Systemic Inflammation in Hemorrhagic Fever with Renal Syndrome Correlates with Hypotension and Thrombocytopenia but Not with Renal Injury

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Systemic inflammation is common in patients with nephropathia epidemica (NE), a European form of hemorrhagic fever. Markers of inflammation were studied in a patient with NE with respiratory insufficiency (patient 1), 18 other patients with NE, and 13 patients with a viral infectious disease other than NE. Neutrophil and monocyte CD11b expression levels, determined by flow cytometry; soluble interleukin (IL)-2 receptor (sIL-2R), IL-6, and IL-8 concentrations, determined by means of Immulite; and soluble E-selectin, determined by ELISA, were higher in patients with NE than in healthy subjects. The findings were not specific for NE and did not correlate with serum creatinine levels, but the findings correlated inversely with mean arterial pressure (sIL-2R and monocyte CD11b expression) and minimum platelet count (sIL-2R, IL-6, neutrophil, and monocyte CD11b expression). Monocyte CD11b expression in patient 1 was extremely high, suggesting that monocytes may contribute to development of lung injury. Severity of inflammation in patients with NE is related to hypotension and platelet consumption but not to renal injury.

Hantaviruses, such as the Hantaan, Puumala (PUU), and Sin Nombre viruses, are enveloped RNA viruses that are serologically related to each other and define a genus within the Bunyaviridae family (reviewed in Kanerva et al. [1]). Hantaviruses are found throughout the world, are each carried by a distinct rodent host, and cause human zoonoses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS) [1]. HFRS in the Far East is caused by the Hantaan and Seoul viruses, and HPS in North America and South America is caused mainly by the Sin Nombre and Andes viruses, respectively. In Europe, 2 hantaviruses, PUU and Dobrava, are known to be human pathogens. Most cases of hantavirus infection in Europe are caused by the PUU virus, which, along with its rodent host, Clethrionomys glareolus, is found in most of Europe and usually causes a mild form of HFRS known also as nephropathia epidemica (NE). In Finland, ~1000 cases of NE are diagnosed annually [2].

Increased endothelial permeability is a common pathognomonic feature for HFRS and HPS [3]. The prognosis of NE is in general favorable, with mortality at <0.2% [4]. The deaths are mainly attributable to irreversible circulatory collapse and brain edema [2]. In contrast, HPS is characterized by progressive respiratory illness with a 30%–50% fatality rate within 6–8 days from the onset of symptoms [5], and a few patients also develop renal dysfunction [6]. On the other hand, renal failure is typical of NE. In addition, minor pulmonary findings occur in 16%–53% of patients with NE [7, 8], whereas only a few patients develop severe lung injury that mimics lung damage in HPS [9], suggesting a common pathogenetic mechanism.

Sudden onset of high fever, increased peripheral blood leukocyte count with left shift, and thrombocytopenia typify NE [10]. Consequently, most patients meet the clinical criteria of systemic inflammatory response syndrome (SIRS) and, by definition, also the criteria of sepsis [11]. SIRS, sepsis, and organ dysfunction are considered to represent a continuum of systemic inflammatory reaction designated by activation of circulating neutrophils and monocytes, the generation of proinflammatory cytokines, such as tumor necrosis factor–α (TNF-α), and the activation of vascular endothelium [12]. At present, the role of systemic inflammation in the development of organ failure in patients with hantavirus infection is unclear. Although hypotension, a common feature of HFRS, worsens the degree of renal dysfunction, it is not necessary for the development of renal failure [13]. More recent studies suggest that pathogenesis of organ dysfunction involves proinflammatory mediators generated by the host in response to the virus [14].

During our previously reported study of markers of systemic...
inflammation in SIRS [15], we encountered a patient with NE complicated by respiratory insufficiency. We refer to this patient as patient 1. The level of cell surface expression of CD11b/CD18, a β2-integrin that serves as a marker of neutrophil and monocyte activation in patients with SIRS [15] and sepsis [16], was extremely high in the patient’s monocytes but virtually normal in his neutrophils. This prompted us to explore markers of systemic inflammation such as phagocyte CD11b expression, circulating levels of interleukin (IL)-6 and IL-8, soluble E-selectin (sE-selectin), a marker of endothelial activation, and soluble IL-2 receptor (sIL-2R), a T cell activation marker, in relation to mean arterial pressure, thrombocytopenia, and renal failure in patients with NE. To evaluate the specificity of the findings, we also studied patients with systemic viral infection other than PUU virus infection.

**Patients and Methods**

**Patients.** The patients were recruited among those treated in the Helsinki University Central Hospital and Aurora Hospital, Helsinki, during the years 1994–1997. NE was diagnosed by immunofluorescence test detecting circulating IgG antibodies of low avidity against the PUU virus [17]. Patient 1 came to our attention unexpectedly during a previous study [15]. We next recruited 23 patients who presented with the clinical picture typical of NE but whose diagnostic antibody test results were not yet available. The test results subsequently proved to be negative in 5 patients, and they were excluded from the study. Thus, the NE group consisted of patient 1 and 18 other patients (13 men and 5 women) with a median age of 38 years (range, 18–67 years). The median length of stay in the hospital was 8 days (range, 3–25 days). Mean arterial pressure (MAP) value was calculated as follows: MAP (mm Hg) = [2 × diastolic arterial pressure (mm Hg) + systolic arterial pressure (mm Hg)]/3.

The other patients with systemic viral infection were as follows. Seven patients, 5 men and 2 women, had acute viral hepatitis (assigned to group H) diagnosed by the presence of serum IgM antibodies against the hepatitis A virus (n = 4) and by IgM antibodies against the core antigen of the hepatitis B virus (n = 3). The median age of the patients was 40 years (range, 27–52 years). Four patients (3 women and 1 man, with a median age of 17 years; range, 16–30 years) had infectious mononucleosis (assigned to group IM) with IgM antibodies against the Epstein-Barr virus. The length of hospital stay was 7 days (range, 4–38 days) in group H and 9 days (range, 5–18 days) in group IM. One man (19 years old) had varicella, which was diagnosed on the basis of his clinical findings. He stayed in the hospital for 5 days. One girl (17 years old; length of hospital stay, 23 days) had viral meningitis with compatible cerebrospinal fluid analysis findings and a 4-fold increase in serum titers of adenovirus antibodies. Healthy subjects (n = 56), represented by hospital staff members, had no signs of infection and were not taking any medication.

**Blood samples.** Two blood samples were collected from each subject by venipuncture. One sample was placed in a prechilled polystyrene tube (Falcon No. 2058; Becton Dickinson Labware, Lincoln Park, NJ) supplemented with 500 μL of pyrogen-free citrate (113 mmol/L; Baxter Healthcare, Thetford, England) and 300 μL of dextran (mol wt, 70,000; 60 g in 1000 mL of physiologic saline; Kabi Pharmacia, Uppsala, Sweden), and the other sample was placed in a glass tube (Venoject VT-100PZX; Terumo Europe, Leuven, Belgium). Immediately after sampling, the tubes were pressed into thawing ice (0°C) to minimize leukocyte activation ex vivo [18]. Serum was separated by centrifugation at 4°C and stored in aliquots at −20°C until use. The polystyrene tube was incubated for 60 min at 0°C. The leukocyte-rich plasma layer was collected into a prechilled polystyrene tube and kept at 0°C until use. The polystyrene tube was incubated at 4°C and stored in aliquots at −20°C until use. The polystyrene tube was incubated for 60 min at 0°C. The leukocyte-rich plasma layer was collected into a prechilled polystyrene tube and kept at 0°C until the cells were labeled with monoclonal antibodies.

**Flow cytometry.** The cell labeling for flow cytometry was as described elsewhere [19, 20]. Briefly, aliquots of leukocyte-rich plasma were double-labeled by the addition of saturating amounts of CD14-FITC (mouse anti-CD14 IgG2b) and CD11b-PE (mouse anti-CD11b IgG2a) or a corresponding control antibody (mouse anti–keyhole limpet hemocyanin IgG2a-PE; Becton Dickinson, San Jose, CA). Contaminating erythrocytes were lysed by the addition of 1/10 diluted ice-cold fluorescence-activated cell sorter (FACS)
The leukocytes were collected by centrifugation at 4°C, washed once in ice-cold PBS, and resuspended in 300 µL of saline supplemented with formaldehyde (final concentration, 0.5%). Finally, the cells were stained with LDS-751 (Exciton, Dayton, OH), a nucleic acid dye [18].

The FACScan flow cytometer and Lysys II software (Becton Dickinson) were used to acquire and analyze the data. Two separate data sets, one for neutrophils (5 × 10^3 LDS-751-positive events) and the other for monocytes (1 × 10^4 CD14-positive events), were acquired for each specimen, as described elsewhere [19, 20]. During the analysis, several electronic gates were used to identify intact neutrophil and monocyte populations. In a forward/side light-scatter dot plot, LDS-751-positive neutrophils were first separated from contaminating mononuclear cells by creating a region, R1. Next, in an LDS-751 fluorescence/side scatter dot plot, a second region, R2, was created to separate neutrophils from cell aggregates and from damaged cells with markedly increased LDS-751 permeability and fluorescence intensity. Finally, the CD11b expression on intact neutrophils (i.e., on cells colocated in the regions R1 and R2) was evaluated by creating a CD11b histogram. CD14-positive monocytes collected in live mode were similarly analyzed. CD11b expression is reported in relative fluorescence units (RFU), that is, as the median channel of the positively fluorescent cell population. In all experiments, >95% of neutrophils and monocytes were CD11b positive.

Serum levels of sE-selectin, IL-6, IL-8, and sIL-2R. Serum sE-selectin levels were determined by an ELISA kit (Bender Med-Systems, Vienna, Austria) with a detection limit of 0.8 ng/mL. Immulite (Diagnostic Products, Los Angeles, CA), a chemiluminescent immunoassay system, was used to determine the levels of IL-6 and IL-8 (detection limits, 5 pg/mL), and sIL-2R (detection limit, 10 U/mL).

Data analysis. The results are presented as median values, and the range is provided. The comparisons between groups were done with Kruskal-Wallis analysis of variance. When the test revealed a significant difference, 2-group comparisons were done with the Mann-Whitney U test with exact P value, and, because there were multiple comparisons, the P values were corrected with the Bonferroni method. The relationships between the markers of inflammation and minimum platelet count, MAP on admission, and maximum serum creatinine level were analyzed with the Spearman rank correlation test and a locally weighted scatter plot smoother (LOWESS). The correlation coefficient (r) and its 95% confidence interval (CI) are presented. The α level was .05 for all statistical tests.

Results

Report of the case. A 66-year-old man with a history of minimal change glomerulonephritis and autoimmune hemolytic anemia (diagnosed when the patient was 51 years old) was admitted in June 1994 to the Medical Emergency Unit of Helsinki University Central Hospital because of fever (39°C) for the past 5 days, dyspnea, dizziness, and vomiting. He took 150 mg metoprolol daily as an antiarrhythmic drug. On admission, his arterial pressure was 95/65 mm Hg, his heart rate was 140 beats per minute, and his respiratory rate was 24 breaths per minute. Radiological examination of the chest was normal. His PaO2 was 7.2 (normal range, 11.3–13.3), and his PaCO2, while breathing ambient air was 3.2 (normal range, 4.6–6.0). The
Neutrophil CD11b expression values (figure 2) of group NE (78 RFU; range, 45–214) and group IM (109 RFU; range, 83–131) were significantly higher than the respective values of the control group (47 RFU; range, 24–85). Among patients with NE, neutrophil CD11b expression correlated positively with monocyte CD11b expression ($r = 0.80; 95\% CI, 0.54 to 0.92; P < .001$) and inversely with minimum platelet count ($r = -0.64; 95\% CI, -0.24 to -0.85; P < .005$).

In group NE, neither monocyte nor neutrophil CD11b expression levels differed significantly between samples taken within 7 days after the rise in fever and those taken later (data not shown).

sIL-2R. The median sIL-2R concentrations of group NE (3641 U/mL; range, 674–9738), group H (3162 U/mL; range, 1259–4492), and group IM (1909 U/mL; range, 1110–8746) were all significantly higher than the respective values of healthy controls (416 U/mL; range, 241–845; figure 3A). The 2 patients with varicella and viral meningitis had clearly increased sIL-2R levels. In group NE, sIL-2R levels correlated negatively with the minimum platelet count (figure 3B) and MAP on admission to the hospital (figure 3C).
**Discussion**

The results show that monocyte and neutrophil CD11b expression and plasma concentrations of sIL-2R, sE-selectin, IL-6, and IL-8 are elevated in patients with NE. Furthermore, CD11b densities and the concentrations of sIL-2R and IL-6 correlated with the minimum platelet count, indicating a relationship between severity of systemic inflammation and platelet consumption, the major cause of thrombocytopenia in NE patients [21]. Monocyte CD11b expression and sIL-2R levels correlated inversely with the initial MAP. This indicates a close relationship, but no causality, between blood pressure and activation of the immune-inflammatory mechanisms. The findings were not specific for NE, which agrees with previous studies showing that patients with acute viral hepatitis and IM have increased levels of proinflammatory cytokines [22, 23] and sIL-2R [24, 25]. To our knowledge, CD11b density and sE-selectin have not been studied in patients with acute hepatitis A or B or in patients with IM. We found increased CD11b expression in the latter. Although it may have had its origin from the Epstein-Barr virus infection itself, the possibility cannot be excluded that enhanced CD11b expression was caused by a secondary bacterial infection [26, 27]. The high sE-selectin levels suggest that vascular endothelium was activated in the patients with hepatitis and IM.

None of the markers studied correlated with the severity of the renal injury, suggesting that the pathogenesis of renal dysfunction in NE may not involve systemic inflammation. Furthermore, hypotension is not a prerequisite for the development of renal failure in patients with Korean hemorrhagic fever [13] or NE [28]. Taken in concert, the findings above support the idea that intrarenal events, not systemic inflammation, play a role in the development of the renal injury [3, 29].

Patient 1 developed a noncardiogenic respiratory failure with
a clinical course mimicking that in HPS. The major abnormality in the markers of inflammation was that the patient’s monocytes were strongly activated, as suggested by their strikingly high CD11b density. This finding has several pathogenetic implications. First, lymphocytes and macrophages but only few neutrophils occur in lung biopsy samples from HPS patients [30] and in bronchoalveolar lavage samples from NE patients [31]. Because phagocytes produce chemokines that attract specific cell types [32], the production of explicit chemokines might explain the mononuclear cell predominance. Second, cellular entry of the PUU virus is mediated by \( \alpha_v\beta_3 \)-integrin [33, 34], a ubiquitous vitronectin receptor expressed on many cell types, including endothelial cells [35], macrophages [36], and monocytes [37]. An unduly strong response to the PUU virus would explain monocyte activation in patient 1. Third, our previous study shows that monocyte CD11b expression is related to the development of organ failure in patients with sepsis [16]. That study and the present one were carried out concurrently, which makes the CD11b density values comparable. Thus, the monocyte CD11b density of patient 1 (311 and 277 RFU) was of the same magnitude as that of a patient with meningococcal septic shock (328 RFU), representing the highest CD11b density among all the patients, as described in detail elsewhere [16].

The possibility that such monocytes, regardless of whether activated by a virus or by invading bacteria, play a role in the pathogenesis of organ dysfunction warrants further study. Finally, in patient 1, the strongly enhanced CD11b expression was most probably not due to a secondary infection because the results of repeated blood culture tests were negative, the maximum IL-6 level (298 pg/mL) was modest, compared with that in the septic shock patients [16], neither circulating TNF-\( \alpha \) nor IL-1\( \beta \) (determined as a part of the previously reported study [15]) was detectable, and enhanced CD11b expression, which occurs on both monocytes and neutrophils during bacterial sepsis [15, 16, 18], was confined to the patient’s monocytes.

Monocyte and neutrophil CD11b expression and circulating levels of sIL-2R, sE-selectin, and IL-6 are increased in patients with NE, denoting the presence of systemic inflammation. Severity of inflammation was not related to renal dysfunction. The results of patient 1 raise the question of whether the activation of mononuclear phagocytes plays a unique role in the development of the lung injury in the patients with NE. This possibility remains to be explored by studying CD11b expression and other aspects of monocyte activation in the patients with NE who develop pulmonary changes and in patients with HPS.

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References


