

Proinflammatory and Anti-Inflammatory Cytokine Balance in Patients With Cirrhotic Hepatitis During Live-Donor Liver Transplant

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Abstract

Objectives: The immune system releases cytokines during the stress response, and the balance between proinflammatory and anti-inflammatory cytokines is important. This prospective study was done to determine which cytokines are responsible for maintaining cytokine balance during live-donor liver transplant surgery.

Materials and Methods: Recipients undergoing live-donor liver transplant surgery due to cirrhotic hepatitis were allocated to a recipient group (n=44), and healthy donors were placed in the donor group (n=45). In donors, blood sampling for cytokine level analysis was performed after anesthetic induction (before the start of surgery, time point 1). In recipients, blood samples were collected before the start of surgery (time point 1), 60 minutes after the start of the anhepatic period (time point 2), and 60 minutes after reperfusion (time point 3). The proinflammatory cytokines measured were interleukin-1 β , interleukin-6, and tumor necrosis factor- α ; the anti-inflammatory cytokines were interleukin-10 and interleukin-4. Cytokines were quantified using sandwich enzyme-linked immunoassays. The time course of proinflammatory and anti-inflammatory cytokine concentrations during surgery in the recipient group was evaluated.

Results: Interleukin-6, interleukin-10 and tumor necrosis factor- α showed significant changes in concentration during surgery, with interleukin-6 reaching levels 40 times higher than the preoperative value at the anhepatic stage. Interleukin-10 reached a peak at the neohepatic phase, with values 60 times higher than the preoperative value. The preoperative concentrations of interleukin-6 and interleukin-10 in the recipient group were higher than those in the donor group with a median of 4.48 vs 1.98 pg/mL ($P < .001$) and 2.98 vs 1.22 ($P = .026$).

Conclusions: Interleukin-6 and interleukin-10 play a major role in cytokine balance before and during live-donor liver transplant surgery.

Key words: Cytokines, Cytokine balance, Interleukin-6, Interleukin-10, Live-donor liver transplant

Introduction

A significant stress response occurs in the body during illness and surgery. Several different systems within the body are involved in this response. The endocrine system releases stress hormones (eg, glucocorticoids), and the autonomic and sympathetic nervous systems also play important roles in this response. Additionally, the immune system has an important function.¹ The immune system releases cytokines during the stress response, and the balance between cytokines is important for an effective response. For example, proinflammatory cytokines enhance cell-mediated immunity and chemotaxis of inflammatory cells, while anti-inflammatory cytokines suppress excessive inflammatory reactions thereby balancing the immune response. In healthy conditions, these 2 cytokines are balanced and thus immunologic homeostasis is maintained.²

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A liver transplant serves as a stressor threatening this homeostasis. Combined with underlying liver failure, the transplant surgery itself is a stressor promoting the secretion of cytokines.³⁻⁵

Studies on the proinflammatory and anti-inflammatory cytokine balance involved in maintaining immunologic homeostasis during liver transplant surgery are rare. We conducted this study to determine which cytokines are involved in maintaining the balance between proinflammatory and anti-inflammatory cytokines before and during liver transplant.

Materials and Methods

This prospective study was done in a cohort of recipients and donors involved in live-donor liver transplants at our hospital between May 2007 and December 2008. Patients with an unstable preoperative cardiovascular status, those with severe hypoxemia due to acute respiratory distress syndrome or hepatopulmonary syndrome, those with evidence of infection (other than viral hepatitis), and minors were excluded. Healthy donors (n=45) were allocated to the donor group, and recipients (n=44) with cirrhotic hepatitis were assigned to the recipient group. The study was approved by the ethics committee of our hospital (approval number: KCMC070T268) and registered with CRiS (identification number: KCT0000038). All protocols confirmed with the ethical guidelines of the 1975 Helsinki Declaration. Each patient gave written, informed consent.

Each patient was given general anesthesia induced with 5 mg/kg thiopental sodium and 1 mg/kg succinylcholine, without premedication, and was intubated. General anesthesia was maintained with isoflurane and medical air in oxygen (FiO₂, 0.4). Vecuronium was intermittently administered to maintain adequate relaxation of the abdomen. Mechanical ventilation was controlled with a tidal volume of 10 mL/kg, and the ventilation rate was adjusted to obtain an end-tidal partial pressure of CO₂ between 35 and 40 mmHg. Living-donor liver transplant was undertaken for patients in recipient group, and a right lobe hepatectomy was performed for donor group patients. Liver allografts were preserved in University of Wisconsin solution. The immunosuppressive regimen in living-donor liver transplant patient was prednisolone 125 mg just

before the end of anhepatic phase. No patient received a venovenous bypass or temporary portocaval anastomoses.

Immediately after anesthesia induction, a 20-gauge angiocatheter was inserted into the right radial artery, and 10 mL of fresh blood was collected for serum cytokine level analysis. In donors, blood sampling was done after anesthetic induction (before the start of surgery: time point 1). In recipients, blood samples were collected after anesthesia induction (time point 1), 60 minutes after the start of the anhepatic period (time point 2), and 60 minutes after reperfusion (time point 3). The collected blood samples were preserved in test tubes in an ice-filled container and centrifuged at 1300 rpm for 5 minutes at 4°C. The separated serum was frozen at -70°C.

Proinflammatory cytokines measured were tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6, and the anti-inflammatory cytokines were IL-4 and IL-10. The cytokines were quantified using a commercial sandwich enzyme-linked immunoassay kit (Quantikine; R & D Systems Inc, Minneapolis, MN, USA). We evaluated differences in cytokine secretions between groups at time point 1, and the time course of proinflammatory and anti-inflammatory cytokine concentrations during surgery in the recipient group.

Parametric data are presented as means \pm SD, and nonparametric data are presented as medians with quartiles. We used the SAS version 8.02 (SAS Institute, Inc., Cary, NC, USA) for statistical analyses. The Mann-Whitney rank sum test was used to compare cytokine levels between the 2 groups, and the Friedman test was performed to reveal changes in cytokine concentrations during the operation. Values for $P < .05$ were considered significant.

Results

Table 1 presents the demographic data of the 89 patients involved in this study. Preoperative concentrations of proinflammatory cytokines IL-6 and TNF- α and the anti-inflammatory cytokine IL-10 in the recipient group were significantly higher than those of the healthy donor group (Figure 1).

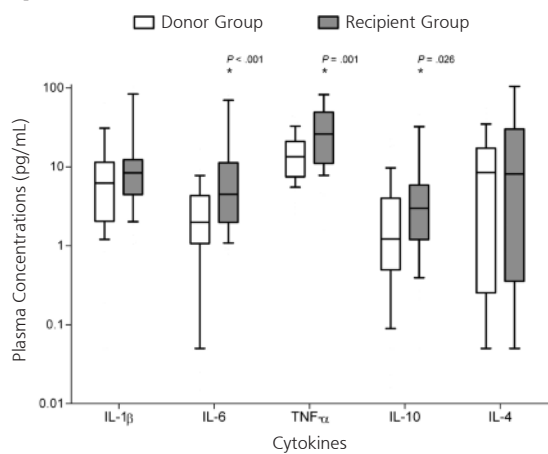
Among the proinflammatory cytokines, IL-6 and TNF- α showed significant changes in concentration during surgery, with IL-6 levels reaching as much as 40 times the preoperative level at the anhepatic stage (Table 2). Levels of the anti-inflammatory cytokine

Table 1. Demographic Data of the Patients

Demographics	Donor Group (n=45)	Recipient Group (n=44)
CTP criteria	NA	6/13/25 (A/B/C)
Underlying disease	NA	
Viral hepatitis		33
Alcoholic hepatitis		11
Age (y)	32.4 ± 4.7	48.5 ± 7.9
Body weight (kg)	70.6 ± 7.3	65.4 ± 7.2
Height (cm)	173.6 ± 9.5	168.2 ± 10.4
Sex (M/F)	39/6	24/20

Abbreviations: CTP, Child-Turcotte-Pugh; F, female; M, male; NA, not applicable

Values are means ± SD, or number.

Figure 1. Comparison of Preoperative Cytokine Concentrations Between Groups

The box plots show the median (line in the middle of the box), interquartile range (box), and 10th and 90th percentiles (whiskers).

IL-10 increased as surgery progressed, reaching peak levels that were 60 times higher than preoperative values at the neohepatic phase. Interleukin-1 β and IL-4 levels did not show significant changes in concentration during surgery (Table 2).

Discussion

Proinflammatory cytokines are secreted in response to stress reactions (eg, sepsis and tissue damage) and

induce hypotension, high fever, and accelerate the production of acute phase proteins.² They also enhance cell-mediated immunity and chemotaxis of inflammatory cells, thus playing a role in protecting the body from external stress.⁶ In contrast, anti-inflammatory cytokines suppress excess inflammatory reactions, thus playing a role in restricting the adverse influence they induce.⁷ Under healthy conditions, these 2 cytokines are balanced and homeostasis is maintained; however, in trauma, sepsis, and neoplasms, such homeostasis is disrupted resulting in excess elevation of proinflammatory cytokines that ultimately increase morbidity and mortality.^{2,8-10}

Our results show that the cytokines released markedly in patients with cirrhotic hepatitis during liver transplant surgery include IL-6 (a proinflammatory cytokine whose concentration increased as much as 40 times compared with its preoperative values), and IL-10 (an anti-inflammatory cytokine whose concentration was 60 times higher than its preoperative value). The preoperative levels of these 2 cytokines were significantly higher in liver transplant recipients compared with healthy donors, suggesting that IL-6 and IL-10 are cytokines playing an important role in cytokine balance in patients with cirrhotic hepatitis before and during liver transplant.

When the proinflammatory and anti-inflammatory cytokine balance is broken owing to excess proinflammatory cytokines released during stress, anti-inflammatory cytokines are usually secreted in response to restore the balance. This process is called *the phase of early proinflammatory and late anti-inflammatory cytokines*.⁸⁻¹⁰ This study demonstrated this condition; the secretion of IL-6 (a proinflammatory cytokine) increased markedly at the anhepatic stage, while the secretion of IL-10 (an anti-inflammatory cytokine) was prominent at the neohepatic stage. Interleukin-6 is a cytokine actively secreted during major surgeries (eg, cardiopulmonary

Table 2. Time Course of Cytokine Concentrations in the Recipient Group

	Time 1	Time 2	Time 3	P Value
IL-1 β	8.42 (4.45, 12.26)	9.72 (3.95, 33.69)	7.86 (3.14, 41.29)	.956
IL-6	4.48 (2.05, 11.10)	176.35 (50.96, 458.83)	135.66 (52.09, 327.30)	<.001
TNF- α	26.02 (11.18, 47.79)	36.14 (15.39, 65.30)	54.65 (21.28, 67.33)	.002
IL-10	2.98 (1.22, 5.74)	95.32 (48.62, 198.16)	184.35 (96.65, 298.80)	<.001
IL-4	8.16 (0.46, 27.69)	13.85 (3.19, 40.82)	7.83 (1.14, 21.40)	.687

Abbreviations: IL, interleukin; TNF, tumor necrosis factor

Values are median with quartile (25%, 75%).

All cytokines concentrations are pg/mL in value.

Time 1: after induction of anesthesia; Time 2: 60 min after the start of the anhepatic stage;

Time 3: 60 min after reperfusion.

bypass),¹¹⁻¹⁴ and its concentration is closely related with postoperative prognosis.^{15,16} Interleukin-10, which is secreted by activated helper T lymphocytes and induced by TNF- α secreted from monocytes,^{17,18} suppresses the release of proinflammatory cytokines such as TNF- α , IL-1 β , and IL-8.¹⁹ This study shows that IL-6 and IL-10 are typical early proinflammatory and late anti-inflammatory cytokines that are secreted during liver transplant surgery.

Preoperative plasma concentrations of IL-6 and TNF- α as proinflammatory cytokines, and IL-10 as an anti-inflammatory cytokine were significantly higher in patients with liver failure than in donors, confirming the difference in preoperative stress response between the 2 groups. This stress results from dysfunction of multiple organs, including the liver, as well as systemic inflammation and the stress response. Measuring plasma cytokine levels could be a basis for indirectly determining the degree of inflammation and stress in a patient with liver failure. However, preoperative differences in cytokine concentrations between healthy subjects and patients with liver failure seen in this study might not be a pure reflection of disease severity, because blood samples were collected in the operating suite after anesthesia induction. Drugs administered to induce and maintain anesthesia, the response to tracheal intubation, and preoperative anxiety about surgery could be stress factors that might interfere with assessing the influence of end-stage liver disease on cytokine concentrations. Reports that anesthesia itself has an effect on the immune response support these concerns.^{2,20,21} Nevertheless, we showed clear differences in preoperative cytokine concentrations between the donor and recipient groups. Moreover, factors associated with anesthesia would have affected both groups equally. Therefore, one reasonably may conclude that differences in cytokine concentrations are a reflection of disease severity.

The time courses of cytokine concentrations in the recipient group during surgery were similar to those of previous studies. Santiago and associates⁴ found that TNF-s and IL-10 levels gradually increased as the operation progressed and reached their maximums after reperfusion, which also was true in our study. Boros and associates²² and Arranz and associates²³ reported that TNF- α and IL-1 did not rise significantly until reperfusion, and that the IL-6 level showed a sharp rise after the initiation of surgery. In

our study, TNF- α concentration increased after reperfusion, and the IL-6 level increased as much as 40 times during anhepatic stage compared with values before surgery, which was similar to the above-mentioned authors' results. However, the concentration of IL-6 declined at the neohepatic phase compared with that at the anhepatic phase, which is attributed to the effect of immunosuppressive regimen that was administered just before the end of the anhepatic phase,²⁴ and the immunoregulatory actions of IL-10 that was secreted markedly at the neohepatic phase.

Care must be taken in interpreting our study results: IL-6 and IL-10 are pleiotropic, that is, they have both positive and negative effects on the clinical outcome. In addition to its original proinflammatory effect, IL-6 also has broad hepatoprotective effects.²⁵⁻²⁸ Even though IL-10 can reduce the magnitude of the inflammatory response and improve outcomes,^{9,29-31} it also can exacerbate T-cell dysfunction, decrease T-cell apoptosis, reduce antimicrobial function, and increase mortality in other less acute bacterial models of sepsis or after thermal injury.³² Therefore, when interpreting the results of this study, the consequence of the increased concentration of IL-6 and IL-10 during surgery should not be limited to regulation of the magnitude of the inflammatory response. The clinical implication of IL-6 and IL-10 in maintaining cytokine balance during liver transplant surgery must be elucidated in future studies.

In conclusion, it appears that the cytokines IL-6 and IL-10 play important roles in maintaining the cytokine balance during live-donor liver transplant. Also, we found a difference in the preoperative stress response between healthy people and patients with cirrhotic hepatitis by measuring differences in the levels of proinflammatory and anti-inflammatory cytokines. These results may contribute to our understanding of the pathophysiology of cirrhotic hepatitis.

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