Evaluation of Systemic Inflammatory Responses in Neonates with Herpes Simplex Virus Infection

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Neonatal herpes simplex virus (HSV) infection is a severe disease with high mortality and morbidity. To investigate the pathogenesis of neonatal HSV infection, we examined inflammatory responses and markers of apoptosis in patients with neonatal HSV infection. Concentrations of inflammatory cytokines and markers of apoptosis were significantly higher in patients with disseminated HSV infection and were correlated with HSV load. It appears that the immunopathological damage that results from host responses to viral infection leads to organ dysfunction in patients with neonatal HSV infection.

Neonatal herpes simplex virus (HSV) infection is a severe disease with high mortality and morbidity [1]. Neonates develop 3 types of infection, which are classified according to the clinical extent of disease: localized skin, eyes, or mouth (SEM) infection; central nervous system (CNS) infection; and disseminated infection, with or without CNS involvement. The prognosis for neonatal HSV infection ranges from 3 to 23 days after birth (mean, 7.2 days). Diagnosis was based on the isolation of HSV or the detection of HSV DNA by use of polymerase chain reaction (PCR) [8]. The HSV type was determined with the type-specific monoclonal antibody or by use of restriction fragment–length polymorphism analysis of amplified products [8]. The HSV type was determined with the type-specific monoclonal antibody or by use of restriction fragment–length polymorphism analysis of amplified products [8]. The HSV type was determined with the type-specific monoclonal antibody or by use of restriction fragment–length polymorphism analysis of amplified products [8]. The HSV type was determined with the type-specific monoclonal antibody or by use of restriction fragment–length polymorphism analysis of amplified products [8].

It is known that viral and bacterial infections cause systemic inflammatory response syndrome (SIRS). A frequent complication of SIRS is the development of multiple organ dysfunction syndrome (MODS) [3]. Investigations into the pathophysiology of SIRS have indicated that proinflammatory cytokines, such as interleukin (IL)–6 and tumor necrosis factor (TNF)–α, play a central role [4]. Many studies have shown that the serum concentrations of proinflammatory and anti-inflammatory cytokines are elevated in patients with SIRS. Concentrations of these cytokines are reported to be elevated in neonatal patients with severe bacterial infection [5] and have been correlated with poor clinical outcome. The occurrence of organ damage is thought to be related to immunopathological damage as the result of overexuberant host responses. Furthermore, some pro-inflammatory cytokines have the potential to enhance apoptosis in organ tissues and endothelial cells. Recently, it has been reported that apoptosis plays a role in the development of MODS in patients with SIRS [6].

Although the criteria for SIRS in neonates have not been established, we hypothesized that neonatal disseminated HSV infection corresponds to SIRS, because the clinical manifestations of these patients were similar and because they often developed MODS. To evaluate the inflammatory responses, we measured the serum concentrations of IL-6 and soluble TNF receptor 1 (sTNF-R1). We also measured the concentrations of mitochondrial aspartate aminotransferase (mAST) and cytochrome c, which are considered to be markers of apoptosis [7].

**Methods.** Informed consent was obtained from the parents of all children in this study. Nineteen patients with neonatal HSV infection were enrolled in this study, all of whom had been referred to the Nagoya University Graduate School of Medicine for the diagnosis of HSV infection. Their ages ranged from 33 to 41 weeks (mean, 38.6 weeks), and their birth weights ranged from 1208 to 4034 g (mean, 2929 g). The day of onset of infection ranged from 3 to 23 days after birth (mean, 7.2 days). Diagnosis was based on the isolation of HSV or the detection of HSV DNA by use of polymerase chain reaction (PCR) [8]. The HSV type was determined with the type-specific monoclonal antibody or by use of restriction fragment–length polymorphism analysis of amplified products [8]. Nine cases were caused by HSV-1, and 10 were caused by HSV-2. The clinical types of neonatal HSV infection were classified according to the National Institutes of Allergy and Infectious Diseases Collaborative Antiviral Study Group [1].

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Figure 1. Comparison of herpes simplex virus (HSV) load (A) and concentrations of interleukin (IL)-6 (B), soluble tumor necrosis factor receptor 1 (sTNF-R1; C), mitochondrial aspartate aminotransferase (mAST; D), and cytochrome c (E) in patients with disseminated (Diss) HSV infection and central nervous system (CNS)/skin, eyes, and mouth (SEM) HSV infection. All of the samples were obtained at diagnosis or within 3 days after the initiation of therapy with acyclovir. Bars, Means and SEs for each group. Dotted lines, Detection limits for each sample. The statistical method used was the Mann-Whitney U test.

Serum HSV load was quantified by use of real-time PCR assay using the TaqMan PCR kit (Applied Biosystems), as described elsewhere [9]. Serum concentrations of IL-6 and sTNF-R1 were determined with 100 µL of serum, using sandwich-type ELISA kits (R&D Systems), as described elsewhere [10]. This assay takes ∼5 h. The normal values for IL-6 and sTNF-R1 are <12.5 pg/mL and 500–1500 pg/mL, respectively.

Serum concentrations of mAST were assayed by use of the inhibition method with proteinase K, at the SRL Laboratory (Tokyo). Serum concentrations of cytochrome c were determined by use of ELISA, at the Eisai Laboratory (Ibaraki, Japan) [11]. The normal values for mAST and cytochrome c are <7 IU/L and <1000 pg/mL, respectively.

Statistical analysis was performed by use of StatView software (version 5.0; SAS Institute). Statistical comparisons of virus loads, serum concentrations of cytokines, and serum concentrations of markers of apoptosis were evaluated by use of the Mann-Whitney U test. Regression analyses were used to compare virus loads, serum concentrations of cytokines, and serum concentrations of markers of apoptosis. P < .05 was considered to be statistically significant.

Results. Of 19 patients with neonatal HSV infection, 7 were classified as having disseminated infection, 10 as having CNS infection, and 2 as having SEM infection. The mean ± SD time of onset was significantly earlier in the patients with disseminated infection, compared with those with CNS or SEM infections (3.9 ± 0.8 vs. 9.1 ± 5.4 days), as reported elsewhere [1]. Other characteristics (birth weight, gestational age, sex, and HSV type) did not differ among the clinical types.

Most of the patients with disseminated infection had high fever, tachycardia, and tachypnea. At admission, 4 of the 7 patients already had DIC. All but 1 patient received therapy with acyclovir. Two patients died of MODS. The remaining patients recovered without apparent sequelae after 1 year of observation.

The serum HSV loads of the patients are shown in figure 1A. The mean ± SE HSV load was significantly higher in patients with disseminated infection (10^5.5 ± 10^5.7 copies/mL) than in patients with CNS and SEM infections (10^5.6 ± 10^5.4 copies/mL).

Serum concentrations of IL-6 and sTNF-R1 were also examined (figure 1B and 1C). The mean ± SE concentration of IL-6 was significantly higher in patients with disseminated infection (550 ± 180 pg/mL) than in patients with CNS and SEM infections (27 ± 22 pg/mL). The mean ± SE concentration of sTNF-R1 was also higher in patients with disseminated infec-
tion (6600 ± 610 vs. 2400 ± 240 pg/mL). Two patients who had extremely high concentrations of IL-6 (>1000 pg/mL) and sTNF-R1 (>7000 pg/mL) subsequently died.

Serum concentrations of mAST and cytochrome c are shown in figure 1D and 1E. Concentrations (mean ± SE) of both of these markers of apoptosis were significantly higher in patients with disseminated infection than in patients with CNS and SEM infections (1100 ± 320 vs. 4.9 ± 0.7 IU/L for mAST; 5000 ± 650 vs. 130 ± 29 pg/mL for cytochrome c).

We then analyzed the correlations between the different parameters. There were positive correlations between HSV load and cytokine concentrations (HSV load vs. sTNF-R1, r = 0.85 and P < .0001; HSV load vs. IL-6, r = 0.68 and P = .003). There were also positive correlations between the concentrations of cytokines and markers of apoptosis. Cytochrome c correlated with sTNF-R1 (r = 0.86 and P < .0001) and IL-6 (r = 0.78 and P < .001). Furthermore, mAST correlated with IL-6 (r = 0.77 and P < .001) and sTNF-R1 (r = 0.78 and P < .001). Representative results are shown in figure 2A (HSV load vs. sTNF-R1) and figure 2B (sTNF-R1 vs. cytochrome c).

The concentrations of cytokines and markers of apoptosis were estimated sequentially and plotted against the day after onset of disseminated HSV infection. The concentrations of sTNF-R1 and cytochrome c decreased with time (figure 2C and 2D). The concentration of sTNF-R1 decreased gradually but remained detectable until 15–20 days after onset. On the other hand, the concentration of cytochrome c decreased more rapidly and was negligible 10 days after onset. As with cytochrome c, the concentrations of IL-6 and mAST decreased rapidly with time (data not shown).

**Discussion.** IL-6 is an important mediator of the early systemic host response to infection; it reaches peak concentrations rapidly after onset, with a half-life of several hours [12]. The concentration of IL-6 has been shown to be an effective prognostic indicator of clinical severity in SIRS and correlates with mortality states. In addition to IL-6, we examined sTNF-R1, which is one of the extramembraneous fragments of the TNF receptor on cells. TNF-α is one of the major proinflammatory cytokines in the cytokine cascade that is manifested in SIRS. However, the detection of TNF-α in patients with SIRS has not been consistent, and correlations with severity and outcome of disease have been poor because the peak concen-

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**Figure 2.** Correlation of soluble tumor necrosis factor 1 (sTNF-R1) concentrations with herpes simplex virus (HSV) load (A) and concentrations of cytochrome c (B). Regression analysis was used for the comparison. Dynamics of sTNF-R1 (C) and cytochrome c (D) of patients with disseminated HSV infection. Lines connect individual data points for each patient.
trations of TNF-α occur rather rapidly after the onset of disease. TNF-α binds to sTNF-Rs that are shed into the circulation [12]. Thus, instead of measuring the concentration of TNF-α, we measured the concentration of sTNF-R1, which is the natural homeostatic regulator of the action of TNF-α. In the present study, most of the patients with disseminated HSV infection had high concentrations of IL-6 and sTNF-R1. The 2 patients who subsequently died had higher concentrations of these cytokines. Furthermore, the concentrations of IL-6 and sTNF-R1 correlated with HSV load. These data suggest that the immunopathological damage caused by the host responses leads to organ dysfunction or death in patients with neonatal disseminated HSV infection and that these immune responses are dependent on the magnitude of HSV infection.

It is known that some of the proinflammatory cytokines have the potential to enhance apoptosis in organ tissues and endothelial cells and that apoptosis plays a role in the development of MODS in patients with SIRS [6]. In particular, TNF-α is the major cytokine implicated in the development of apoptosis, either by signaling through TNF-R1 or via other mechanisms. TNF-α has been shown to mediate apoptosis in endothelial cells, hepatocytes, and other cell types that are dysfunctional in SIRS [6]. To evaluate apoptosis in the patients, we measured the concentrations of cytochrome c and mAST. Both cytochrome c and mAST are mitochondrial proteins. It has been shown that TNF-α causes rapid release of cytochrome c from mitochondria, and convergent evidence suggests that the release of cytochrome c from mitochondria is a critical step in the apoptotic process. Early during the apoptotic process, mitochondrial depolarization occurs, and cytochrome c and mAST are released from the mitochondria into cytoplasm [7]. In the present study, the concentrations of cytochrome c and mAST were significantly higher in patients with disseminated infection than in patients with CNS and SEM infections and correlated with HSV load and concentrations of cytokines. This result suggests that, as the result of host immune responses, apoptosis is associated with the severity of neonatal HSV infection. In fact, apoptosis of liver cells subsequent to HSV infection is shown in mice [13]. In addition, TNF-α is an important factor in the development of endothelial injury and DIC in patients with SIRS. It has been reported that 34% of patients with disseminated infection show DIC [1]. In the present study, 4 of the 7 patients with disseminated infection had DIC at admission. The frequent observation of DIC in patients with disseminated infection might be associated with systemic cytokine responses.

It is not clear why neonates with disseminated HSV infection show high inflammatory responses. Previous studies have shown that deficiencies in the neonatal immune response permit disseminated HSV infection. NK cells and antibody-dependent cellular cytotoxicity are critical components of the early immune response to viral infection [14]. It has been shown that concentrations of transplacentally acquired neutralizing antibodies and antibody-dependent cellular cytotoxicity are lower in patients with disseminated HSV infection. On the other hand, Schultz et al. reported that the cytokine-producing capacity of macrophages was higher in neonates than in adults [15]. It might be speculated that, during disseminated infection, macrophage or other immune cells are activated in an abnormal fashion to eradicate HSV. These uncontrolled immune responses may result in damage to the organs of the patients.

Our findings are important for the development of a strategy for treating neonatal HSV infection. Although an intact inflammatory response is essential for the maintenance of normal host defense mechanisms, the modulation of inflammatory responses or apoptosis could be an appropriate target for therapeutic intervention. Although further studies are necessary, the combination of antiviral and anticytokine or antiapoptotic therapies may improve the prognosis of neonatal disseminated HSV infection.

References


