High Levels of Cytokine-Producing Cells in the Lung Tissues of Patients with Fatal Hantavirus Pulmonary Syndrome

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Hantavirus pulmonary syndrome (HPS) is characterized by the rapid onset of pulmonary edema and a high case-fatality rate. Hantavirus antigens have been demonstrated in pulmonary capillary endothelial cells, but the mechanisms causing capillary leakage remain unclear. Immunohistochemical staining was used to enumerate cytokine-producing cells (monokines: interleukin [IL]-1α, IL-1β, IL-6, and tumor necrosis factor [TNF]-α; lymphokines: interferon-γ, IL-2, IL-4, and TNF-β) in tissues obtained at autopsy from subjects with HPS. High numbers of cytokine-producing cells were detected in the lungs and spleen tissues of HPS patients, but only low numbers in the livers and kidneys. A modest increase in the numbers of cytokine-producing cells was detected in the lungs of patients who died with non-HPS acute respiratory distress syndrome (ARDS), and very few (or no) cytokine-producing cells were detected in the lungs of patients who died of causes other than ARDS. These results suggest that local cytokine production may play an important role in the pathogenesis of HPS.

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Materials and Methods

Specimens. For the present study, we selected 6 patients diagnosed with fatal HPS at the University of New Mexico Health Sciences Center (UNM) from whom frozen (< 80°C) tissue blocks
obtained at autopsy were available for immunohistochemical staining (table 1). Hantavirus infection was confirmed in each case by detection of antibody to SNV antigens in antemortem blood specimens [18], detection of hantavirus gene sequences in autopsy tissues by polymerase chain reaction [8], and/or immunohistochemical detection of hantavirus antigens in autopsy tissues [8]. The interval between admission to UNM and death for these patients ranged from 2 hours to 2 days. For comparison, we selected for study 6 patients with non-HPS diseases from whom frozen lung tissue blocks were available, 2 with non-HPS ARDS and 4 without non-ARDS controls. Tissue samples of kidney, liver, and spleen were also available for study from 3 of these patients. All autopsies were performed 2–20 h after death. Frozen sections (8 μm thick) of lung, kidney, spleen, and liver from HPS patients were prepared and stored at −20°C until examination.

Tests for specificity of cytokine staining. Positive control slides were prepared as described above. Irrelevant isotype-matched MAbS were used to assess nonspecific staining. Positive and negative control slides were included in each staining procedure, and the cytokine-producing cells were enumerated in patient specimens only when the appropriate staining characteristics were observed in both the positive and negative control slides. We also performed immunoabsorbion tests for all cytokines examined; purified re-
Pathologic Findings

Lung. Histologically, a mild to moderate interstitial pneumonitis with variable degrees of congestion, edema, and mononuclear cell infiltration was observed in all lungs from HPS cases. The interstitial infiltrate was composed of a mixture of small and enlarged mononuclear cells with the appearance of immunoblasts. Variable numbers of inflammatory cells were demonstrated within the alveoli. Neutrophils were rarely seen in the interstitium, alveoli, or bronchioles. Pulmonary vessels contained neutrophils and atypical lymphocytes with the appearance of immunoblasts. Focal hyaline membranes were observed in 5 of 6 cases. No viral inclusions or type II pneumocyte hyperplasia were identified. Proteinaceous pleural effusions were found in 5 of 6 patients.

Lung tissues from the non-HPS ARDS patients (A1, A2) showed diffuse alveolar damage with focal hyaline membrane formation; the lung tissue of patient A1 also showed pneumonia. There was no evidence of hyaline membrane formation in the lung tissues from the other control patients (C1–C4). However, these lung tissues showed the following abnormalities: acute bronchitis and mild acute pneumonia (patient C1), pulmonary edema (patient C2), and moderate acute pneumonia (patient C4).

Kidney. All of the kidneys from HPS patients and non-HPS patients were histologically normal. Nephrosclerosis was noted in 1 non-ARDS control patient.

Liver. Infiltration of lymphocytes in the portal triads was observed in 3 of 6 HPS patients. No piecemeal necrosis or lobular hepatocyte necrosis was noted. Mild steatosis was noted in the liver of 1 HPS patient. One non-HPS ARDS patient had cirrhosis and a history of alcohol abuse. Livers from the other non-HPS patients were histologically normal.

Spleen. Splenomegaly (weight >200 g) was observed in 1 patient with HPS. Varying numbers of immunoblasts infiltrating into the red pulp and the periarteriolar white pulp of spleen were identified in 4 of 6 HPS patients. Both non-HPS ARDS patients had splenomegaly. No abnormalities were present in the spleens from non-ARDS control cases.

Cytokine Production

Patients without ARDS. Cytokine production was examined in non-ARDS cases (C1–C4) to define a basal level of cytokine-producing cells. No or few cytokine-producing cells were detected in the lungs, kidneys, livers, and spleens of all non-ARDS cases (figures 1, 2A).

Patients with HPS. Cytokine-producing cells were detected in lung, kidney, liver, and spleen tissues of HPS patients (figures 1, 2C, 2D, 3, and 4). Intensely stained cytokine-producing cells were seen throughout the lung tissues of these patients (figures 2C, 3). Monokines (IL-1α, IL-1β, IL-6, and TNF-α) were detected both in small cells within the alveolar walls and in larger cells in the alveolar air spaces (figures 2C, 3A), whereas lymphokines (IFN-γ, IL-2, IL-4, and TNF-β) were detected mainly in small cells located within the alveolar walls (figure 3B, 3C).

Cytokine-producing cells were rare in the kidney. In most kidney specimens examined, there were only a few positively staining cells in the interstitium. However, in case H5, the kidney showed a moderate number of cytokine-producing cells not only in the interstitium but also in the glomeruli (figure 4A). The level of cytokine-producing cells in the kidney of patient H5 was higher than that in the lung of the same patient (figure 1).

The number of cytokine-producing cells in the livers of HPS cases was limited. Most of the liver tissues examined showed no or few cytokine-producing cells, except for those from cases H3 and H4, in which the liver tissues showed moderate numbers
Figure 1. Nos. of cytokine-positive cells in lung, kidney, liver, and spleen sections of study subjects with hantavirus pulmonary syndrome (H1–H6, filled symbols), adult respiratory distress syndrome (A1, A2, symbols with cross hairs), and other non–acute respiratory distress syndrome diseases (C1–C4, open symbols). Individual subjects are represented by single symbol throughout: H1 (circle), H2 (square), H3 (triangle up), H4 (triangle down), H5 (diamond), H6 (hexagon), A1 (diamond), A2 (hexagon), C1 (circle), C2 (square), C3 (triangle up), and C4 (triangle down).

of IL-1α, IL-1β, IL-6, and IL-2–producing cells (figures 1, 4B). Cytokine-producing cells were detected along the hepatic sinusoids. Few cytokine-producing cells were identified within portal triads that contained mononuclear inflammatory cells.

In the spleens, intense immunostaining was observed in most of the HPS cases (figure 1). The spleen tissues of patients H5 and H6 had the highest numbers of cytokine-producing cells (figure 4C). Cytokine-producing cells were found predominantly in the red pulp.

Patients with non-HPS ARDS. Cytokine production was examined in the tissues of 2 patients with ARDS unrelated to hantavirus infection in order to determine whether the pattern of cytokine production seen was specific to HPS. Cytokine-producing cells were detected in some of the tissues from these
Figure 2. Tumor necrosis factor-α immunostaining in tissues of patients with hantavirus pulmonary syndrome (HPS), non-HPS adult respiratory distress syndrome (ARDS), and non-ARDS illnesses. Lung tissue from patient with (A) non-ARDS illness (patient C1, 0 cells/mm²), (B) non-HPS ARDS (patient A1, 0 cells/mm²), and (C) HPS (patient H2, 307 cells/mm²). D, Kidney tissue from patient with HPS (patient H2, 0 cells/mm²). All photomicrographs are at ×100 magnification, bar = 50 μm.

Figure 3. Cytokine immunostaining in lung tissues of patients with hantavirus pulmonary syndrome. A, Interleukin (IL)-1β (patient H2, 578 cells/mm²). B, Interferon-γ (patient H3, 8 cells/mm²). C, IL-2 (patient H3, 79 cells/mm²). All photomicrographs at ×100 magnification, bar = 50 μm.
patients; however, the numbers of cytokine-producing cells in the lungs of these patients were usually lower than in most of the patients with HPS (figures 1, 2B).

Statistical Comparison of Findings among Groups

Figure 1 shows the numbers of cytokine-producing cells in the various tissues of HPS cases and non-HPS cases. The numbers of cytokine-producing cells in the lungs of HPS patients were significantly higher than those in the lungs of control patients ($P<.01$ for IL-2, IL-4, and IL-6; $P<.05$ for IFN-$\gamma$, TNF-$\beta$, IL-1$\alpha$, and TNF-$\alpha$). The numbers of cytokine-producing cells in spleen tissues of HPS patients were also significantly higher than those of the control patients ($P<.05$ for IFN-$\gamma$, IL-2, TNF-$\beta$, IL-1$\beta$, and IL-6). There were no statistically significant differences between patients with HPS and with non-HPS ARDS because of the small sample size.

Discussion

In the present study, we demonstrated specific localization of cytokine-producing cells in the lungs of patients who died of HPS. The numbers of cytokine-producing cells in the lungs of HPS patients were higher than in the kidneys and livers of the same patients. This localization of cytokine-producing cells to the lung parallels the distribution of hantavirus antigens, as reported by Zaki et al. [8]. We detected cells producing predominantly monocyte-derived cytokines, such as IL-1$\alpha$, IL-1$\beta$, IL-6, and TNF-$\alpha$, as well as cells producing predominantly lymphocyte-derived cytokines, such as IFN-$\gamma$, IL-2, IL-4, and TNF-$\beta$. We did not perform detailed studies to characterize the cells producing each cytokine; however, on morphologic grounds alone it appeared that lymphocytes in addition to monocytes and macrophages were involved in cytokine production in the lungs of these subjects.

Cytokine production by both activated T lymphocytes and monocytes is likely to be important in the pathogenesis of HPS, which is characterized by a capillary-leak syndrome in the lungs. Similar mechanisms have been postulated in the pathogenesis of DHF, which is also characterized by a capillary-leak syndrome, although capillary leakage in DHF is diffuse and generally spares the lungs [19]. TNF-$\alpha$ is thought to play an important role in causing capillary leakage [20, 21], and IL-2 has also been shown to increase vascular permeability in vivo [22]. IFN-$\gamma$ produced by activated T lymphocytes is known to enhance TNF-$\alpha$ production and exacerbate shock in vivo [23]. Therefore, it is notable that we detected cells producing TNF-$\alpha$, IL-2, and IFN-$\gamma$ in the lungs of these subjects with HPS.

Prior to 1993, human hantavirus infections had been associated only with HFRS and nephropathia epidemica, both characterized predominantly by renal impairment. Several studies demonstrated impaired endothelial cell function in the kidney
during hantavirus infection of mice and humans [24, 25]. It has also been reported that serum cytokine levels and the expression of activation antigens on peripheral blood lymphocytes are increased in patients with HFRS [15, 26–29]. Thus, although the major organ affected differs in HPS and HFRS, activation of cytokine production and T cell function appears to be involved in the pathogenesis of both diseases.

One patient with HPS in this study (H5), who had renal impairment during acute infection, had higher numbers of cytokine-producing cells in the kidney than in other organs, including the lung. Zaki et al. [8] also demonstrated SNV antigens in the medulla and glomeruli of kidney tissues of some patients with HPS. The presence of SNV antigens and cytokine-producing cells in the kidney may thus cause renal impairment and proteinuria in some patients with HPS. Concurrent pulmonary and renal manifestations are characteristic of HPS due to Bayou and Black Creek Canal viruses in the southeastern United States [30, 31]. Our patient H5 was infected in New Mexico by a strain of SNV (demonstrated by polymerase chain reaction amplification of viral sequences from blood [32]), and a few other SNV infections have been complicated by renal failure and proteinuria.

Two other patients with HPS in the present study (H3, H4) had high numbers of cytokine-producing cells in their liver tissues. These cytokine-producing cells were located mainly within sinusoids, suggesting that these cells may be in the general circulation. Few cytokine-producing cells were seen in the portal triads.

Several studies [33–35] have reported that trauma or cardio-pulmonary resuscitation induces cytokine expression in the lung, resulting in acute pulmonary damage. Therefore, to determine whether the finding of high numbers of cytokine-producing cells was specific for HPS, we examined lung tissues obtained from patients who died of causes other than HPS. We found few cytokine-producing cells in the lungs of patients who died without clinically significant acute lung disease. We found moderately increased levels of cytokine-producing cells in the lungs of 2 patients with non-HPS ARDS. The numbers of cytokine-producing cells in the lungs of these patients was somewhat lower than in subjects with HPS, although this difference was not statistically significant. It is difficult for us to draw firm conclusions from the data from non-HPS ARDS subjects because the number of patients studied was small and samples were obtained late in disease. However, the suggestion that local cytokine production in the lung may be involved in the development of non-HPS ARDS is in agreement with a previous study showing high cytokine levels in bronchoalveolar lavage fluid of patients with non-HPS ARDS [36].

In conclusion, we have demonstrated markedly increased numbers of cytokine-producing cells in the lungs of HPS patients. In 1 HPS patient who developed massive proteinuria, many cytokine-producing cells were observed in the kidney as well. These findings suggest that T lymphocyte activation and cytokine production play important roles in the pathogenesis of HPS as well as in the renal dysfunction in hantavirus infection.

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References