Histology and Serum Cytokine Responses in an Imported *Rickettsia slovaca* Infection, Germany

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Abstract. *Rickettsia slovaca*, a spotted fever group rickettsial pathogen, causes a syndrome consisting of scalp eschar and neck lymphadenopathy following tick bite. We analyzed the histologic skin reaction in the eschar, showing a prominent eosinophilic infiltration, as well as the presence of B lymphocytes and CD4- and CD8-positive T cells. Examination of the serum cytokine responses over time demonstrated an initial proinflammatory cytokine elevation followed by normalization.

Rickettsia slovaca is a spotted fever group (SFG) rickettsial pathogen transmitted by *Dermacentor* ticks throughout Europe.¹ The intracellular bacterium causes tick-borne lymphadenopathy (TIBOLA), also known as *Dermacentor*-borne necrosis, erythema, and lymphadenopathy (DEBONEL), or scalp eschar and neck lymphadenopathy following tick bite (SENLAT).^{1,2} Peak incidences of the disease occur during March–May and September–November, associated with the activity of *Dermacentor* ticks.² We here describe the clinical and histologic findings and serum cytokine responses in a traveler returning from southern France to Germany infected with *R. slovaca*.

On March 21, 2016, a 62-year-old female German patient was seen in an outpatient dermatology center in Hamburg with a painful inflamed lesion on her scalp and cervical lymphadenopathy (Figure 1). A skin biopsy was taken from the periphery of the lesion, and the patient received oral cefuroxime and topical fusidic acid treatment as a scalp abscess was suspected. Five days before, during a vacation in southern France, she had been bitten by a tick on her scalp. The symptoms had developed on her journey back to Hamburg, 3 days before her visit to the dermatology center. One day after she was examined in the dermatology center, a facial edema occurred. After another day, the antibiotic treatment was switched to moxifloxacin and a blood sample was taken (day 4 after symptom onset, day 7 after the tick bite). Full blood count and liver and kidney function tests were normal, but C-reactive protein levels were elevated (9 mg/L; normal < 5 mg/L). Another 2 days later, a maculopapular exanthema and fever of 39°C developed for 2 days, followed by arthralgia of the knees and back pain. On day 7, after symptom onset (day 10 after the tick bite), a second blood sample was obtained and serological testing for borreliosis was negative. Immunofluorescence tests for rickettsiosis with SFG rickettsial antigens (*Rickettsia conorii*, *Rickettsia africae*, and *Rickettsia rickettsii*) and typhus group rickettsial antigens (*Rickettsia typhi* and *Rickettsia prowazekii*) were also negative. In a further blood sample obtained on day 10 after symptom onset (day 13 after the tick bite), low immunoglobulin G (IgG) antibody titers of 1:40 (cut-off < 1:40) against SFG rickettsial antigens were obtained, and a beginning seroconversion was suspected. Borreliosis serology remained negative. Doxycycline was not administered

as the patient felt well again and no longer had fever. A few days later, in the beginning of April, the scab from the scalp lesion was molecularly analyzed for rickettsial infection. Following DNA extraction (DNeasy Tissue kit; Qiagen, Hilden, Germany), real-time polymerase chain reaction (PCR) targeting the pan-rickettsial *ompB* gene³ yielded positive results. Blast analysis (www.blast.ncbi.nlm.nih.gov) of sequenced rickettsial *ompB*,³ *ompA*,⁴ and *gltA*⁵ genes after successful conventional PCR amplification revealed 99% nucleotide sequence homology with *R. slovaca*. Histologic examination of the skin biopsy showed a dense inflammatory infiltrate composed of lymphocytes, histiocytes, and numerous eosinophils in the dermis. Approximately 80% of the lymphocytes were T lymphocytes and 20% were B lymphocytes as demonstrated by anti-CD3 and anti-CD20 immunostaining, respectively. Immunohistochemistry with anti-CD4 and anti-CD8 revealed that CD4 cells constituted roughly 60% of the T lymphocyte infiltrate, whereas CD8 cells formed 40% of the T lymphocytes in the inflamed lesion (Figure 2). Two months later (day 86 after the tick bite), the patient was seen again in good health, and IgG titers of 1:640 were noted against SFG rickettsial antigens. No seroconversion against typhus group rickettsiae occurred. The patient was not investigated for seroconversion to other diseases. Serum cytokine levels were analyzed by bead-based LEGENDplex assay (BioLegend, London, United Kingdom) from all three stored samples (Figure 3). The levels of interferon- γ (IFN- γ), IFN- γ -induced protein-10 (IP-10), and macrophage inflammatory protein-1 α (MIP-1 α) were strikingly elevated during the acute phase of infection (days 10 and 13 p.i.) when compared with healthy controls, and normalized in the reconvalescent phase (day 86 p.i.) or decreased over time. In contrast, levels of interleukin (IL)-1 β , IL-8, and MIP-1 β were only slightly increased during the acute phase, followed by a decline to lower normal levels in the reconvalescent phase. IL-21 levels were initially low and returned to normal concentrations during reconvalescence. Vascular endothelial growth factor and monocyte chemoattractant protein-1 concentrations were at the lower end in comparison to that in healthy controls. The serum concentrations of IL-2, IL-4–6, IL-9, IL-10, IL-12p70, IL-13, IL-17A and F, IL-22, tumor necrosis factor- α (TNF- α), regulated on activation, normal T cell expressed and secreted (RANTES), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) were similar between the patient and controls (data not shown).

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FIGURE 1. Clinical presentation of the patient with SENLAT (scalp eschar and neck lymphadenopathy following tick bite) due to *Rickettsia slovaca*. (A) Scalp eschar. The arrow points at the typical eschar with a necrotic center and inflamed surrounding tissue hidden underneath the scalp hair. (B) Cervical lymphadenopathy. The arrow points at an enlarged lymph node of the neck. (C) Exanthema on the leg. (D) Prominent maculopapular exanthema on the forehead.

In the case described, the patient demonstrated the typical clinical picture of SENLAT. The reason why women are at higher risk and why *Dermacentor* ticks preferably bite on the

scalp remains unclear.² The prominent eosinophilia seen in the rickettsial lesion by histopathological examination might be an allergic reaction to tick saliva, or, more likely, rather

FIGURE 2. Histology of the eschar region in the *Rickettsia slovaca* infection. (A) A prominent eosinophil inflammatory infiltrate is seen besides lymphocytes and histiocytes. The biopsy was taken from the inflamed tissue surrounding the necrotic center of the eschar. Hematoxylin and eosin stain, original magnification $\times 50$. (B) Immunohistochemistry for CD3. Roughly 80% of the lymphocytes stained with the T lymphocyte marker CD3. Original magnification $\times 50$. (C) Immunohistochemistry for CD20. Approximately 20% of the lymphocytes are positive for the B lymphocyte marker CD20. Original magnification $\times 50$. (D) Immunohistochemistry for CD4 (left) and CD8 (right). The ratio of CD4:CD8 was 60:40. Original magnification $\times 75$.

FIGURE 3. Serum cytokine level changes during *Rickettsia slovaca* infection. Twenty sera from healthy blood donors were analyzed in parallel. In the acute phase of the infection (days 10 and 13 p.i.), increased levels of interferon- γ (IFN- γ), IFN- γ -induced protein-10 (IP-10), and macrophage inflammatory protein-1 α (MIP-1 α) are visible, which returned to baseline levels during reconvalescence (day 86 p.i.), or decreased over time. Similarly, an incremental concentration of interleukin (IL)-1 β , IL-8, and MIP-1 β is seen in the acute phase, followed by normalization during reconvalescence. The concentrations of vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein-1 (MCP-1) remained within the lower range of normal controls during the study period. The levels of IL-21 were low initially and returned to normal concentrations during reconvalescence.

typical for *R. slovaca* as the biopsy was taken 5 days after the tick bite from the inflamed tissue at some distance to the inoculation site. In *R. conorii* eschars of Mediterranean Spotted Fever (MSF), macrophages and T and B lymphocytes are numerous,^{6,7} but some eosinophilic infiltration has also been noted.⁸ In African tick bite fever (ATBF) caused by *R. africae*, predominantly neutrophils were detectable in the lesion.⁶ The results for cytokine measurements in human SFG rickettsioses are inconsistent,^{9,10} possibly because of different time points of sampling. An increase in IFN- γ concentrations, as determined here, was also described for MSF, but not for ATBF^{9,10}; TNF- α increases, as known from MSF and ATBF^{9,10} were not noted in our patient. IL-8 and MIP-1 α increases, as shown here, also occur in ATBF.¹⁰ The most prominent changes in our case were the elevations of IFN- γ , IP-10, and MIP-1 α in the acute phase of infection, followed by normalization or decreasing levels, respectively, during reconvalescence. The level variations of the other cytokines measured are likely not relevant; however, they follow the same kinetics. The initial proinflammatory cytokine elevations reflect the activation of immune cells, such as

monocytes/macrophages and endothelial cells which are known to be infected by rickettsiae, and attraction of T cells.¹¹

In our study, the histological skin reaction and cytokine level changes were described based on a single subject only. Future studies should address the aspects in more detail to shed more light on the pathogenesis of *R. slovaca* infection. This case demonstrates the typical late seroconversion seen in rickettsioses. In addition, the opportunity of molecular testing for the definitive diagnosis of rickettsial pathogens by PCR from eschars⁶ is emphasized which can be done noninvasively using scab material.

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