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Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome

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ABSTRACT

Proinflammatory cytokines are related to the pathogenesis of enterohemorrhagic *Escherichia coli* (EHEC) infection and hemolytic-uremic syndrome (HUS). We assessed the kinetics of the release of cytokines such as neopterin, interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α and the soluble forms of type I and II TNF receptors during EHEC O111-induced HUS (EHEC O111/HUS). Fourteen patients with EHEC O111/HUS were enrolled in this study. Serum concentrations of all cytokines other than TNF-α were significantly elevated in patients with severe HUS compared with those in patients with mild HUS. Although serum concentrations of TNF-α were not significantly higher in patients with severe HUS, most patients with acute encephalopathy showed elevated TNF-α levels. Serum concentrations of these cytokines rapidly and markedly increased, and massive hypercytokinaemia developed 1 day before the diagnosis of HUS in patients with severe HUS. Changes in the number of white blood cells and concentration of serum lactate dehydrogenase were significantly larger between the onset of hemorrhagic colitis and the time of the diagnosis of HUS in patients with severe HUS compared with those in patients with mild HUS. Proinflammatory cytokines play an important role in the pathogenesis of EHEC infection and development of severe complications, including HUS and encephalopathy. Monitoring the cytokine profile may be useful for assessing disease activity of EHEC O111 infections.

1. Introduction

Hemolytic-uremic syndrome (HUS) is a multisystem disease characterised by the triad of microangiopathic hemolytic anaemia, thrombocytopaenia and renal failure. HUS occurs after a prodrome of hemorrhagic colitis (HC) caused by Shiga toxin-producing *Escherichia coli*, also known as enterohemorrhagic *E. coli* (EHEC) [1,2]. Neurological complications involving the central nervous system occur in approximately 20% of patients with HUS [3]. Common clinical symptoms include alterations of consciousness and seizures, and morbidity and mortality are also high in affected patients [4].

The pathophysiology of EHEC-associated HUS remains obscure. Endothelial cell damage is the main histopathological feature of

Abbreviations: EHEC, enterohemorrhagic *Escherichia coli*; HUS, hemolytic-uremic syndrome; EHEC O111/HUS, EHEC O111-induced HUS; HC, hemorrhagic colitis; IL, interleukin; LDH, lactate dehydrogenase; LPS, lipopolysaccharides; TNF, tumour necrosis factor; sTNF-RI, soluble forms of TNF receptor type I; sTNF-RII, sTNF-R type II; TP, total protein; WBC, White blood cells.

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HUS. Increasing experimental evidence suggests that immune responses of the host to Shiga toxin and/or lipopolysaccharide (LPS) are involved in the pathophysiology of EHEC infections [5–7].

Proinflammatory cytokines are related to the pathogenesis of EHEC infection and HUS [8–15]. A recent study reported that these cytokines are useful for predicting neurological complications in patients with HUS [15].

The most prevalent serotype is EHEC O157; however, EHEC O111 can also cause these complications [16,17]. An outbreak of EHEC O111 occurred in Toyama and other prefectures in Japan between late April and early May 2011. The courses in some patients were extremely aggressive, and some were fatal [16]. We completely realised the importance of establishing a monitoring system with useful clinical markers for predicting severe clinical outcomes in patients with EHEC O111 infection in the course of this outbreak of EHEC O111.

To assess the kinetics of the release of cytokines in patients with EHEC O111-induced HUS (EHEC O111/HUS) and clarify the importance of the cytokine profile in the pathogenesis of EHEC O111/HUS, we measured serum concentrations of cytokines such as neopterin, interleukin (IL)-6, IL-8 and tumour necrosis factor (TNF)-α and the soluble forms of TNF receptor types I (sTNF-RI) and II (sTNF-RII) in patients with EHEC O111/HUS. Furthermore, we evaluated which of these clinical markers is important for predicting severe clinical outcomes in patients with EHEC O111/HUS.

2. Methods

2.1. Patients and samples

Serum samples were obtained from 14 patients during the HUS phase in the outbreak of EHEC O111 that occurred in Toyama and other prefectures in Japan between late April and early May 2011. Samples from seven patients with EHEC O111 infection were serially obtained from the HC phase to the HUS phase. The clinical characteristics of the patients with EHEC O111/HUS are shown in Table 1. EHEC O111 infection was diagnosed when one or both of the following criteria were fulfilled: bloody diarrhoea, vomiting or bowel cramps with microbiological identification of EHEC O111 infection. The presence of HUS was defined by thrombocytopaenia (platelet count of <150,000/mm^3), hemolytic anaemia and acute renal dysfunction. Acute renal dysfunction was defined as renal injury evidenced by hematuria, proteinuria or elevated creatinine levels: ⩾1.0 mg/dl in children <13 years old or ⩾1.5 mg/dl in patients ⩾13 years old or a ⩾50% increase over baseline [18]. The presence of acute encephalopathy was defined by some behavioural abnormalities or neurological symptoms with pathological magnetic resonance imaging findings. The severity of HUS was classified according to Gianantonio's criteria: mild, no anuria; moderate, <7 days of anuria; severe: ⩾7 days of anuria [19]. One patient (case 8) did not have anuria but suffered from acute encephalopathy, acute lung injury and acute pancreatitis. Therefore, this patient was classified as severe. One patient (case 4) died of acute encephalopathy with diffuse brain edema 2 days after the diagnosis of HUS. This patient was also classified as severe.

Eleven patients were classified as severe (Table 1), no patient was classified as moderate and three patients were classified as mild. Eight patients presented with complications due to acute encephalopathy. Two patients died of acute encephalopathy with diffuse brain edema. Some patients had neurological sequela despite aggressive treatment, including continuous renal replacement therapy, plasma exchange and anti-inflammatory therapy with steroids and intravenous immunoglobulin. Serum samples were separated, divided into aliquots, frozen and stored at −80 °C until analysis. This study was approved by the institutional review board of Kanazawa University, and all specimens were used after the receipt of informed consent.

Table 1
Clinical characteristics of patients with EHEC O111/HUS. EHEC O111/HUS, *Escherichia coli* O111-induced hemolytic-uremic syndrome.

Case	Age (years)	Sex	VT1	VT2	ARF	Encephalopathy	Severity	Outcome
1	7	F	+	+	+	+	Severe	Alive
2	7	M	–	+	+	+	Severe	Died
3	1	M	–	+	+	+	Severe	Alive
4	14	M	–	–	+	+	Severe	Died
5	26	F	+	+	+	+	Severe	Alive
6	7	F	–	+	+	+	Severe	Alive
7	16	F	–	+	+	+	Severe	Alive
8	13	M	+	+	–	+	Severe	Alive
9	23	M	–	–	+	–	Severe	Alive
10	16	F	–	+	+	–	Severe	Alive
11	17	F	–	+	+	–	Severe	Alive
12	8	F	–	+	–	–	Mild	Alive
13	6	F	–	+	–	–	Mild	Alive
14	16	M	+	+	–	–	Mild	Alive

2.2. Quantification of serum cytokines

Serum concentrations of neopterin, IL-6, IL-8, TNF-α, sTNFRI and sTNFRII were evaluated using commercial enzyme-linked immunosorbent assays according to the manufacturer's instructions (neopterin: IBL, Hamburg, Germany; IL-6, sTNFRI, sTNFRII and IL-8: R&D Systems, Inc., Minneapolis, MN, USA).

2.3. Statistical analysis

Within-group comparisons were analysed using the Mann–Whitney *U*-test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Massive hypercytokinaemia expressed during HUS in patients with severe EHEC O111/HUS

Serum concentrations of neopterin, IL-6, IL-8, TNF-α, sTNFRI and sTNFRII were determined in patients with EHEC O111/HUS to investigate the relevance of proinflammatory cytokines in the pathogenesis of EHEC O111/HUS. As shown in Fig. 1A, serum concentrations of all cytokines, except TNF-α, were significantly elevated during the diagnosis of HUS in patients with severe HUS compared with those in patients with mild HUS. Although serum concentrations of TNF-α were not significantly elevated in patients with severe HUS compared with those in patients with mild HUS, four of eight patients with acute encephalopathy showed elevated TNF-α levels during the diagnosis of HUS. In addition, seven of the eight patients with acute encephalopathy showed elevated TNF-α levels during the HUS phase (Fig. 1B). Because many proinflammatory cytokines are associated with the pathogenesis of EHEC O111/HUS, we believe that monitoring these cytokine profiles, rather than any individual cytokine level, may be more useful for evaluating disease activity. Consequently, we show these cytokine profiles using a radar chart (Fig. 1C and D). Patients with severe HUS developed more severe hypercytokinaemia during the HUS phase than patients with mild HUS.

3.2. Massive hypercytokinaemia developed just after the onset of HUS

Serum concentrations of these proinflammatory cytokines were serially monitored in seven patients with EHEC O111/HUS to investigate the kinetics during the clinical course of EHEC O111/HUS.