Introduction. Observing the effects of high-volume hemofiltration (HVHF) treatment on the monocytes apoptosis, antigen presentation, and secretion function, this study investigated the mechanism of HVHF effect on immunity homeostasis in multiple organ dysfunction syndrome (MODS) in an animal model.

Materials and Methods. Lipopolysaccharides were administered in 12 Beagle dogs in order to induce MODS. Six dogs were randomly assigned to receive HVHF treatment for 12 hours (HVHF group) and the rest did not receive any treatment (the MODS group). The expression of DLA-DR, apoptosis, and cytokine levels were measured at 7 time points: normal condition (T1), after operation (T2), and zero, 3, 6, 9, and 12 hours after endotoxin injection (T3 to T7, respectively).

Results. Apoptosis of CD14+ mononuclear cell increased in early and late stages gradually in the MODS group and began to decline gradually after the HVHF treatment. There was a significant difference between the two groups at time points T2 to T7 ($P < .01$). After HVHF, the impaired expression of dog leukocyte antigen-DR showed an improvement trend in the HVHF group, which was significant better at T5 and T7 than that in the MODS group ($P < .05$). Interleukin-4 secretion increased significantly with HVHF and was significantly higher at time points T4 to T7 as compared with the MODS group ($P < .01$).

Conclusions. High-volume hemofiltration can alleviate the mononuclear cell apoptosis, improve antigen-presenting function and secretion function, inhibit the release of inflammatory factors, and maintain immune homeostasis, and consequently alleviate symptoms of MODS effectively.
High-volume hemofiltration (HVHF) has shown beneficial effects in severe sepsis and multiple organ failure, improving hemodynamics and fluid balance, which is helpful to alleviate the inflammatory conditions. Indications of the HVHF are a wide spectrum, from treatment of acute liver or kidney failure to treatment of necrotic pancreatitis and even all the organs related to the immune system. The association of plasma cytokine reduction and cellular immune function recovery in MODS and HVHF treatment needs further investigation.

It has been confirmed that HVHF (6 L/h) can clear the plasma myocardial inhibitory factor of septic shocked pig, and effectively remove a variety of inflammatory mediators of infection in MODS, especially the large and medium-sized molecular.

Both sepsis and MODS are characterized by an influx of macrophages and neutrophils into the plasma, with concurrently increased peripheral blood monocyte activation. Infection and inflammation are strongly implicated in the pathology of many infectious diseases, with HVHF considered a promising candidate for its prevention or treatment. In this study, we investigated changes in plasma monocytes in response to lipopolysaccharides, inflammation agonists used to trigger MODS in vivo, and the effects of HVHF on the monocyte function and immunity homeostasis for the treatment of dogs with multiple organ failure.

MATERIALS AND METHODS

Animal Model

A total of 12 male and female Beagle dogs (15 ± 2 kg body weight) were provided by the Experimental Animal Center of Xinjiang Medical University. At least 2 weeks before the experiment, 12 Beagle dogs were standardized fed in constant temperature environment. The dogs were kept off food and water for 24 hour before the experiment. They were randomly assigned to the MODS group (n = 6) and HVHF group (n = 6) after modelling. The study was approved by the Ethics Committee of Xinjiang Medical University.

Hemorrhagic shock was caused using the method of Wiggers. The mean arterial pressure was maintained between 6.0 kPa and 7.3 kPa for 1 hour, preceding the blood loss and rapid transfusion an amount of Ringer solution equal to 2 volumes of the blood loss. Twelve hours later, the endotoxin (Escherichia coli serotype 0111:B4, Sigma, USA) was intravenously administered at 0.125 mg/kg/h for 12 hours. The dogs in the MODS group received no treatment, while the HVHF group received HVHF treatment for 12 hours.

High-volume Hemofiltration

Intelligent bedside renal replacement therapy machines (Prisma, Sweden) were used. With a membrane area of 0.9 m², polyacrylonitrile membrane AN-69 filter blood filter had strong adsorption and permeability. Molecules weighing from 20 Da to 30000 Da were filtered. The vascular access was established by an indwelling double-lumen catheter in the right internal jugular vein or the femoral vein. The blood flow was set at 180 mL/min; replacement fluid velocity, at 200 mL/kg/h; and the treatment time, at 12 hours. Replacement fluid formula included bicarbonate, 3.35 mmol/L; calcium, 2.3 mmol/L; sodium, 143.6 mmol/L; potassium, 3.79 mmol/L; chloride, 116.7 mmol/L; magnesium, 1.57 mmol/L; and glucose, 6 mmol/L. The circuit was primed with heparinized saline (5000 U of heparin in 1000 mL of normal saline), and HVHF was started with an initial dose of 1000 U/kg, and followed by an intravenous infusion of 500 U/h.

Specimen Collection Time

Biochemical index were observed at 7 time points in this study: normal condition (T1), after operation (T2), and zero, 3, 6, 9, and 12 hours after endotoxin injection (T3 to T7, respectively). Specimens were collected into the heparinized tubes at these time points for immediate processing.

Isolation of Peripheral Monocytes

Peripheral blood was suspended in phosphate-buffered saline (PBS) containing 0.2 mmol/L of ethylenediaminetetraacetic acid disodium, and it was put on lymphocyte separation liquid, which made the cell suspension and separation liquid volume ratio of 2:1. The solution was centrifuged at 2000 rpm for 40 minutes. A glass straw aspirated membranoid individual nucleus cells was used and washed 3 times in PBS. The peripheral blood mononuclear cells were suspended in serum containing 5.0 mol/L of ethylenediaminetetraacetic acid disodium.
acid after incubation for 20 minutes, and were washed 2 times in PBS. Isolated peripheral blood mononuclear cells were suspended in RPMI-1640 (Gibco, USA) and incubated for 20 minutes. Hank solution was used to remove nonadherent lymphocytes. Adherent peripheral blood mononuclear cells with PBS containing 0.2 mmol/L of ethylenediaminetetraacetic acid were washed 3 times in Hank solution. The peripheral monocytes were prepared for analysis.

**Apoptosis of Monocytes**

Annexin V assay was used to measured cell apoptosis. Mononuclear cells (5 × 10^5 cells/mL) were washed with PBS once. The cells in the sediment were incubated with 20µL of Annexin V (Boehringer, Germany), 20 µL of propidium iodide, and 1 mL of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid at room temperature for 15 minutes. Then, 0.4 mL of incubation solution was added and the cells were detected using flow cytometry.

**Monocyte Antigen Presentation Function**

The monocytes antigen presentation function was determined by measurement of dog lymphocyte antigen (DLA)-DR expression. Flow cytometry was used to detect the positive rate of human leukocyte antigen-DR CD14+ cells.

**Cytokine Measurement**

Interleukin (IL)-1β and IL-4 were measured in supernatant by an enzyme-linked immunosorbent assay (R&D, USA).

**Statistical Analysis**

All statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc, Chicago, Ill, USA). Continuous variables were presented in as mean ± standard error of mean. Comparison of the mean values between the two groups was done using independent sample t test. A repeated measurement analysis of variance test was performed to assess changes between the pretreatment period and posttreatment period. P values less than .05 were considered significant.

**RESULTS**

**Changes in Peripheral Blood Cells**

There were no significant differences between
the HVHF group and MODS group concerning the baseline peripheral leukocytes, neutrophils, lymphocytes, and monocytes counts and percentages (Table 1). After treatment with HVHF, leukocyte count and neutrophil percentage decreased significantly in the HVHF group, as compared with MODS group at time points T5 to T7. Lymphocytes percentage and monocytes count and percentage increased and monocytes count and percentage were significantly higher in the HVHF group than that in the MODS group at time points T5 to T7.

**Monocytes Apoptosis and Antigen-presenting Function**

Using immunomagnetic beads, mononuclear cell suspension was sorted to obtain mononuclear cells. As shown in Table 2, the isolated mononuclear cells identified by positive staining of CD14+, the average positive rate of CD14+ was above 85%. Apoptosis of CD14+-mononuclear cell in early and late stages increased gradually in the MODS group and began to decline gradually after the HVHF treatment. There was a significant difference between the two groups at time points T2 to T7 ($P < .01$).

Before the experiment, the expression of DLA-DR of CD14+ mononuclear cells was relatively low. After hemorrhagic shock, the expression of DLA-DR decreased. In the pathological process of the MODS, DLA-DR expression level continued to be relatively low and the difference was significant compared with T1 ($P < .01$). After HVHF treatment, expression of DLA-DR showed an upward trend in the HVHF group. Compared with the MODS group, there were significant differences at T5 and T7 ($P < .05$).

**Monocytes Secretion Function**

Table 3 shows that at time point T1, the levels of monocyte-secreted IL-1β and IL-4 were higher after the stimulation by lipopolysaccharides. While the monocytes had active secretory function in normal state, secretion of IL-1β and IL-4 stimulated by lipopolysaccharides were decreased and maintained at a lower level in the MODS group, the difference of which was significant compared with T1 ($P < .01$).

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### Table 2. Changes in Monocytes Apoptosis and Dog Leukocyte Antigen (DLA)-DR Expression in the High-volume Hemofiltration (HVHF) and the Multiple Organ Dysfunction Syndrome (MODS) Groups

<table>
<thead>
<tr>
<th>Time Point</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>85.08 ± 7.75</td>
<td>82.55 ± 3.42</td>
<td>13.86 ± 3.09</td>
<td>11.20 ± 1.92</td>
<td>12.71 ± 2.78</td>
<td>1.33 ± 1.25</td>
<td>6.52 ± 1.47</td>
<td>11.38 ± 3.95</td>
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</tr>
<tr>
<td>T2</td>
<td>82.55 ± 3.42</td>
<td>88.36 ± 3.62</td>
<td>26.90 ± 5.33</td>
<td>11.05 ± 3.05*</td>
<td>11.40 ± 1.73</td>
<td>0.58 ± 0.64*</td>
<td>1.33 ± 0.61</td>
<td>1.33 ± 0.42†</td>
<td></td>
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</tr>
<tr>
<td>T3</td>
<td>86.40 ± 5.77</td>
<td>85.75 ± 5.56</td>
<td>31.11 ± 8.44†</td>
<td>10.06 ± 1.92*</td>
<td>11.13 ± 2.40</td>
<td>1.85 ± 1.58*</td>
<td>2.15 ± 0.93†</td>
<td>1.92 ± 0.26‡</td>
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</tr>
<tr>
<td>T4</td>
<td>87.03 ± 3.42</td>
<td>88.80 ± 5.23</td>
<td>32.18 ± 3.51†</td>
<td>11.15 ± 1.24*</td>
<td>11.88 ± 2.07</td>
<td>3.55 ± 3.56*</td>
<td>3.47 ± 1.81‡</td>
<td>2.87 ± 0.65‡</td>
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<tr>
<td>T5</td>
<td>85.75 ± 5.56</td>
<td>86.93 ± 2.69</td>
<td>35.88 ± 3.85†</td>
<td>11.75 ± 2.43*</td>
<td>11.15 ± 0.75</td>
<td>1.83 ± 1.43*</td>
<td>2.10 ± 0.96†</td>
<td>4.63 ± 1.88‡</td>
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<tr>
<td>T6</td>
<td>85.80 ± 6.34</td>
<td>84.13 ± 5.61</td>
<td>36.60 ± 5.87†</td>
<td>9.51 ± 2.08*</td>
<td>13.86 ± 4.73</td>
<td>4.00 ± 3.41*</td>
<td>3.02 ± 1.46†</td>
<td>4.95 ± 1.31‡</td>
<td></td>
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<tr>
<td>T7</td>
<td>86.91 ± 5.06</td>
<td>86.55 ± 3.14</td>
<td>38.60 ± 7.08†</td>
<td>10.56 ± 2.52*</td>
<td>12.75 ± 2.30</td>
<td>2.83 ± 1.93*</td>
<td>1.77 ± 1.01†</td>
<td>7.43 ± 3.89‡</td>
<td></td>
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</tr>
</tbody>
</table>

* $P < .01$ compared to MODS group  
† $P < .01$ compared to T1  
‡ $P < .05$ compared to T1  
§ $P < .05$ compared to MODS group

### Table 3. Changes in Monocytes Secretory Function in the High-volume Hemofiltration (HVHF) and the Multiple Organ Dysfunction Syndrome (MODS) Groups

<table>
<thead>
<tr>
<th>Time Point</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
<th>MODS</th>
<th>HVHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>654.17 ± 16.78</td>
<td>655.63 ± 15.44</td>
<td>232.59 ± 9.59</td>
<td>239.06 ± 14.27</td>
<td>94.27 ± 5.58†</td>
<td>99.58 ± 7.27†</td>
</tr>
<tr>
<td>T2</td>
<td>482.08 ± 8.75*</td>
<td>415.02 ± 18.26*</td>
<td>162.29 ± 9.88*</td>
<td>146.79 ± 6.91*</td>
<td>119.81 ± 9.37†</td>
<td>139.23 ± 7.26†</td>
</tr>
<tr>
<td>T3</td>
<td>343.86 ± 12.45†</td>
<td>360.66 ± 13.68†</td>
<td>98.44 ± 7.15*</td>
<td>99.58 ± 7.27†</td>
<td>110.07 ± 2.52*</td>
<td>12.75 ± 2.30</td>
</tr>
</tbody>
</table>

* $P < .01$ compared to MODS group  
† $P < .01$ compared to T1  
‡ $P < .05$ compared to MODS group
With the ongoing HVHF treatment, spectrum of monocyte secretion changed correspondingly. In the HVHF group, IL-4 secretion increased significantly but did not reach the level before the experiment, and compared with the MODS group, there were significant differences at time points T4 to T7 ($P < .01$). The level of IL-1 was maintained low and was not significantly different between the two groups.

**DISCUSSION**

The early stage of MODS is the systemic inflammatory response syndrome. Inflammatory response will go out of control without timely treatment, transforming protective effect of cell factor into damage effect. Apoptosis of cells, immune hypofunction, shock, and organ damage are the subsequent outcomes MODS. Mononuclear cell is one of the important immune cells, and its function changes reflects the immune function to a certain extent. High-volume hemofiltration acts mainly through increased input of conversion liquid to strengthen the removal of large solute convection reaction in the body. Recent studies showed that HVHF could alleviate the MODS through some special mechanisms. In the present study, we observed the effects of HVHF as the treatment of MODS in dogs on the monocyte function, shedding light on the alleviating effect of HVHF on the immune system and its mechanism in MODS.

Cell apoptosis is mediated by genes. Inflammatory immune cells normally involve in apoptosis phenomenon and play an important role in maintaining the balance of immune system. Endotoxin, ischemia, hypoxia, and excessive inflammation can cause the abnormal apoptosis of mononuclear cells in the pathogenesis of MODS. In our experiment, apoptosis of CD14+ mononuclear cell in early and late stages increased gradually in the MODS group and began to decline gradually after HVHF treatment. In the peripheral blood, the monocytes number and percentage in the HVHF group were significantly higher than that in the MODS group 6 to 12 hours after endotoxin injection. The experiment proved that HVHF treatment could effectively reduce the apoptosis of monocytes and significantly increase the number of monocytes, which play an important role in MODS. While the mononuclear cells are activated by endotoxin, pro-inflammatory and anti-inflammatory cytokines release synchronously. Monocytes become mature and enter into the blood circulation to gather in the inflammatory area; the whole process needs to have a certain amount of cytokines and chemokines to reach an equilibrium condition in the microenvironment; otherwise, the mononuclear cells will start apoptosis. Van Bommel and colleagues pointed out that continuous hemofiltration could remove cytokines and cytokine inhibitory factors by convection or adsorption. However, with low volume hemofiltration, there were no significant changes in plasma levels of cytokines or other parameters; thus, HVHF was advocated to reduce the levels of plasma cytokines and cell inhibitory factors. Recent studies have shown that CRRT can exclude inflammatory mediators and is related with the convection, which is the main mechanism in HVHF.

Mononuclear cell DLA-DR expression plays a crucial role in the process of lymphocyte antigen presenting and the start of immune response. In severe infection or trauma, the inflammatory medium is released massively, the expression of DLA-DR on monocytes decrease significantly, and the immunity homeostasis disorder and cell damage develop, leading to the development of MODS. The decreased expression of DLA-DR antigen in MODS was possibly related to the stimulated autonomic nerve humoral regulation system provoked by pro-inflammatory cytokines and anti-inflammatory mediators. While the endogenous and exogenous endotoxins explode in MODS, expression of DLA-DR antigen stimulated by interferon-$\gamma$ can be inhibited by endotoxin. Meanwhile, the significantly increased levels of interferon and endorphin, which plays a negative regulatory role on DLA-DR antigen expression, is another reason that leads to reduction in DLA-DR antigen expression. At the end of the disease, mononuclear cells in peripheral tissues or their DLA-DR antigen synthesis function are consumed too much that is the third reason expression of DLA-DR antigen is reduced. In our MODS model, DLA-DR expression maintained a relatively low level, significantly lower than baseline. After HVHF treatment, expression of DLA-DR showed an upward trend in the HVHF group. Compared with the MODS group, there were significant differences 6 to 12 hours after endotoxin injection. After removal of excess pathogenic medium, HVHF
helped to keep balance of water and electrolyte and maintain a stable internal environment, thus the mononuclear cells in inhibitory state recovered gradually, presentation function improved, and MODS was alleviated.

Interleukin-1β is produced by macrophages and monocytes, and it plays an important role in early stage of systemic and local inflammatory reaction. Interleukin-4 is produced by T helper 2 cells, mast cells, and basophils. As an anti-inflammatory mediator, it has a variety of immune functions, such as the inhibition of produce and release of the inflammatory cells and inflammatory cytokines. In normal condition, monocytes has active secretory function. In the pathologic process of MODS, IL-1β and IL-4 release of monocytes, stimulated by lipopolysaccharides, decreased and maintained at a lower level, demonstrating that MODS may partially inhibit the reaction of immune cells. With the ongoing HVHF treatment, in the HVHF group, IL-4 secretion increased significantly but did not reach the level before the experiment. The level of IL-1 was maintained low in both groups. These data indicate that HVHF treatment patterns clean the inhibition substance of IL-4. The purification effects of some inhibitory substances may have certain significance to the clinical treatment, which need further study in future. In this study, there are still some limitations; the study objects were 12 Beagle dogs, and the sample size was small. On the other hand, the indicators concerning about the immune system and HVHF were less, only partially but not fundamentally illuminating the mechanism of HVHF in alleviating MODS. Finally, high costs always limit the application of HVHF in clinic.

CONCLUSIONS

High-volume hemofiltration can alleviate the mononuclear cell apoptosis, antigen-presenting function, and secretion function, the potential mechanism including peripheral additional cytokines elimination, monocyte function regulation, inhibiting inflammatory factors level, and maintaining immune homeostasis, thereby relieving MODS symptoms effectively. However, HVHF is nonselective and therefore removes water soluble vitamins, amino acids, trace elements, etc. This potential side effect which needs to be further studied. Meanwhile, it is necessary to monitor serum electrolytes, blood glucose, serum phosphorus, serum calcium, acid-base balance, and antibiotics dose in the process of HVHF treatment. In addition, input of large doses of replacement fluid may reduce the patients’ temperature which may mask signs of infection.

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CONFLICT OF INTEREST

None declared.

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