Clinical Study

Upregulation of TLR2/4 Expression in Mononuclear Cells in Postoperative Systemic Inflammatory Response Syndrome after Liver Transplantation

Ziqing Hei, Xinjin Chi, Nan Cheng, Gangjian Luo, and Shangrong Li

Department of Anesthesiology, Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, China

Correspondence should be addressed to Ziqing Hei, heiziqing0530@hotmail.com

Received 28 November 2009; Revised 25 April 2010; Accepted 26 April 2010

Academic Editor: Philipp Lepper

Copyright © 2010 Ziqing Hei et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. To explore the relationship between Toll-like peripheral blood mononuclear cells (PBMC) and systemic inflammatory response syndrome (SIRS) in postoperative patients of liver transplantation (LT).

Methods. Blood samples of 27 patients receiving LT were collected at T1 (after induction of anaesthesia), T2 (25 minutes after the beginning of anhepatic phase), T3 (3 hours after graft reperfusion), and T4 (24 hours after graft reperfusion). The expression of TLR2/4 on PBMC and serum concentration of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8 were measured. The patients were divided into SIRS group (n = 12) and non-SIRS group (n = 15) for analysis.

Results. Blood loss and transfusion were higher in the SIRS group than in the non-SIRS group. Both the preanhepatic and anhepatic phase were significantly longer in the SIRS group. The TLR2/4 expression on PBMC as well as serum TNF-α, IL-1β, and IL-8 were significantly higher at T3 and T4 than that at T1 and T2 in the SIRS patients. The expression of TLR4 on PBMC is positively correlated to serum TNF-α, IL-8. Expression of TLR2/4 on PBMC and serum concentrations of TNF-α, IL-1β, did not differ among the 4-time points in non-SIRS patients.

Conclusions. Upregulation of TLR2/4 expression on PBMC may contribute to the development of postoperative SIRS during perioperative period of LT.

1. Introduction

Orthotopic liver transplantation (OLT) is the most effective and the best therapeutic solution for final stage liver diseases. More than 60,000 patients receive OLT every year worldwide [1]. Systemic inflammatory response syndrome (SIRS) often accompanies sepsis, trauma, hypoxia, and ischemia-reperfusion injury (IRI) [2–4]. The activation of mononuclear phagocytes, and consequently release of massive amount of proinflammatory cytokines may lead to multiorgan dysfunction syndrome (MODS) [3, 4]. SIRS is a common feature after major surgery [5, 6]. In recent years, SIRS has been interpreted as a warning sign for postoperative complications and organ failure [7–9]. Specifically, longer SIRS duration has been associated with poor outcomes after surgery [5, 6, 8, 9].

Toll-like receptors (TLRs) play an important role in many pathophysiological processes such as inflammation and IRI [10–14]. TLR2 and TLR4 are members of the TLRs family [13, 14], and could initiate inflammatory responses to various stimuli [2, 15].

A previous study in this laboratory revealed increased expression of TLR2/4 in mononuclear and proinflammatory cytokines in liver transplantation [16]. Based on this finding, we speculate that TLR2/4 may also contribute to the development of SIRS during OLT. In the current study, we examined the expression of TLR2/4 on PBMC in a group of OLT patients with SIRS, and compared the results to that in a group of patients without SIRS.

2. Patients and Methods

This study was approved by the Research Ethics Board of The Third Affiliated Hospital, Sun Yat-sen University. Written informed consent was obtained from all patients prior to the enrollment.
2.1. Study Population. Twenty-seven patients (24 males and 3 females) with end-stage liver diseases undergoing modified piggyback liver transplantation were enrolled. Among these patients, 12 had hepatitis B cirrhosis, 8 had small liver cancer (tumor diameter < 3 centimeter) on a hepatitis B background, 4 had chronic severe hepatitis B and the remaining 3 had drug-related acute liver failure. Liver function based on modified Child-Pugh classification [17] was A in 10 patients, B in 5 patients, and C in the remaining 12 patients. Physical status of the patients was III or IV according to the American Society of Anesthesiologists (ASA) classification (Table 1).

The status of organ donors was cardiac death in 8 cases, brain death in 10 cases, and living relatives in 9 cases. Warm ischemia for donation after cardiac death was 3 to 4.5 minutes (Table 2). There was no warm ischemia in other cases.

2.2. Anesthesia. Anesthesia was induced with intravenous (i.v.) fentanyl and propofol. Tracheal intubation was facilitated with rocuronium. The lungs were mechanically ventilated with oxygen (50%). Partial pressure of carbon dioxide (PetCO2) was maintained at 30–35 mmHg. Anesthesia was maintained with isoflurane and intermittent i.v. of fentanyl and propofol. Tracheal intubation was facilitated with oxygen (50%).

2.3. Surgical Procedure. All patients received modified piggyback liver transplantation with venous reformation and no veno-venous bypass (VVBP). Surgical management of first and second hepatic hilums is similar to classic orthotopic liver transplantation but without short hepatic vein disposal. After disconnection of the first hepatic hilum, vena cava was interrupted by a satinsky clamp from the back of the liver. Vena cava of second hepatic hilum was blocked by a Klintmalm liver clamp. The liver was then removed. The openings of hepatic veins on the anterior wall of the vena cava were connected to form an open inverted triangular cuff. The posterior wall of the donor inferior vena cave (IVC) was incised to fashion a wide-open inverted triangular cuff that matched the IVC opening in the recipient. The openings were closed with 4–0 Prolene suture. The graft was flushed with 400 to 800 mL cold FFP. Donor infrahepatic vena cava was ligated, and the portal vein was anastomosed. The clamps were then removed to allow reperfusion. Hepatic artery and bile duct anastomoses were completed [18–20].

The entire procedure consisted of an anhepatic phase (from vascular clamping to reperfusion of portal vein and inferior vena cava) and a neohepatic phase (from reperfusion of donor liver to the end of operation).

2.4. Collection of General Data. The demographic data as well as the Child-Turcotte-Pugh (CTP) scores, ASA classification, duration of the operation, volume of blood loss and input were collected. Duration of postoperative mechanical ventilation, alanine aminotransferase (ALT), aspartate aminotransferase (AST), prothrombin time (PT), blood urea nitrogen (BUN), and serum creatinine (SCr) were also recorded.

2.5. Collection of Blood Samples. Whole blood (4 mL) was collected at T1 (after induction of anesthesia), T2 (25 minutes after the beginning of anhepatic phase), T3 (3 hours after graft reperfusion), and T4 (24 hours after graft reperfusion). Two mL of blood sample was collected in EDTA tubes for analysis of TLR2/4 immediately with flow cytometry. The remaining two mL of blood sample was collected in dry tubes for TNF-α, IL-1β, and IL-8 assay.

2.6. Analysis of TLR2/4 Expression. Twenty μL alphycoy cyanin (APC) antihuman CD14 (eBioscience) plus 20 μL fluorescein isothiocyanate (FITC) antihuman Toll-like receptor 2 (eBioscience, San Diego, California, USA) or phycoerythrin (PE) antihuman Toll-like receptor 4 (eBioscience) were added to 100 μL of EDTA treated blood. The mixture was incubated for 20 minutes in the dark at ambient temperature. After which, they were mixed with 2 mL of RBC Lysis Buffer (eBioscience) in the dark for 15 minutes and then centrifuged for 5 minutes at 300 g. After rinsing twice, the supernatant was discarded and sample was preserved at 4°C in the dark. Samples were quantified with FACs Calibur flow cytometry (Becton Dickinson, Franklin Lakes, New Jersey, USA). The isotype controls were FITC mouse IgG2a and PE mouse IgG2a (eBioscience).

2.7. Cytokine Assay. TNF-α, IL-1β, and IL-8 were measured with ELISA (Rapidbio, West Hills, California, USA).

2.8. Perioperative SIRS Monitoring. Patients’ temperature, heart rate, respiratory rate, and white blood cell count were assessed every 6 hours for 7 days after the operation. The diagnosis of SIRS was based on the presence of two or more of the following criteria [21], verified by an ICU physician as well as an anesthesiologist: (1) temperature >38°C or <36°C,
Table 2: Postoperative SIRS in patients receiving different categories of transplant.

| Features | Total | SIRS | non-SIRS |
|----------|-------|------|----------|
| n        | 27    | 12   | 15       |
| Gender (M : F) | 24 : 3 | 11 : 1 | 13 : 2 |
| Age (years) | 47 ± 11 | 48 ± 11 | 47 ± 12 |
| Weight (kilogram) | 64 ± 10 | 61 ± 8 | 66 ± 11 |
| CTP score (A : B : C) | 10 : 5 : 12 | 4 : 2 : 6 | 6 : 3 : 6 |
| Ascites (mL) | 622 ± 1305 | 775 ± 983 | 500 ± 1538 |
| Urine (mL) | 1541 ± 699 | 1455 ± 567 | 1609 ± 801 |
| Volumes of blood loss (mL) | 3011 ± 1286 | 3617 ± 1380* | 2527 ± 1004 |
| Concentrated red blood cell (mL) | 989 ± 536 | 1225 ± 554* | 800 ± 453 |
| Pre-anhepatic phase (min) | 105 ± 44 | 125 ± 55* | 89 ± 24 |
| Anhepatic phase (min) | 46 ± 22 | 55 ± 30* | 38 ± 8 |
| Neohepatic phase (min) | 252 ± 46 | 242 ± 26 | 259 ± 57 |
| Total operation time (min) | 402 ± 65 | 422 ± 65 | 386 ± 63 |

Mean ± SD or median (Q). *P < .05, compared with non-SIRS.

(2) heart rate >90 per minute, (3) respiratory rate >20 per minute or PaCO₂ < 32 mmHg, and (4) white blood cell count >12,000/mL, <4,000/mL, or >10% immature (band) forms.

2.9. Data Analysis. The data are expressed as mean ± standard deviation. One-Way ANOVA was used to analyze the difference between the different phases in the same group. Independent-samples t-test was used to analyze the difference between the SIRS and non-SIRS groups. Data of nonnormal distribution are expressed as median (interquartile range) [Median (Q)], and were analyzed by Wilcoxon signed ranks test. Spearman correlation analysis was used to determine the relationship between different measures. P < .05 is considered statistically significant. All data were processed by SPSS12.0 for windows (SPSS Inc., Chicago, Ill, USA).

3. Results

3.1. General Data. Twelve out of 27 patients developed SIRS after OLT (at 6 to 78 hours), and two died of lung infection. There were no significant differences between two groups on graft origination, duration of cold or warm ischemia (Table 2). Blood loss and concentrated red blood cell (RBC) transfusion during the operation were larger in the SIRS group than in the non-SIRS group. The pre-anhepatic phase and anhepatic phase lasted longer in SIRS patients (Table 3). CTP score, age, gender, body weight, ascites, urinary production, the length of neohepatic phase or the entire operation did not differ between the SIRS and non-SIRS groups (Table 3).

Duration of postoperative mechanical ventilation in the SIRS group was significantly longer than non-SIRS group. Hepatic function, renal function, and infection (respiratory tract) after the surgery also did not differ between the 2 groups (Table 4).

3.2. Difference of TLR2/4 Expression on PBMC between SIRS and Non-SIRS Groups. The baseline TLR2 on PBMC was 74% (interquartile range: 25%) and 80% (interquartile range: 28%) in SIRS and non-SIRS groups (P > .05, Figure 1). Baseline TLR4 was 12% (interquartile range: 8%) and 18% (interquartile range: 21%) in SIRS and non-SIRS groups (P > .05, Figure 2). TLR 2 expression was significantly higher at T3 and T4 in comparison to T1 and T2 in the SIRS patients but not in the non-SIRS group.
Figure 1: Representative FACS plots of TLR2 staining on PBMC. (a) SIRS; (b) non-SIRS. The non-specific binding is relatively small relative to specific binding as defined by the isotype controls. Dotted line represents the isotype control.

Figure 2: Representative FACS plots of TLR4 staining on PBMC. (a) SIRS; (b) non-SIRS. The non-specific binding is relatively small relative to specific binding as defined by the isotype controls. Dotted line represents the isotype control.

(Figure 3). Similar changes were found for TLR4 expression (Figure 4).

The expression of TLR2/4, and particularly relative increase at T3/4 over T1, in two died patients were higher than the average.

3.3. Difference of the Serum Levels of TNF-α, IL-1β and IL-8 between SIRS and Non-SIRS Groups. Baseline TNF-α, IL-1β, and IL-8 was 90 (interquartile range: 118), 34 (interquartile range: 239), and 163 (interquartile range: 181) pg/mL in SIRS group, and 96 (interquartile range: 488), 38 (interquartile range: 161), and 64 (interquartile range: 173) pg/mL in the non-SIRS group, respectively. Serum TNF-α, IL-1β, and IL-8 was significantly higher at T3 and T4 in comparison to T1 and T2 in the SIRS group, but not in the non-SIRS group (Figures 5, 6, and 7).

3.4. Correlation Analysis. There was no relationship between TLR2/4 and CTP score. The expression of TLR4, but not TLR2, was positively correlated to serum TNF-α
compared with T1/T1.

KappaB and activator protein-1 (AP-1) in response to surgical trauma, hemorrhage, and ischemia-reperfusion injury. Incidence of postoperative SIRS in our study is 44%. To surgical trauma, hemorrhage, and ischemia-reperfusion injury [2–4, 21]. A cardinal feature of SIRS is the activation of inflammatory cells such as monocyte-macrophages, neutrophils, and massive release of proinflammatory cytokines [2, 4]. SIRS is common in OLT due to surgical trauma, hemorrhage, and ischemia-reperfusion injury. Incidence of postoperative SIRS in our study is 44%. TLR2/4 on immune cells can activate nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) in response to a variety of pathological conditions, which in turn initiate or amplify inflammation, and ultimately, organ injury [11–14]. Lipopolysaccharide (LPS) can induce TLR4 gene expression in granulocyte and endothelial cells, and activate NF-κB and the production of TNF-α, IL-6, and IL-8 [22]. TLR4 antibody can inhibit activation of NF-κB and production of inflammatory cytokines [23]. Previous studies also demonstrated that high expression of TLR4 is positively correlated with ischemia-reperfusion injury [24]. Importantly, the transcriptional and translational signal of TLR2/4 in mononuclear cell was upregulated significantly in SIRS patients [25]. Although there was no significant difference in CTP scores between the SIRS and non-SIRS groups, the expression of TLR2/4 on PBMC and serum proinflammatory cytokines at T3 and T4 were significantly higher in the SIRS group, suggesting that high expression of TLR4 in OLT patients is associated with SIRS.

Previous studies indicated that cytokine, endotoxin are involved in the regulation of TLR2/4 expression [26–30]. Within the context of liver transplantation, factors that could

Table 4: Postoperative clinical characteristics in SIRS versus non-SIRS.

|                          | SIRS (n = 12) | non-SIRS (n = 15) |
|--------------------------|--------------|-------------------|
| Duration of mechanical ventilation (h) | 19 (44) * | 11 (9) |
| ALT (U/L)                | 530 ± 268    | 655 ± 410         |
| AST (U/L)                | 136 (153)    | 60 (112)          |
| PT (s)                   | 17 ± 4       | 16 ± 2            |
| BUN (mmol/L)             | 19 ± 11      | 13 ± 6            |
| Cr (μmol/L)              | 102 ± 56     | 92 ± 40           |
| Pulmonary infection      | 9/12         | 6/15              |

Mean ± SD or median (Q), *P < .05, compared with the non-SIRS group. ALT = alanine aminotransferase; AST = aspartate aminotransferase; PT = prothrombin time; BUN = blood urea nitrogen; SCr = serum creatinine. Duration of postoperative mechanical ventilation in the SIRS group was significantly longer than non-SIRS group. Hepatic function, renal function and pulmonary infection after the surgery also did not differ between the 2 groups.

(r = 0.310, P = .029), and IL-8 (r = 0.304, P = .025) in the SIRS group but not in the non-SIRS group. In the SIRS patients, the increase of TLR4 at T4 was positively correlated with the length of anhepatic phase (r = 0.688, P = .013).

4. Discussion

The current study demonstrated that expression of TLR2/4 on PBMC and concentration of inflammatory cytokines after liver transplant reperfusion were significantly higher in patients with SIRS than those without.

SIRS is an inflammatory state caused by serious trauma, and infection [2–4, 21]. A cardinal feature of SIRS is the activation of inflammatory cells such as monocyte-macrophages, neutrophils, and massive release of proinflammatory cytokines [2, 4]. SIRS is common in OLT due to surgical trauma, hemorrhage, and ischemia-reperfusion injury. Incidence of postoperative SIRS in our study is 44%.

TLR2/4 on immune cells can activate nuclear factor kappa B (NF-κB) and activator protein-1 (AP-1) in response...
upregulate TLR2/4 may include prolonged surgery, massive blood loss and transfusion, liver ischemia/reperfusion, translocation of enteric microbes during portal vein occlusion and reopening, and ischemia-reperfusion injury of graft [31–33]. Specifically, blood loss, the length of prehepatic phase and anhepatic phase may be the most important factors for upregulation of TLR2/4 expression in PBMC after OLT.

In conclusion, our findings suggest that upregulation of TLR2/4 on PBMC could initiate SIRS after major surgery such as OLT.

Acknowledgments

This study was supported by grants from the National Natural Science Foundation of China (no. 30972858), Natural Science Foundation of Guangdong Province, China (no. 7001567), and the Science and Technology Project Foundation of Guangdong Province, China (no. 2007B031511009). Z. Hei and X. Chi contributed equally to this study.

References

[1] M. Manyalich, A. N. Costa, and G. Paze, “2008 International donation and transplantation activity. IRODaT preliminary data,” Organs, Tissues & Cells, vol. 12, pp. 83–88, 2009.
[2] K. Zacharowski, P. A. Zacharowski, and A. Koch et al., “Toll-like receptor 4 plays a crucial role in the immune-adrenal response to systemic inflammatory response syndrome,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 16, pp. 6392–6397, 2006.
[3] A. E. Baue, “MOF, MODS, and SIRS: what is in a name or an acronym?” Shock, vol. 26, no. 5, pp. 438–449, 2006.
[4] N. Matsuda and Y. Hattori, “Systemic inflammatory response syndrome (SIRS): molecular pathophysiology and gene therapy,” Journal of Pharmacological Sciences, vol. 101, no. 3, pp. 189–198, 2006.
[5] T. A. Hassen, S. Pearson, P. A. Cowled, and R. A. Fitridge, “Preoperative nutritional status predicts the severity of the systemic inflammatory response syndrome (SIRS) following major vascular surgery,” European Journal of Vascular and Endovascular Surgery, vol. 33, no. 6, pp. 696–702, 2007.
[6] K. Kasahara, Y. Yajima, and C. Ikeda et al., “Systemic Inflammatory Response Syndrome and postoperative complications after orthognathic surgery,” The Bulletin of Tokyo Dental College, vol. 50, no. 1, pp. 41–50, 2009.
[7] S. J. Hanssen, J. P. Derikx, and I. C. Vermeulen Windsant et al., “Visceral injury and systemic inflammation in patients undergoing extracorporeal circulation during aortic surgery,” Annals of Surgery, vol. 248, no. 1, pp. 117–125, 2008.
[8] I. Dimopoulou, A. Armaganidis, and E. Douka et al., “Tumour necrosis factor-alpha (TNFa) and interleukin-10 are crucial mediators in post-operative systemic inflammatory response and determine the occurrence of complications after major abdominal surgery,” Cytokine, vol. 37, no. 1, pp. 55–61, 2007.
[9] D. Mokart, M. Leone, and A. Sannini et al., “Predictive perioperative factors for developing severe sepsis after major surgery,” British Journal of Anaesthesia, vol. 95, no. 6, pp. 776–781, 2005.
[10] K. Takenaka, E. Ogawa, H. Wada, and T. Hirata, “Systemic inflammatory response syndrome and surgical stress in thoracic surgery,” Journal of Critical Care, vol. 21, no. 1, pp. 48–53, 2006.
[11] D. N. Cook, D. S. Pisetsky, and D. A. Schwartz, “Toll-like receptors in the pathogenesis of human disease,” Nature Immunology, vol. 5, no. 10, pp. 975–979, 2004.
[12] N. J. Gay, M. Gangloff, and A. N. R. Weber, “Toll-like receptors as molecular switches,” Nature Reviews Immunology, vol. 6, no. 9, pp. 693–698, 2006.
[13] R. Salomão, P. S. Martins, and M. K. C. Brunialti et al., “TLR signaling pathway in patients with sepsis,” Shock, vol. 30, no. 1, pp. 73–76, 2008.
[14] T. Kawai and S. Akira, “TLR signaling,” Seminars in Immunology, vol. 19, no. 1, pp. 24–32, 2007.
[15] J. C. Leemans, G. Stokman, and N. Claessen et al., “Renal-associated TLR2 mediates ischemia/reperfusion injury in the kidney,” Journal of Clinical Investigation, vol. 115, no. 10, pp. 2894–2903, 2005.
[16] X.-J. Chi, J. Cai, and C.-F. Luo et al., “Relationship between the expression of Toll-like receptor 2 and 4 in mononuclear cells and postoperative acute lung injury in orthotopic liver transplantation,” Chinese Medical Journal, vol. 122, no. 8, pp. 895–899, 2009.
[17] G. Tarantino, V. Citro, and P. Esposito et al., “Blood ammonia levels in liver cirrhosis: a clue for the presence of portosystemic
collateral veins,” BMC Gastroenterology, vol. 9, article 21, pp. 1–11, 2009.

[18] Y. M. Wu, M. Voigt, and S. Rayhill et al., “Suprahepatic venacavaplasty (cavaplasty) with retrohepatic cava extension in liver transplantation: experience with first 115 cases,” Transplantation, vol. 72, no. 8, pp. 1389–1394, 2001.

[19] A. Mehrabi, Z. A. Mood, and H. Fonouni et al., “A single-center experience of 500 liver transplants using the modified piggyback technique by Belghiti,” Liver Transplantation, vol. 15, no. 5, pp. 466–474, 2009.

[20] G. H. Chen, M. Q. Lu, and C. J. Cai et al., “Prophylaxis and treatment of operation-correlated complications in orthotopic liver transplantation,” Zhonghua Wai Ke Za Zhi, vol. 44, no. 5, pp. 295–297, 2006.

[21] R. C. Bone, R. A. Balk, and F. B. Cerra et al., “Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis,” Chest, vol. 101, no. 6, pp. 1644–1653, 1992.

[22] S.-L. Fu, Y.-H. Hsu, and P.-Y. Lee et al., “Dioscorin isolated from Dioscorea alata activates TLR4-signaling pathways and induces cytokine expression in macrophages,” Biochemical and Biophysical Research Communications, vol. 339, no. 1, pp. 137–144, 2006.

[23] E. Czeslick, A. Struppert, A. Simm, and A. Sablotzki, “E5564 (Eritoran) inhibits lipopolysaccharide-induced cytokine production in human blood monocytes,” Inflammation Research, vol. 55, no. 11, pp. 511–515, 2006.

[24] A. Shimamoto, A. J. Chong, and M. Yada et al., “Inhibition of Toll-like receptor 4 with eritoran attenuates myocardial ischemia-reperfusion injury,” Circulation, vol. 114, no. 1, pp. I270–I274, 2006.

[25] L. Armstrong, A. R. L. Medford, K. J. Hunter, K. M. Uppington, and A. B. Millar, “Differential expression of Toll-like receptor (TLR)-2 and TLR-4 on monocytes in human sepsis,” Clinical and Experimental Immunology, vol. 136, no. 2, pp. 312–319, 2004.

[26] A. Visintin, A. Mazzoni, J. H. Spitzer, D. H. Wyllie, S. K. Dower, and D. M. Segal, “Regulation of Toll-like receptors in human monocytes and dendritic cells,” Journal of Immunology, vol. 166, no. 1, pp. 249–255, 2001.

[27] D. Bosisio, N. Polentarutti, and M. Sironi et al., “Stimulation of Toll-like receptor 4 expression in human mononuclear phagocytes by interferon-γ: a molecular basis for priming and synergism with bacterial lipopolysaccharide,” Blood, vol. 99, no. 9, pp. 3427–3431, 2002.

[28] M. Muzio, N. Polentarutti, D. Bosisio, M. K. P. Prahladan, and A. Mantovani, “Toll-like receptors: a growing family of immune receptors that are differentially expressed and regulated by different leukocytes,” Journal of Leukocyte Biology, vol. 67, no. 4, pp. 450–456, 2000.

[29] Y. Mita, K. Dobashi, and K. Endou et al., “Toll-like receptor 4 surface expression on human monocytes and B cells is modulated by IL-2 and IL-4,” Immunology Letters, vol. 81, no. 1, pp. 15–77, 2002.

[30] M. T. Abreu, E. T. Arnold, and L. S. Thomas et al., “TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells,” The Journal of Biological Chemistry, vol. 277, no. 23, pp. 20431–20437, 2002.

[31] T. Ikeda, K. Yanaga, K. Kishikawa, S. Kakizoe, M. Shimada, and K. Sugimachi, “Ischemic injury in liver transplantation: difference in injury sites between warm and cold ischemia in rats,” Hepatology, vol. 16, no. 2, pp. 454–461, 1992.

[32] A. Katsargyris, C. Klonaris, A. Alexandrou, A. E. Giakoustidis, I. Vasilieou, and S. Theocharis, “Toll-like receptors in liver ischemia reperfusion injury: a novel target for therapeutic modulation?” Expert Opinion on Therapeutic Targets, vol. 13, no. 4, pp. 427–442, 2009.

[33] E. Abdala, C. E. Baia, and S. Mies et al., “Bacterial translocation during liver transplantation: a randomized trial comparing conventional with venovenous bypass vs. piggyback methods,” Liver Transplantation, vol. 13, no. 4, pp. 488–496, 2007.