Immune Parameters in Hemorrhagic Fever with Renal Syndrome during the Incubation and Acute Disease: Case Report

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We describe immune parameters in a Croatian soldier who presented with mild flu-like symptoms and interstitial inflammatory infiltrate in the lungs on an X-ray during the incubation phase of hemorrhagic fever with renal syndrome (HFRS). Enzyme-linked immunosorbent assay (ELISA) IgM and polymerase chain reaction (PCR) were negative. Two weeks later, he developed HFRS caused by the Puumala virus. We performed two-color immunofluorescence cytometry with monoclonal antibodies identifying the activation markers on T cells. Serum samples were also examined by enzyme immunoassay (EIA) for the presence of interleukins IL-2 and IL-6 and their soluble receptors (sR). The analysis of early and late activation markers during the period of incubation revealed a small increase in the percentage of helper (CD4⁺CD25⁺) T cells and no significant increase in total activated (HLA-DR⁺TCR⁺) and cytotoxic (CD8⁺CD71⁺) T cells as compared with healthy controls. In the serum, only the concentration of soluble IL-6 receptor was increased. However, when the patient developed HFRS, all activation markers on T cells increased. Concentrations of sIL-2Rα and IL-6 remained increased two and six days after HFRS onset, respectively, whereas sIL-6R increased six days after HFRS onset. IL-2 concentration did not change. Our case indicates that rapid, modern diagnostic tools are necessary in the diagnosis of infectious diseases and their differential diagnosis. Immunological tests, which provide information on the patient immune status and especially on early changes in immune parameters, may contribute to the improvement of the diagnosis, prognosis, and therapy of HFRS.

Key words: Croatia; hemorrhagic fever with renal syndrome; interleukin-6; Puumala virus; receptors, interleukin-2; receptors, interleukin-6; T-Lymphocytes; war

Hemorrhagic fever with renal syndrome is a systemic infectious disease caused by hantaviruses (1). Hemorrhagic fever with renal syndrome is endemic in Croatia and the greatest outbreak of the disease was recorded during 1995 (1-5). Recent research confirmed that Puumala and Dobrava viruses were the main causative agents of hemorrhagic fever with renal syndrome in Croatia (4). Hemorrhagic fever with renal syndrome encompasses a broad spectrum of clinical presentations, ranging from inapparent or mild illness to fulminant hemorrhagic processes (3,4). Although hantaviruses predominantly affect kidneys, mild to moderate pulmonary involvement is common with Puumala virus infection (3,4,6). Several studies also showed that alterations in the renal function and blood pressure could be expected in patients who recovered from hemorrhagic fever with renal syndrome (7,8). Immunoreactions have been suggested to play important role in the pathogenesis of the disease, but it has not been thoroughly investigated yet. Clinical studies have implicated the role of proinflammatory cytokines (9-11) and peripheral blood mononuclear cell phenotype changes (12,13) in the pathogenesis of hemorrhagic fever with renal syndrome. However, there are no data on clinical, virological or immunological developments in patients with hemorrhagic fever with renal syndrome during the incubation period. To the best of our knowledge, this is the first report describing clinical findings and parallel virological and immunological examinations during the incubation phase of hemorrhagic fever with renal syndrome.

Case Report

A 36-year-old Croatian soldier was admitted to the Zagreb University Hospital for Infectious Diseases in May 1995, during an outbreak of hemorrhagic fever with renal syndrome in Croatia. He developed fe-
ver (39.5°C), headache, backache, visual impairment (confirmed by ophthalmological examination), and renal failure. Creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatinine phosphokinase (CPK) were increased. A serum sample was examined by IgM and IgG enzyme-linked immunosorbent assay (ELISA) test. Analysis of the RNA by polymerase chain reaction (PCR) was carried out with primers targeting the 5 RNA segment of Murinae- and Arvicolinae-borne hantaviruses. Puumala virus infection was confirmed. Two weeks earlier, several soldiers in the patient’s military unit presented with hemorrhagic fever with renal syndrome after a contact with rodents in the endemic region of hemorrhagic fever at the Mala Kapela Mountain. At that time, the patient was in a group of 40 soldiers who were free from symptoms of hemorrhagic fever with renal syndrome but were examined by IgM ELISA test for evidence of inapparent hantavirus infection. All of them were serologically negative. At that time, the patient had mild flu-like symptoms, and chest X-ray showed interstitial inflammatory infiltrate in the left lung. The results of ELISA IgM, IgG, and PCR analysis for hantaviruses were negative. ELISA IgM, IgG, and IgA tests for Mycoplasma pneumoniae and Coxiella burnetii (in disease phases 1 and 2), IgM and IgG for Legionella pneumophila, and indirect immunofluorescent antibody (IFA) IgM, IgG, and IgA assay for Chlamydia psittaci and Chlamydia pneumoniae were also performed; all results were negative. After examination, the patient stayed at home and had no further contact with rodents. The same immune parameters were measured in the period of incubation (15 days before the onset) and after the onset of hemorrhagic fever with renal syndrome. Two-color immunofluorescence cytometry was performed with monoclonal antibodies identifying T cell activation markers (CD25, CD71, and HLA-DR) (Fig. 1). Serum samples were also examined with enzyme immunoassay for the presence of interleukins IL-2 and IL-6 and their soluble receptors (sR) (Fig. 2). The analysis of early and late activation markers during the period of incubation revealed a small increase in the percentage of helper (CD4+CD25+) T cells and no significant increase in total activated (HLA-DR+ TCR+) and cytotoxic (CD8+CD71+) T cells as compared with healthy controls (Fig. 1A). In serum, only the concentration of soluble IL-6 receptor was increased (Fig. 2). However, when the patient developed hemorrhagic fever with renal syndrome, all activation markers on T cells increased (Fig. 1B). The sIL-2Rα and IL-6 remained elevated two and six days after hemorrhagic fever with renal syndrome onset; sIL-6R increased in the second serum sample (Fig. 2), whereas IL-2 showed no increase at all (data not shown). Clinical and laboratory examinations performed three and four years after the hemorrhagic fever with renal syndrome showed no signs of chronic disease (data not shown).

Discussion

We described immune parameters in a Croatian soldier during the incubation of hemorrhagic fever with renal syndrome and after the development of the disease. He was infected with Puumala virus and developed moderate symptoms of the disease. It is not
always easy to distinguish mild or moderate hemorrhagic fever with renal syndrome from other infectious diseases. Our patient was in a group of soldiers tested for specific IgM to hantaviruses in search for some inapparent cases of hemorrhagic fever with renal syndrome. They were all in the same military unit where several soldiers suffered from hemorrhagic fever with renal syndrome, and they all spent some time in the known endemic region of the Mala Kapela Mountain. It is clear that the patient was in the incubation period of the hemorrhagic fever with renal syndrome when we first tested him. Pulmonary disorders are common in Puumala virus infections, and this virus usually causes mild or moderate hemorrhagic fever with renal syndrome (3,4,6). We supposed that his flu-like symptoms and unilateral interstitial infiltrate confirmed by chest X-ray two weeks before the onset of the disease could be a result of Puumala virus replication and consequent local inflammation of the lungs. Although the pathogenesis of pulmonary infiltrates is not clear, direct viral cytopathic effect during local replication, acute local inflammation with consequent leukocyte recruitment and infiltration, vascular permeability, and immune complexes have been implicated in the pathogenesis of the lung alterations (3). To the best of our knowledge, this is the first description of patient with hemorrhagic fever with renal syndrome in the period of disease incubation.

Several studies published over the last decade showed that alteration in renal function and blood pressure could be expected in patients who had recovered from hemorrhagic fever with renal syndrome (7,8). However, four years after our patient had experienced hemorrhagic fever with renal syndrome, no signs and symptoms of any chronic sequel that could be linked to hemorrhagic fever with renal syndrome were found. Still, it is difficult to make conclusions on the basis of a single case.

We found significant differences between the immune parameters measured in the incubation period of hemorrhagic fever with renal syndrome and after the development of the disease. We have previously described increased expression of both early and late T cell activation antigens, (CD25, CD71, and HLA-DR) in patients with hemorrhagic fever with renal syndrome (13), but no such findings were observed in our patient during the incubation period. Increased sIL-2Rα, IL-6, and sIL-6R are characteristics of the developed disease (our unpublished data); however, only sIL-6R concentration was increased in the serum of our patient during incubation period. Huang et al (12) found sIL-2Rα to correspond with the degree of illness in patients infected with Hantaan virus, and we observed a similar pattern for IL-6 and its receptor in our patient. Other authors have also pointed to the role of cytokines in the immunopathogenesis of hemorrhagic fever with renal syndrome (9-11). Recently, sIL-6R-mediated signaling has been recognized as an important intermediary in both the resolution of inflammation and support of transition between the early, predominantly neutrophilic stage of an infection, and the more sustained mononuclear cell influx (14). Further studies are necessary to clarify whether the measurement of sIL-6R in the incubation period of hemorrhagic fever with renal syndrome in target population could be of prognostic value for subsequent development of the disease.

The presented case provides evidence that rapid, modern diagnostic tools are necessary in the diagnosis of infectious diseases and their differential diagnosis. Modern immunologic tests, which provide information on the patient immune status and especially on early changes in immune parameters, may contribute to the improvement of the diagnosis, progno-

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**Figure 2.** The level of interleukin (IL)-2 soluble receptor alpha (sRα) (top), IL-6 (middle), and IL-6sR (bottom) in the patient’s serum on day 15 before the onset of hemorrhagic fever with renal syndrome, and 2 and 6 days after he had developed hemorrhagic fever with a renal syndrome (Puumala virus infection). Dashed line – normal values for healthy controls (according to the manufacturer: IL-2sRα – 2132 pg/mL; IL-6 – 10 pg/mL; IL-6sR – 46 ng/mL); arrow indicates the onset of hemorrhagic fever with renal syndrome.
sis, and therapy of hemorrhagic fever with renal syndrome.

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References


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