ranging from 0 to 11, were determined on the basis of laboratory data and major symptoms on admission.

2.4. Statistical analyses

Analysis of the correlation between the clinical severity score and cytokine levels in acute phase serum of patients was performed, and the paired Student's *t*-test was used for statistical analyses using statistical software (Microsoft Excel 2003). A comparison was considered statistically significant if the *p*-value was <0.05. This study was approved by the Institutional Review Board of the Faculty of Medical Science of the University of Fukui.

3. Results

3.1. Profiles, symptoms, and laboratory data of nine patients with tsutsugamushi disease

Patient characteristics are shown in Tables 2 and 3. Each patient exhibited a persistent fever above 38.0 °C and eschar, a characteristic site for a mite bite, where the pathogen was inoculated. Laboratory data showed that alanine aminotransferase (ALT) increased more than 40 IU/l in 89% (8/9) of patients and lymphadenopathy was detected in 56% (5/9) of patients. Some patients had severe complications: DIC, meningeal symptoms, and severe myalgia. Severity scores, with possible total points ranging from 0 to 11, were determined on the basis of laboratory data and major symptoms on admission. The median score for the nine patients in this study on admission was 4 (range 2–6; mean ± standard deviation 3.9 ± 1.5). The rationale for using this scoring system was to ensure that the increased complexity of disease in patients not currently being treated was consistently represented in evaluations and descriptions. The patients were separated into two groups. One was a higher severity score group that showed a score ≥ 5, and the other was a lower severity score group with a score <5. Three patients belonged to the higher severity score group, and six patients belonged to the lower severity score group. The mean scores of the higher and lower severity groups were 5.7 and 3.0, respectively.

The IgG antibody titers were more than 1:320 during the clinical phase of each patient, and IgM antibody titers also increased. Only one patient was diagnosed with Gilliam serotype infection, the others were diagnosed as Karp serotype infection (Table 3).

3.2. Cytokine levels in serum during anti-rickettsial treatment of patients with tsutsugamushi disease

The serum levels of IL-10 (normal range <3.2 pg/ml) and IL-12p40 (normal range <3.2 pg/ml) were elevated in all patients, above the normal limits, during the acute phase of the disease. Serum TNF-α levels were elevated above the normal upper limit (0.12 pg/ml) in 89% (8/9) of patients, and IFN-γ levels were elevated above the normal level (20.9 pg/ml) in 44% (4/9) of patients. The levels of these four cytokines decreased significantly in the convalescent phase (Figures 1–3). However, IL-4 did not show any changes in either the acute or convalescent phases (Figure 3). The mean concentrations of IFN-γ, IL-4, IL-10, IL-12p40, and TNF-α in serum from the higher and lower severity score