Original Paper

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Plasma Cytokine Levels in Hemolytic Uremic Syndrome

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Abstract

The cytokines tumor necrosis factor-α (TNF-α) and its soluble TNF receptors 55 and 75 (sTNFR55, sTNFR75), interleukin-1β (IL-1β) and interleukin-6 (IL-6) were measured in plasma from 13 patients with the hemolytic uremic syndrome (HUS) on admission. No significant changes in the plasma levels of TNF-α and IL-1β were detected in the HUS patients as compared to the plasma levels of the control groups. Levels of IL-6 were significantly elevated in the plasma of those HUS patients who had extrarenal manifestations, consisting of seizures, loss of consciousness, coma and pancreatic necrosis. Although the exact function of IL-6 in the plasma of HUS patients is still unknown and the group of HUS patients is small, plasma IL-6 is associated with the severity and outcome of the disease. Plasma levels of sTNR55 and sTNFR75 were significantly elevated in all HUS patients compared to the healthy controls, but they were also elevated in the children with chronic renal failure. This indicates that elevated levels of circulating sTNFR should be carefully interpreted when kidney failure exists.

Introduction

Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia, thrombocytopenia and acute renal failure. The most common form of HUS seen in children is the epidemic form, which is preceded by an acute, often bloody, gastroenteritis [1]. In the last decade it has become clear that infections with verocytotoxin-producing *Escherichia coli* strains are the main cause of HUS in childhood [2, 3]. Although the exact pathogenesis of HUS is still unknown, it is generally accepted that endothelial cell activation and damage play a central role. Cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6) are known to play an important role in infectious and inflammatory diseases, and have been extensively studied in diseases such as sepsis, severe infectious purpura and Kawasaki disease [4–6]. Elevated levels of cytokines are also found in plasma taken in the acute phase of patients with thrombotic thrombocytopenic purpura (TTP), a syndrome which is in many aspects familiar with HUS [7].

The aim of this study was to determine whether circulating cytokines are detectable in the blood of HUS patients. For this purpose, we measured TNF-α and its soluble receptors sTNFR55 and sTNFR75, IL-1β and IL-6 in the plasma of 13 patients admitted to the hospital with the epidemic form of HUS.

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Table 1. Laboratory indices of patients with HUS on the day of admission

Patient No.	Age months	Hb mmol/l	Platelets 10^9/l	WBC 10^9/l	Urea mmol/l	Creatinine µmol/l
1	14	3.9	276	22.3	20.2	106
2	21	4.9	45	20.4	33	295
3	6	4.7	25	25.5	29.6	350
4	53	5.3	56	31.5	36	770
5	112	5.5	55	10.9	67	1,020
6	36	4.2	138	17.7	40	640
7	54	3.7	203	8.8	63.2	928
8	10	4.1	56	23.2	61.6	514
9	41	3.7	130	12	45	178
10	22	3.9	37	19.5	26.9	288
11	36	9.2	304	24.1	6.8	54
12	22	7.5	11	53.6	68.2	335
13	40	5.7	30	33.6	17.2	157
Normal values children		6–9	140–440	4–11	3–7	30–90

The laboratory indexes are given for each HUS patient. On the second day after admission the laboratory parameters of patient 11 were as follows: Hb 4.5 mmol/l, platelets 25 × 10^9/l, urea 10.5 mmol/l and creatinine 130 µmol/l. Patients 10, 11, 12 and 13 had, in contrast with the other HUS patients, severe extrarenal manifestations, consisting of seizures, loss of consciousness, and coma. Pancreatic necrosis was also diagnosed in patient 10.

Patients and Methods

Patients

Thirteen children (4 females/9 males, mean age 38 ± 28 months; range 6–112 months) were diagnosed as having HUS, because of the presence of hemolytic anemia with burr cells in the peripheral blood smear, renal failure and thrombocytopenia [8]. HUS was preceded by mostly blood-stained diarrhea. Antibodies against the most common verocytotoxin-producing *E. coli* (VTEC) serotype O157, which indicates an infection with VTEC, were detected in the plasma of 7 of 12 patients [9].

The main laboratory indexes of the patients on admission are displayed in table 1. On the day of admission to the hospital, before any treatment was given, blood from these 13 children was collected into EDTA-containing tubes, centrifuged and the plasma was stored in small aliquots at −70 °C until assays were performed. Plasma of 8 age-matched healthy children and 8 children with chronic renal failure taken before dialysis served as controls.

Methods

TNF-α was assessed by radioimmunoassay (RIA), as previously described [10]. This RIA measures total TNF-α (both free and complexed to its receptors), as demonstrated by the lack of interference of the addition of up to 5 ng/ml recombinant sTNFR55 and sTNFR75 to sera containing known amounts of TNF-α. The sensitivity of this assay is 100 pg/ml. IL-1β assay was also performed with a RIA [11]. Before application, both RIAs (TNF-α and IL-1β) were thoroughly validated towards linearity and determination of matrix effects. When 100 µl of plasma was tested, recovery experiments of the respective cytokine resulted in recovery values exceeding 90%, both at the low and the high concentration range tested. IL-6 was measured by an ELISA as described by Barrera et al. [12]; materials were a gift from Dr. J. Wijdenes (Innotherapy, Besançon, France). The sensitivity of this ELISA is 20 pg/ml.

Soluble TNF receptors (sTNFR55 and sTNFR75) were measured by ELISA (Hoffmann-La Roche, Basel, Switzerland). These assays measure total circulating (both free and bound) receptor concentrations, since addition of up to 10 ng/ml recombinant TNF-α to plasma containing known amounts of sTNFR does not influence these assays. The sensitivity of these assays is 100 pg/ml.

Statistical Analysis

Results are expressed as mean ± SD. The significance of differences between the various groups of patients was determined by using the Mann-Whitney test. Spearman's rank test was used for determining the correlation between cytokine levels and the laboratory indexes in the HUS group.

Results

The individual data of cytokine levels in the plasma of the HUS patients are displayed in table 2. Only patients 10, 11, 12 and 13 had severe extrarenal manifestations, consisting of seizures, loss of consciousness and coma. Patient 10 also developed necrosis of the pancreas. The latter 3 patients (11–13) died due to cerebral complications in the acute phase of the disease. No significantly increased levels of TNF-α and IL-1β were found in the

Table 2. Plasma cytokine levels in patients with HUS

Patient No.	TNF-α ng/ml	IL-1β ng/ml	IL-6 pg/ml	sTNFR55 ng/ml	sTNFR75 ng/ml
1	0.15	<0.10	<20	3.5	9.3
2	0.10	<0.10	<20	13.2	9.0
3	0.14	<0.10	30	45.1	6.4
4	0.15	<0.10	<20	38.5	39.8
5	0.15	<0.10	<20	17.2	10.9
6	0.12	<0.10	<20	26.4	35.7
7	0.13	<0.10	<20	11.1	4.3
8	0.15	<0.10	20	NM	NM
9	0.13	<0.10	<20	10.1	20.6
10	0.15	<0.10	275	22.0	24.7
11	0.13	<0.10	160	28.2	34.0
12	NM	NM	262	32.1	33.2
13	0.14	<0.10	410	20.2	22.4
Patients 1–9	0.13±0.02	<0.085	<30	20±16++	17±14+
Patients 10–13	0.13±0.05	<0.10	277±103**	26±6+	29±6+
Control (n = 8)	0.12±0.02	0.07±0.02	<20	1.4±1	4.2±1
CRF (n = 8)	0.14±0.04	0.08±0.05	<20	17±6++	27±7+

Plasma cytokine levels were determined on admission in patients with HUS. Patients 10, 11, 12 and 13 had, in contrast with the other HUS patients, severe extrarenal manifestations, consisting of seizures, loss of consciousness, and coma. Pancreatic necrosis was also diagnosed in patient 10. The data are expressed individually and as the mean ± SD of the various groups. To compare the data of the different groups of patients and healthy controls the Mann-Whitney test was used.

$^{+}$ $p < 0.05$, $^{++}$ $p < 0.001$ as compared to the control group; ** $p < 0.01$ as compared to HUS patients (and the control group). NM = Not enough material for the test available.

plasma of patients with HUS as compared to the control groups (table 2). Only the 4 patients who had extrarenal manifestations (severe form of HUS) had significantly elevated levels of IL-6 (277 ± 103 pg/ml), as compared to the control group of healthy children, whereas the IL-6 levels in the plasma of the other HUS patients (<30 pg/ml, mild form of HUS) were not significantly elevated. Significantly increased levels of both soluble TNF receptors were found in all HUS patients (sTNFR55 22 ± 12 ng/ml and sTNFR75 21 ± 13 ng/ml) as compared to the control group of healthy children (sTNFR55 1.4 ± 1 ng/ml and sTNFR75 4.2 ± 1 ng/ml). There was no difference in the plasma concentrations of sTNFR55 and sTNFR75 between the mild and severe forms of HUS. However, both sTNFRs were also increased in the plasma of the children with chronic renal failure (17 ± 6 ng/ml for sTNFR55 and 27 ± 7 ng/ml for the sTNFR75), which suggest that renal failure per se, and associated with a decrease in clearance causes high levels of sTNFR in the plasma.

Plasma levels of TNF-α, IL-1β or sTNFR75 did not correlate with hemoglobin, platelets, white blood cell count, urea or creatinine. Only sTNFR55 and IL-6 showed a mild correlation with hemoglobin ($r = 0.56$ and $r = 0.52$, respectively) and white blood cell count (both $r = 0.60$, Spearman's correlation) in the total HUS group.

Discussion

The role of circulating cytokines and soluble TNF receptors in the pathogenesis of HUS was investigated in this study. No elevated levels of TNF-α, IL-1β and IL-6 were found in plasma of HUS patients, who had no extrarenal manifestations during the disease. In the study done by Fitzpatrick et al. [13] in which IL-8 and TNF-α were measured in the plasma of 16 HUS patients, only 1 patient had an elevated concentration of TNF-α in the plasma. However, the occurrence of these cytokines in the plasma during the initial phase of the disease cannot be excluded, because these cytokines have a short half-life

(less than 1 h) and may therefore no longer be detectable in the plasma taken on admission in the hospital. On the other hand, Siegler et al. [14] reported that although TNF-α is not detectable in plasma, it is elevated in the urine of HUS patients, suggesting that TNF-α may be locally produced in the kidney and have a local effect in the kidney of HUS. Elevated levels of urinary cytokines have also been found in other kidney diseases and are believed to play a role in local inflammatory and infectious processes in the kidney [15]. Evidence for locally produced cytokines in the kidney has also been obtained by immunostaining and in situ hybridization [16] and from in vitro experiments with renal cells [17, 18]. Evidence for a local role of TNF-α in HUS was recently given by Harel et al. [19], who gave Shiga-like toxin I to transgenic mice bearing a chloramphenicol acetyltransferase (CAT) reporter gene with the TNF-α promoter. When giving Shiga-like toxin I to these mice, CAT activity, which reflects the degree of the activation of TNF-α synthesis, was induced within the kidney, but not in other tissues. Increased production of the cytokines TNF-α, IL-1β and IL-6 can also be found in the media of cultured human monocytes after stimulation with verocytotoxin-1 [20]. A possible role for the locally produced TNF-α in the kidney could be to increase the susceptibility of the endothelial cells locally for verocytotoxin by inducing more verocytotoxin receptors on the cell surface [21].

Elevated levels of IL-6 were only found in the plasma of those HUS patients who had extrarenal manifestations, consisting of seizures, loss of consciousness, coma and pancreatic necrosis. In inflammatory conditions IL-6 is synthesized by a wide variety of cell types (macrophages, endothelial cells and fibroblasts) upon stimulation with TNF-α, IL-1 and other agents. The major effects of IL-6 in an infection are the induction of acute-phase proteins in hepatocytes and the final differentiation of B cells into antibody-producing cells. We have shown before that IL-6 did not induce the receptor of verocytotoxin on cultured human endothelial cells and therefore did not increase the susceptibility of the endothelial cells for verocytotoxin, in contrast to TNF-α and IL-1 [21]. Although the exact function of IL-6 in the plasma HUS patients is still unknown and the group of HUS patients is small, plasma IL-6 is associated in our study with the severity and/or outcome of the disease. Closely related to HUS is thrombotic thrombocytopenic purpura (TTP), a syndrome which in many aspects is familiar to HUS (micro-angiographic hemolytic anemia, thrombocytopenia and renal impairment), but in which generalized symptoms (fever and neurological complications) are always present. In the plasma of all the 13 patients of TTP, examined by Wada et al. [7], elevated circulating levels of TNF-α, IL-1β and IL-6 were found. These data suggest that in patients with TTP and in severe cases of HUS, who also develop extrarenal manifestations, circulating cytokines may play a role in the pathogenesis of the disease.

Recently, two immunologically distinct soluble receptors sTNFR55 and sTNF75 have been identified [22]. These soluble receptors represent forms of the extracellular domain of the cell surface receptors for TNF-α and studies done in healthy volunteers indicate that TNFα itself is involved in the induction of sTNFR release [22, 23]. Recent studies have shown that in sepsis and in malaria elevated levels of sTNFR are present [22, 24, 25]. Although both sTNFR were elevated in the plasma of HUS patients, they were also elevated in the group of children with chronic renal failure. This implies that these elevated sTNFR levels cannot only be explained by increased activity of TNF-α or by other substances with proteolytic activity, like elastase, but may be caused by insufficient kidney function. In favor of this hypothesis is the recent report by Bemelmans et al. [26], who demonstrated in a murine model that kidney malfunction induces an increase in the amount of sTNFRs in the plasma.

In conclusion, no elevated levels of circulating TNF-α, IL-1β and IL-6 were detected in the mild form of HUS. On the other hand, elevated levels of IL-6 in the plasma were detected in HUS patients who displayed severe extrarenal manifestations. Probably locally produced cytokines may be more important in the pathogenesis of HUS than the circulating concentration. However, when in HUS extrarenal manifestations occur, circulating cytokines may have played or play a role in the pathogenesis. Results of elevated circulating sTNFR as shown in this study should be carefully interpreted when kidney failure exists.

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