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Original Article

The relationship between autophagy and apoptosis of lamina propria dendritic cells from the small intestine on the immunodynamics of sepsis mice

Qing-Qing Wu[1*], Shun-Wen Zhang[2*], Jiang-Dong Wu[3], Jie Zhang[1], Jiang-Tao Dong[1], Da-Long Zhang[1], Peng Guo[1], Su-Yu Tang[1], Wan-Jiang Zhang[3], Fang Wu[3]

[1]The First Affiliated Hospital, Shihezi University School of Medicine, Shihezi 832002, Xinjiang, China; [2]The First School of Clinical Medicine, Nanjing Medical University, Nanjing 211166, Jiangsu Province, China; [3]Department of Pathophysiology, Shihezi University School of Medicine/The Key Laboratory of Xinjiang Endemic and Ethnic Diseases/Collaborative Innovation Center of High Incidence of Zoonoses Communicable Disease Prevention in The Western Region, Shihezi 832002, Xinjiang, China. [*]Equal contributors.

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Abstract: *Objective:* To explore the relationship between autophagy and apoptosis of dendritic cells in the lamina propria (LPDCs) from the mouse small intestine in the regulation of immune function against sepsis. *Methods:* EDTA, DTT, and Type IV collagenase were used to isolate the LPDCs. The positive rate of LPDCs was identified by flow cytometry with CD11c antibody marker. Autophagy-associated proteins Beclin-1 and LC3B were detected by Western blot. Rate of LPDCs apoptosis and MHC-II expression were measured by flow cytometry. Meanwhile, the expression of MHC-II in the peripheral blood was measured. Secretion of IL-10, IL-1β, and TNF-α in the peripheral blood serum was detected by ELISA, which was also used to measure the amount of IL-10 and IL-12 secreted in the LPDCs supernatant. *Results*: Expression levels of the LPDC autophagy-associated protein Beclin-1 and LC3B in LPS-induced mice were increased at 1.5 h, compared with the un-induced control group. Peak values of these molecules were observed at 6 h after the induction. Then, the expression levels thusautophagy decreased significantly ($P<0.05$). The apoptosis of the LPDCs began to increase at 1.5 h, reached a maximum activity at 24 h, and then declined. *Conclusions*: (1) The level of autophagy in small intestine LPDCs is correlated with the immune response ability at different stages of sepsis. LPDCs autophagy plays an important role in immune protection of sepsis. (2) During the development of sepsis, the autophagy and apoptosis of murine small intestine and murine lamina propria dendritic cells (LPDCs) are reciprocally regulated. (3) The change in autophagy and apoptosis of LPDCs are related to immunosuppression of sepsis.

Keywords: LPDCs, immunosuppression, autophagy, immunodynamics, apoptosis

Introduction

Immunodynamic abnormality is an important parameter in the pathophysiology of sepsis to elucidate this disease [1]. The development of sepsis follows certain immunodynamic changes and different stages of immune responses [2]. The negative extreme of the immunodynamics of sepsis is the complete loss of immunity, namely, immunoparalysis. The immunodynamics of sepsis enters into the immunosuppressive state as the disease progresses. The key outcome in this process is the major immune disorder of the body, not only because of the high inflammation state caused by multiple system organ damages, but also because of the developed runaway immune activations as a result of severe immunosuppression and immune attack [3]. Sepsis can damage the immune system of the body by reducing survival time, generating harmful products and altering cellular functions of the homeostasis cells [4]. Decreased number of immune cells and deterioration of cellular functions are the manifestations of immunosuppression in the patient body [1]. Thus, the treatment of sepsis cannot achieve a fundamental breakthrough if the issue of sepsis immunosuppression is not resolved. Sepsis also causes intestinal inflammation and barrier dysfunction. With the devel-

opment of clinical nutrition and, critically, ill medicine in the function of the gut barrier, numerous studies have shown that the digestive tract is the largest digestion organ in the body. The intestinal barrier function limits the bacteria and endotoxin in the small intestine under normal circumstance [5]. Abnormal immune cell function may damage the intestinal barrier. Thus, the gut bacteria translocate more easily, and bacterial endotoxins are absorbed more quickly. This event leads to systemic inflammatory response syndrome (SIRS), systemic infection, and multiple organ dysfunction syndrome. Sepsis can lead to the loss of dendritic cells (DCs) in the spleen and blood, among others [6].

During infection, pathogens enter the body, initiating both adaptive immunity and innate immunity [7]. DCs are important immune modulatory cells in the body and participate in innate and adaptive immune responses by secreting different cytokines. DCs are specialized antigen presenting cells whose main function is to ingest, process and present antigens, initiating specific immune responses. Several studies have indicated the key role that DCs may play in sepsis-induced immune suppression [8]. For example, DCs can induce initial T cell activation [9]. Thus, DCs are the initiators of specific body immune response. They are not only killed by systemic inflammatory response, but also contribute to the development of immune suppression during sepsis [6, 8]. Immature DCs have a strong ability to gobble up the intake antigen (including *in vitro* processing) or by some factors to stimulate their differentiation into mature DCs [10, 11]. As an important part of the immune system, DCs are highly significant in the understanding of the occurrence and development of immune dysfunction in sepsis. Previous studies demonstrated that several groups of DCs are present in the lamina propria of the small intestine. However, DCs, as an ethnic group, are also found in different tissues and organs. These DC groups collectively contribute to the maintenance of gut homeostasis by regulating the balance between active immunity and tolerance, and play a key role in protecting the intestinal immune system against pathogen invasion while avoiding the pathologic inflammation [12-15]. The gastrointestinal tract is an important organ for sepsis. Studies have shown that engraftment and translocation of bacteria and toxins occur easily in the gastrointestinal tract during sepsis [16]. The gastrointestinal tract is likely to be the location of infection in patients with secondary infection of sepsis and multiple organ failure. So far, studies on the effects of autophagy of lamina propria dendritic cells (LPDCs) from the mouse small intestine on sepsis remain unknown.

Recently, it is found the differential homing in these distinct sub-compartments of the gut mucosal immune system. It showed that the colonic CD11c+ APCs imprint CD8+ T cell preferentially home to the colon, in contrast to those from the small intestine that imprint CD8+ T cell homing to the small intestine, and that the differences are related to the variable ability of APCs to induce α4β7-integrin and CCR9 expression on T cells [17, 18]. A CD11c+ cell-directed signaling block increased infection of DC only. It has also been suggested that abnormal CD11c+ mononuclear phagocytes such as DCs are involved in the destruction of immune tolerance in the body during low-grade inflammation in irritable bowel syndrome (PI-IBS) after infection [19], which can lead to chronic inflammatory disease development. A study tested the hypothesis that CD11c+ lamina propria mononuclear phagocytes (CD11c+ LPMP) contribute to the increased mucosal permeability and visceral hypersensitivity in a PI-IBS mouse model. CD11c+ LPMP was isolated and purified by digesting intestinal tissue and magnetically activated cells [19]. They demonstrated that CD11c+ LPMP from this PI-IBS mouse model not only has the ability to transfer intestinal inflammation to normal mice but also causes intestinal dysfunction characterized by epithelial barrier breakdown and visceral hyperalgesia.

Apoptosis of DCs in sepsis leads to immunosuppression, resulting in the noneradication of the primary infection and absence of new secondary infection [8, 20]. Apoptosis induced by sepsis is the main mechanism for the reduction of DCs [21]. Caspase-3-induced apoptosis of DCs was observed in a mouse model of sepsis, and inhibition of DC apoptosis was found to alleviate the sepsis and immunosuppression caused by lipopolysaccharide (LPS) [22]. The apoptosis of mature DCs increases the spread of infection; similarly, the apoptosis of immature DCs may also cause the spread of infection [23].

Autophagy is a kind of metabolic process of material degradation in a class of cells. Autophagy is activated in DCs and plays a protective role in sepsis [24, 25]. Numerous studies have shown that autophagy is activated at the start of sepsis, followed by subsequent injury stages [26, 27]. Autophagy may have an important effect in infected cells and mediate trafficking innate and adaptive immunity required events. It helps cells maintain their stability and cope with adverse conditions. Autophagy is widely used in the physiological function of DCs. In vitro-induced differentiation experiments on autophagy may influence monocyte differentiation of DCs. DCs exhibit a strong ability to devour pathogens and participate in the identification, binding and phagocytic processes of viruses and bacteria. These phenomena are closely related to the presence of antigens. If inhibiting autophagy-associated protein (ATG) affects the DC function, changes in the ATG in DCs can significantly increase the susceptibility to diseases. Autophagy is an important homeostatic process in eukaryotic cells, which is critical for the survival of the cells. Damaged cytosolic components are removed and recycled in double-membrane vacuoles, called autophagosomes, that are characterized by the recruitment of microtubule-associated protein 1 light chain 3 (LC3) conjugated to phosphatidylethanolamine (LC3-II) to their membrane. These autophagosomes then fuse with lysosomes and are digested.

There is a complex interrelationship between autophagy and apoptosis. The association between autophagy ('self-feeding') and apoptosis ('suicide') remains the subject of extensive ongoing research. These two cellular processes share common components that regulate and modify each other's activity and can be triggered by common stimuli including starvation, oxidative stress, and cytokines. Apoptosisis the regulator of autophagy activation and thought to be the mechanism of immunosuppression and multiple organ dysfunction after sepsis. Cellular autophagy is also activated in sepsis and helpsmaintaining cell homeostasis and limiting cell damage and death. The three processes that regulate apoptosis by autophagy are as follows: I) Regulating apoptosis through specific autophagy proteins; II) Activating caspase on autophagosome membranes, which depends on autophagosome formation; III) The regulation of apoptosis by autophagy degradation requires autophagosome formation and lysosomal activity. The two mechanisms by which apoptosis regulates autophagy are as follows: I) Direct regulation of autophagy by specific apoptotic proteins; II) Regulation of autophagy by activated cysteine proteases, which requires an overall activation of the apoptotic process.

Recent studies have suggested that immune dysfunction is an important factor in the treatment of sepsis and for the high fatality rate of this disease [28]. The congenital immune system of a sepsis organism involves immune activation and excessive activation of innate immune cells. These processes cause serious and persistent inflammation, characterized by runaway immune responses and an explosive release of a large number of cytokines and inflammatory mediators, referred to as a "cytokine storm" and "waterfall" of inflammatory mediators, in a short period of time [29]. TNF-α first appears during inflammation and is the most important inflammatory mediator. Then, neutrophils and lymphocytes are activated, vascular endothelial cell permeability is increased, and metabolism of other active organizations is regulated. Finally, other cytokines are synthesized and released [30]. Interleukin (IL-1β) is a pro-inflammatory cytokine and widely involved in various pathologic injury processes, such as human tissue destruction and edema [31]. IL-10 is a multi-functional negative regulation factor [32] and an anti-inflammatory factor that plays a role in reducing inflammatory response and antagonizing inflammatory mediators. The immune response can be induced by the cytokine expression of MHC-II type molecule. It plays a protective role in immune function. Therefore, the MHC-II molecule expression is the symbol of antigen oral ability [33].

The study of the antigen presenting cells such as LPDCs and autophagy mechanism in sepsis will provide a theoretical basis for the experiments in further studies. The results will also facilitate a new direction for sepsis immunosuppression diagnosis and provide a new early treatment for sepsis. In this study, LPS experimental animal models of infection were established. We measured the changes of autophagy related proteins and cytokines over a time course of 36 hours after LPS injection. The models showed the effect of autophagy on the changes in the immune function in sepsis. The

results indicate the change of relationship between autophagy and apoptosis during the immune period of sepsis.

Materials and methods

Laboratory animals and materials

Animal origin and management: Clean-grade C57Bl6 mice, aged 7-10 weeks, with a body mass of 200 ± 20 g, were purchased from Xinjiang Medical University (experimental animal production license No. XJYK (New 0013-2012)). Sixty rats were randomly divided into the LPS + 1.5 h, LPS + 6 h, LPS + 12 h, LPS + 24 h, LPS + 36 h and normal control groups before the operation. The mice were housed in a special cage with 10 randomly selected rats per cage.

Main reagents such as FITC anti-mouse/rat CD11c, FITC anti-mouse/rat MHC class II, LPS, RIPA and PMSF were purchased from Sigma. The reagent for apoptosis was purchased from the fir golden bridge biotechnology Company, Beijing. The cytokine proteinsIL-1β, IL-10, TNF-α, IL-12 were obtained from Cloud-Clone Corp. LC3B and Beclin-1 were procured from CST.

Processing methods

The mice were fasted for 12 h and allowed access to drinking water before the experiment.

Sepsis group: The abdomen of the experimental mice was intraperitoneally injected with LPS at a dose of 10 mg/kg. The mice were divided into the LPS + 1.5 h, LPS + 6 h, LPS + 12 h, LPS + 24 h and LPS + 36 h groups, and each mouse was injected with LPS solution with a volume of about 180-250 μL. After the LPS injection, the animals were observed for diarrhoea, piloerection, incontinence, malaise, eating and drinking activities. The success rate of making animal model was 100%. There was no difference in the body weight of sepsis mice from each group. Normal control group: The normal control group was injected with the same dose of normal saline.

Specimen collection and image staining, counting under the microscope

The mice were sacrificed by draining the ophthalmic vein blood. Then, image staining and counting peripheral blood dendritic cells were performed under a microscope.

Detection of MHC-II of peripheral blood mononuclear cells

The peripheral blood was collected in sterile EDTA containing blood vessels and then added with FITC-MHC-II. Flow cytometry was used to detect the MHC-II in the peripheral blood mononuclear cells of the sepsis mice.

Detection of the inflammatory factors

ELISA was used to detect the inflammatory factors in the peripheral blood secreted by sepsis mice. The peripheral blood was removed from the eyes of the sepsis mice using ophthalmic forceps, placed in an EP tube, centrifuged (12,000 × g, 10 min) and then stored at 70°C for further use.

Extraction and identification of LPDCs

The mice were killed by cervical dislocation, submerged in 75% ethanol for 3 min, placed in a sterile paper, and then fixed in supine position. Briefly, (1) The small intestine was gathered and rinsed with 10% PBS solution. The intestines were opened longitudinally. The bowel mesenteric lymph nodes and adipose tissue were carefully removed, cut into small pieces, and washed thoroughly in PBS. The segments were incubated in the PBS containing 1 mM EDTA-Na2, 1 mM dithiothreitol (DTT) and 10% NCS for 40 min on a shaker at 37°C to remove the epithelial layer [14]. They were then incubated with 100 U/ml collagenase IV and 10% NCS in PBS at 37°C on a shaker for 45 min. (2) Digested tissue was passed through 70-μm cell strainers and the cells were washed twice before storage in 5 ml PBS and 10% NCS on ice until use. (3) Fluorescein is othiocyanate-labeled (FITC)CD11C antibody of 5 μL was used to mark cells at 4°C to avoid light flow during cytometry test for a positive rate after 30 min incubation [34, 35]. (4) High-purity LPDCs were isolated from the small intestine, which were used to conduct follow-up experiments. Then, the LPDCs were stained, imaged and counted under a microscope.

Detection of cytokinesand MHC-II of LPDCs

Enzyme-linked immunosorbent assay was also used to detect the secretion of IL-12 and IL-10 in LPDCs supernatant. Flow cytometry was used to detect the expression levels of MHC-II of LPDCs in the sepsis mice.

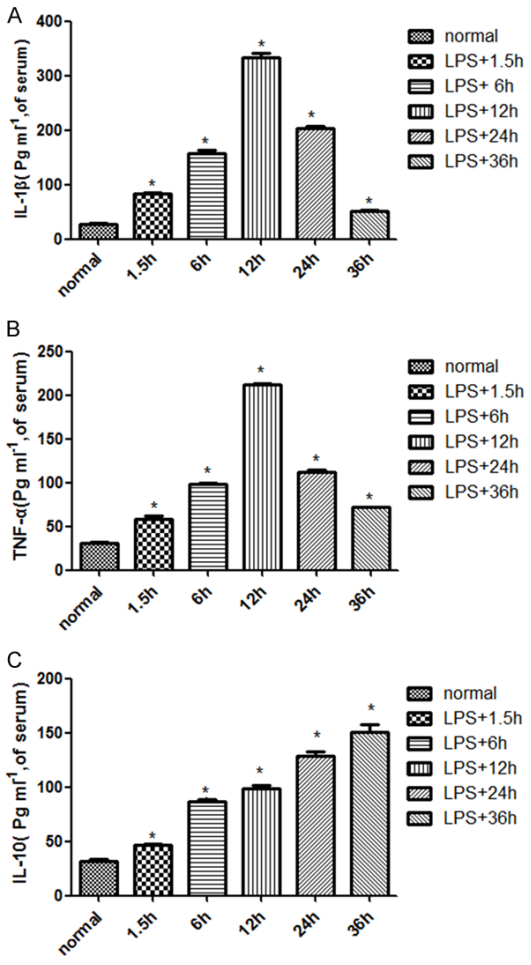

Figure 1. Expression of IL-1β (A), TNF-α (B) and IL-10 (C) in the peripheral blood serum of each group. Note: *indicates a significant difference compared with the normal group (P<0.05).

Detection of the expression of Beclin-1 and LC3B-II

Western blot was used to measure the expression levels of Beclin-1 and LC3 in each group of mice. After boiling of LPDCs in 10% sodium dodecyl sulfate for minutes, the total protein of LPDCs was extracted and then incubated with anti-Beclin-1 antibody and polyclonal anti-goat antibodies. LC3B was treated similarly, but 12% sodium dodecyl sulfate and anti-LC3B antibody used instead. After washing with TBST, the specimens were subjected to anti-chemiluminescence in a dark room and then developed. The film was fixed and then subjected to gel imaging. The expression of LC3B-II and Beclin-1 were proportional to the calculated LC3B II/β-actin and Beclin-1/β-actin expression, and represented as the absorbance ratio.

Statistical analysis

The SPSS 17 software was used for data analysis. Data are presented as mean ± SD with 10 replicates for each treatment at each time point. Different time points were compared with the single factor ANOVA, and the two groups were compared by using t test and curve fitting regression analysis. Differences at P<0.05 were statistically significant.

Results

Animal model

There were individual differences in mice. LPS (10 mg/kg) caused twitching and excitement at 1.5 hours after intraperitoneal injection. After 6 hours, the breathing was cramped and the activity was reduced. The hair was erected and the water drinking was reduced. After 12-hour, the mice showed hair collapse, loose stools, decreased activity, and decreased skin temperature. After 24 hours, most mice showed no signs of life. A few surviving mice showed respiratory distress, incontinence, trembling, and skin temperature decreased earlier. At 36 hours, all the remaining mice died and the sepsis mice were successfully modeled. The mice in the control group had normal activities and could drink water without clinical manifestations of sepsis.

The survival rate was 100% for the animals in the control group even after 36 h injection. There were large differences among injected animals. The LPS + 1.5 h group had 100% survival rate, the LPS + 6 h group, the LPS + 12 h group, the LPS + 24 h group and LPS + 36 h group had 92.44%, 51.21%, 10.03% and 0% survival rate, respectively. We also observed that there is no obvious difference in the body weight between injected and un-injected animals.

IL-1β and TNF-α expression in peripheral serum

The concentrations of IL-1β and TNF-α in peripheral serum were significantly higher at 1.5 h and peaked at 12 h and then continued to decline in the LPS + 24 h and 36 h groups (P<0.05) (**Figure 1A** and **1B**).

The concentration of IL-10 in the peripheral serum significantly elevated in the sepsis groups

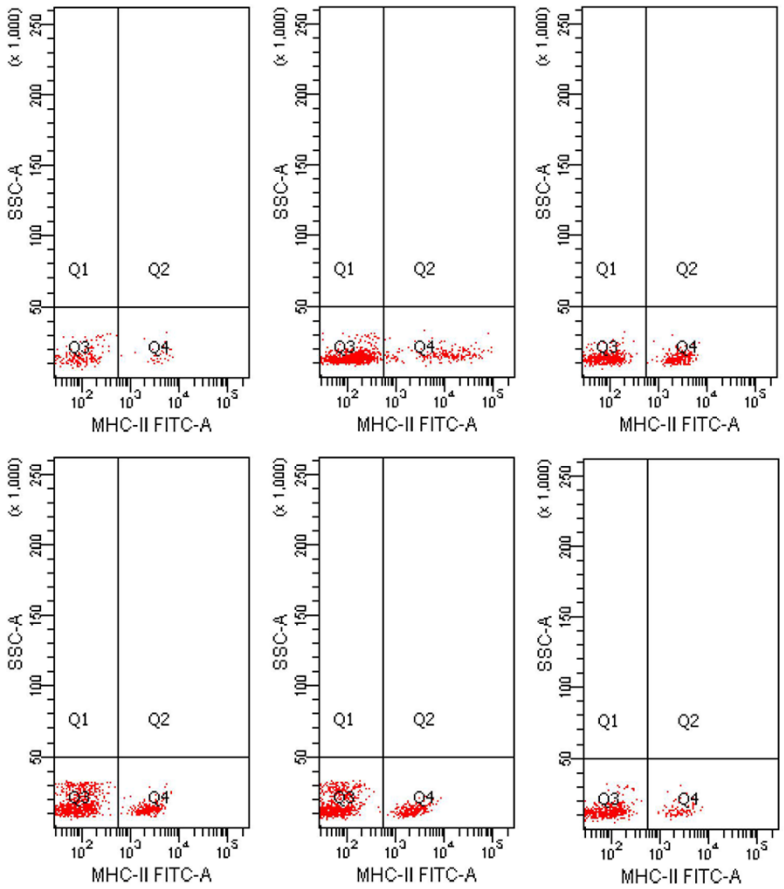

Figure 2. Flow cytometry of MHC-II in the peripheral blood mononuclear cell of each group. Note: From left to right and from top to bottom: normal control group, LPS + 1.5 h, 6 h, 12 h, 24 h, and 36 h groups were sequentially performed. The Q4 quadrant reflects the expression of positive rate of peripheral blood mononuclear cells by FITC-labeled MHC Class II antibody).

compared with the normal group (P<0.05) (**Figure 1C**).

Mononuclear cells MHC-II expression in peripheral blood

The peripheral blood mononuclear cells MHC-II was significantly higher in the LPS + 1.5 h and LPS + 6 h groups than that in the normal group (P<0.05). The peak was at 6 h and then continued to decline (P<0.05) (**Figures 2** and **3**).

Identification of the LPDCs from small intestine

Separation test was difficult to perform because of the small number of DCs in the intestinal mucosa. We developed a new protocol for the separation and used EDTA + DTT and collagenase-IV digestion for the intestinal tissue in mice. LPDCs in mice were prepared in a single cell suspension and identified with anti-CD11c antibody markers for the purity of LPDCs to be used in follow-up tests. The positive rate was 90% ± 1% (**Figure 4**).

Inflammatory mediator and MHC-II expressionin the LPDCs homogenate

The concentrations of IL-10 in the LPDCs homogenate were significantly elevated in the LPS groups compared with the normal group (P<0.05) (**Figure 5A**). The secretion of IL-12 in the LPDCs homogenate was significantly higher at 1.5 h after the LPS injection. The concentrations peaked at 6 h and then continued to decline at 12 h to 24 h (P<0.05) (**Figure 5B**). The expression of MHC-II in the LPDCs was significantly higher at 1.5 h after LPS injection than that in the normal group (P<0.05). The peak was at 6 h and then continued to decline (**Figures 6** and **7**).

LPDCs autophagy expression

The autophagy related proteins Beclin-1 and LC3-II were significantly increased at 1.5 h and peaked at 6 h after the LPS injection compared with that in the normal group. The expression then declined (P<0.05) (**Figure 8**).

LPDCs apoptosis expression

The expression of apoptosis in the LPDCs was significantly increased at 1.5 h compared with that in the normal group after LPS injection (P<0.05). It peaked at 24 h and then declined (**Figures 9** and **10**).

Counting dendritic cells in peripheral blood and LPDCs

The number of dendritic cells in peripheral blood was significantly higher at 1.5 h after the

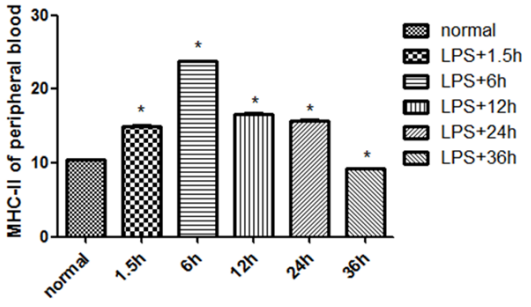

Figure 3. The expression of MHC-II in the peripheral blood mononuclear cell of each group. Note: *indicates a significant difference compared with the normal group (P<0.05).

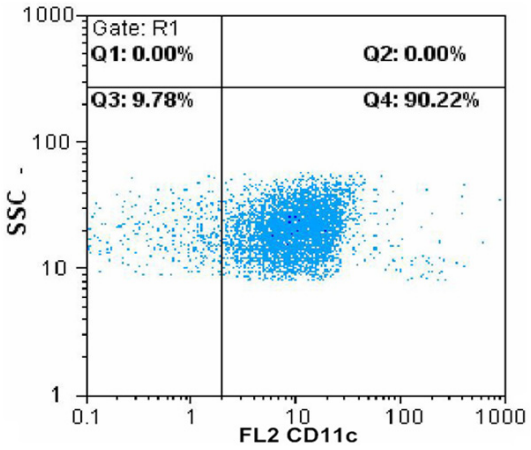

Figure 4. Expression of CD11c positive rate in the LPDCs from the mouse small intestine. As shown in Q4, the purity of LPDCs was 90.22%.

LPS injection than that in the normal group (P<0.05). The peak was reached at 6 h and then continued to decline in each group of mice (**Figure 11A**). The number of LPDCs from the mouse small intestine was significantly higher at 1.5 h after LPS injection than that in the normal group in each group of mice (P<0.05). The peak was reached at 12 h and then continued to decline (**Figure 11B**).

Discussion

This study showed that pro-inflammatory response was strongly increased at 12 h after the LPS-injection in sepsis mice (**Figure 1A** and **1B**). It indicates that the body's immune response is to promote the inflammatory response phase at this stage. However, the secretion of IL-10 continued to increase in the peripheral

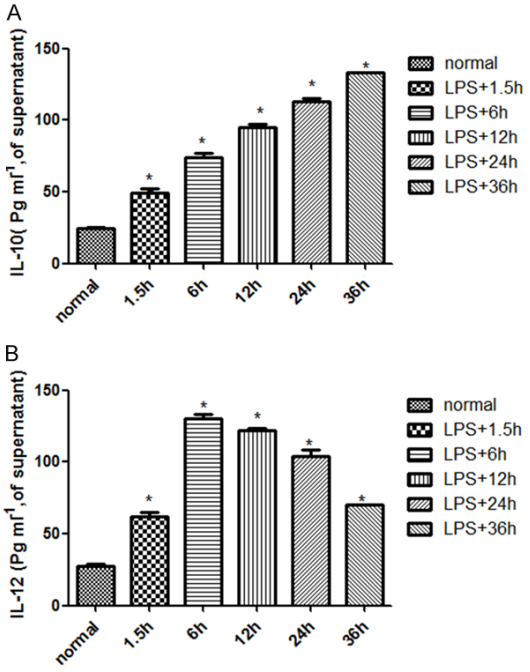

Figure 5. Expression of IL-10 (A) and IL-12 (B) in the supernatant of the LPDCs homogenate from the mouse small intestine of each group. Note: *indicates a significant difference compared with the normal group (P<0.05).

blood serum of the experimental groups after LPS injection (**Figure 1C**). IL-10 suppresses the release of inflammation factors, such as TNF-α caused by a strong pro-inflammatory response [32]. Consequently, the inflammation intensity is diminished, and the runaway sexual inflammation that damages the body is reduced. These events indicate that after 12 h of LPS injection, the body is entering into the compensatory anti-inflammatory response syndrome (CARS) and an endotoxin tolerance stage. These lead to severe immune suppression (immunosuppression) or immune paralysis (immunoparalysis) state, further resulting in serious secondary infection [2]. Our results indicate that high inflammation is not existed alone, inflammatory reaction is at the same time, the sepsis body starts compensatory anti-inflammatory responses.

Sepsis is characterized by the initial overwhelming production of cytokines that promote inflammation, followed by immunosuppression. There is an increasing evidence that autophagic activity increases in the high-power phase of sepsis. In the CLP mouse model, hepatocytes and cardiomyocytes show a rather high LC3-II: LC3-I

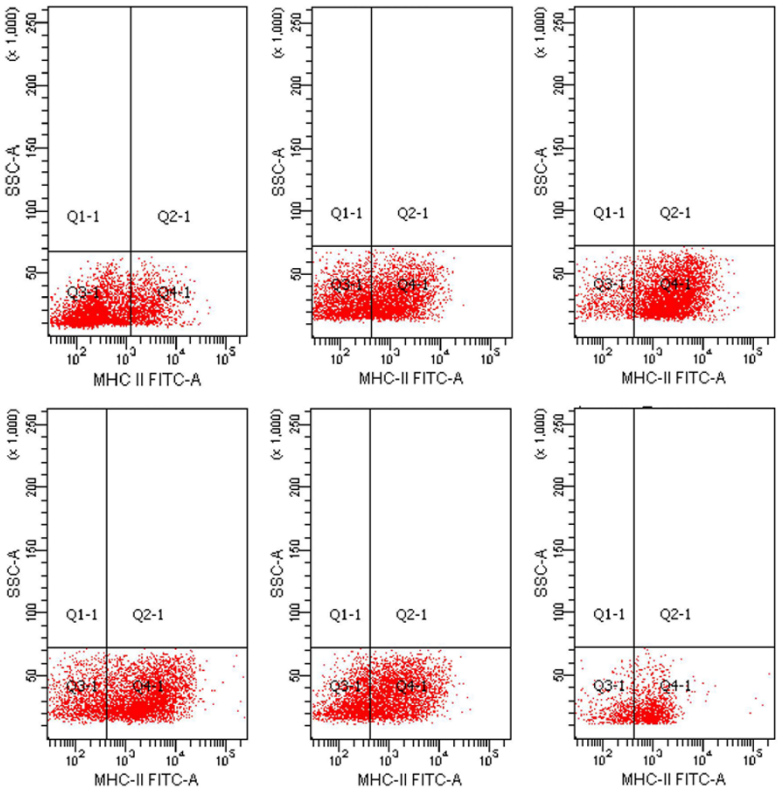

Figure 6. Flow cytometry of MHC-II in the LPDCs from the mouse small intestine of each group. Note: From left to right and from top to bottom: the normal control group and LPS + 1.5 h, 6 h, 12 h, 24 h, and 36 h groups were sequentially performed. The Q4-2 quadrant reflects the expression of positive rates of LPDCs by FITC-tagged MHC Class II antibodies.

ratio (autophagy inducing marker), accompanied by an increase in the number of autophagosomes [24, 36]. In the heart and spleen, the LC3-II: LC3-I ratio was observed to increase with an initial autophagic kinetics with the same pattern, followed by a 24 h decrease [24]. These suggest that autophagy is suppressed after a short period of excessive activity in sepsis. Autophagy associated with immunosuppression remains to be elucidated. Respiratory failure usually occurs in the early stages of sepsis. Expression of autophagy-related proteins (eg, LC3-II, ATG2) decreased early in respiratory failure (at 4 hours) and lasted up to 24 hours [37]. This decrease is associated with increased expression of pro-apoptotic proteins such as Bax and cleaved Capase-3. Experimental induction of autophagy by rapamycin reduces apoptosis and pro-inflammatory cytokines. Lc3 over-expression improves survival and attenuates lung injury through increasing autophagosomal clearance in septic mice [38]. And over-expressing LC3 mice eliminated apoptosis, inflammation, and neutrophil infiltration. Exposure of C57BL/6 mice to LPS for 24 hours showed abnormal mitochondria in the tibialis anterior and soleus muscles, with the increase in the number of autophagosomes, LC3B and ATG14, ATG12, and BECN1 upregulation [39, 40]. In addition, induction of autophagy may increase the rate of protein catabolism. The anti-apoptotic protein Bcl-2 regulates autophagy and apoptosis by binding to autophagy proteins Beclin-1 and Bcl-2 associated proteins (and others) [41, 42]. Beclin-1 represents an important upstream signaling mediator of autophagosome nucleation and interacts with a multiprotein regulatory signalosome. The Bcl-2 binding domain of Beclin-1 serves as an intersection between autophagy and apoptotic signaling pathways at the level of autophagy induction. It has recently been demonstrated that the Beclin-1/Bcl-2 interaction negatively regulates autophagy, providing evidence for the association between autophagy and apoptosis [43].

In this study, we detected the expression levels of the autophagy-associated protein LC3-II and Beclin-1 of LPDCs and observed the highest expression at 6 h in the sepsis mice (**Figure 8**). The expression of LC3-II and Beclin-1 of LPDCs with MHC-II from peripheral blood mononuclear cell had the correlation indices of R^2=0.785 (P=0.019) and R^2=0.605 (P=0.069), respectively. The level of autophagy in small intestine LPDCs is correlated with the immune response ability at different stages of sepsis. The trend of autophagy levels was also the same as that of MHC-II on LPDCs. A previous [44] study and also our data (unpublished) show that autophagosomes can fuse with MHC-II loading compartments. We conclude that the effects of LPDCs antigen presentation correlate to the upregulation and downregulation of the autoph-

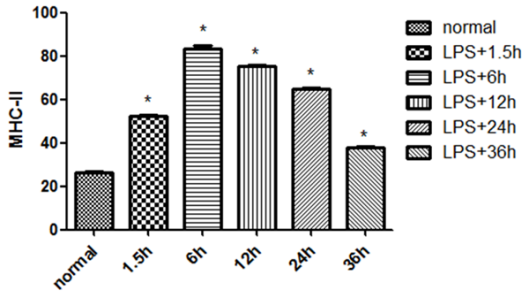

Figure 7. Expression of MHC-II of the LPDCs from the mouse small intestine of each group. Note: *indicates a significant difference compared with the normal group (P<0.05).

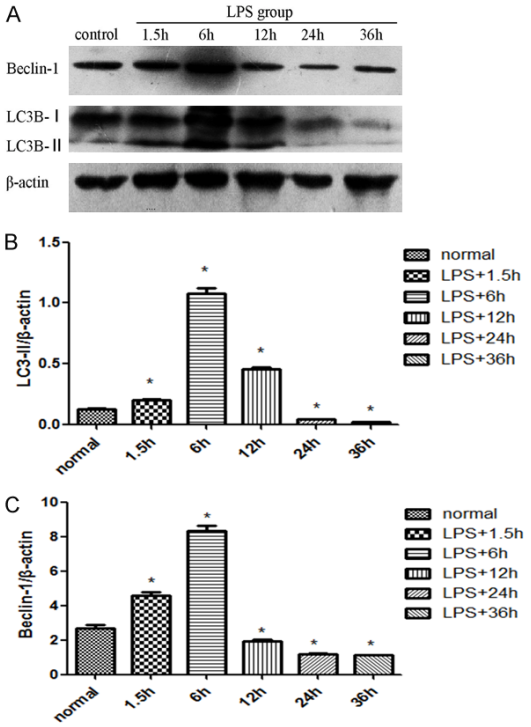

Figure 8. Expression levels of the autophagy-related protein LC3-II and Beclin-1 in the LPDCs from the mouse small intestine of each group. A. Western blot; B. LC3II/β-actin; C. Beclin-1/β-actin. Note: *indicates a significant difference compared with the normal group (P<0.05).

agy of LPDCs. These events show that the autophagy of LPDCs is dependent on immune function and plays a protective role in the sepsis mice. However, the relationship between the autophagy of LPDCs and the immune function may also involve some other underlying mechanisms, including inflammation, lamina proprial barrier dysfunction [5], gut bacteria translocation [16], DC antigen presentation by regulating the secretion of inflammatory cytokines [45], lymphocyte apoptosis [46] and self-apoptosis antagonism of DCs [47] and so on.

During the maturation of DCs, the peripheral tissues of the contact antigen migrate into the secondary lymphoid organs, which are exposed to T cells and stimulate the immune response [9]. DC phagocytosis involves antigen presentation and secretion of various cytokines in senescent cells. The distribution of molecules as MHC class II (MHC-II) type is limited and mainly expressed in DCs, such as antigen-presented cells and sperm cells. Several activated cells also have MHC-II molecules. To provide such information outside the cell, after entering an organization and gobbling up by the DCs, pathogen fragments with MHC and alerts the T helper cells about the immune response [48, 49]. We detected MHC-II in the peripheral blood in each phase to comprehensively assess the antigen presentation during inflammation and demonstrated that antigen presentation ability was weakened after 6 h (**Figures 2** and **3**). It suggests that the body's immune response is increased first and then weakened in sepsis. In acquired immunity, we speculate that MHC-II is an important link that causes immunosuppression on sepsis mice.

IL-12 is an interleukin and naturally produced by DCs in response to antigenic stimulation. It is involved in the differentiation of naive T cells into Th1 cells and known as a T cell-stimulating factor because it can stimulate the growth and function of T cells. IL-12 stimulates the production of TNF-α from T cells [50, 51]. We demonstrated that IL-12 generation of DCs, which leads to the metastasis of Th1/Th2 to Th2, aggravated the immunosuppression after 6 h of the LPS-injection (**Figure 5B**). The intestinal DCs mainly release IL-10, inducing the Th2 reaction to prevent the occurrence of destructive inflammation and maintain the stability of the intestinal environment [32]. The expression of IL-10, as an immunosuppressive factor, continued to increase and reach a peak at 36 h after the LPS injection (**Figure 5A**). At the same time, the release of cytokines in the LPDCs homogenate significantly changed, indicating that the pro-inflammatory cytokines reduced the generation of IL-12 and anti-inflammatory medium increased the secretion of IL-10. These reflect the level of inflammatory response in the

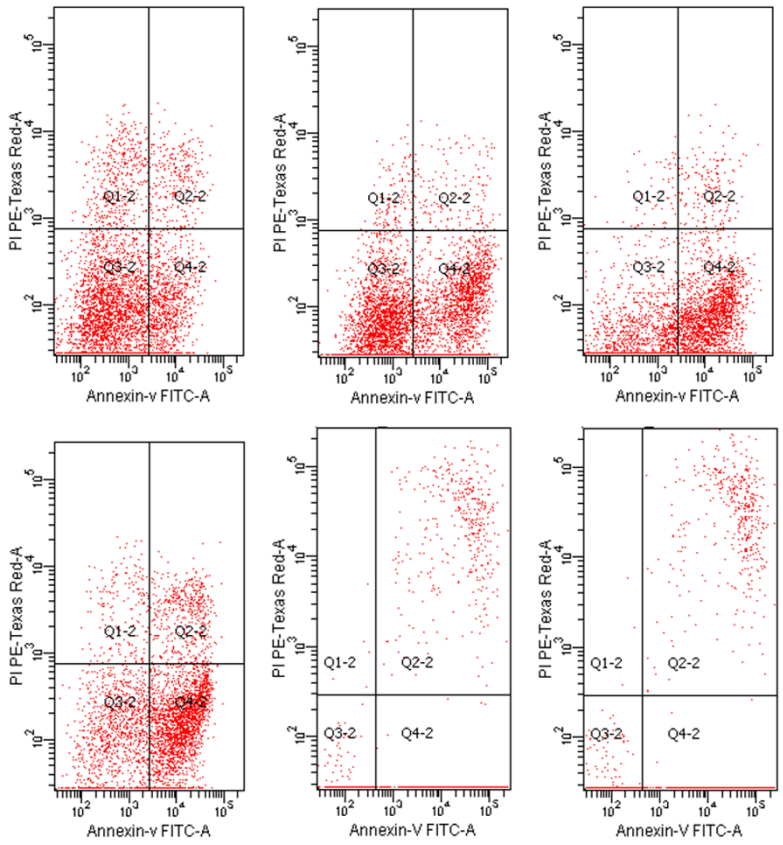

Figure 9. Flow cytometry of apoptosis in the LPDCs from the mouse small intestine of each group. Note: Q1-2 is a necrotic area, Q2-2 reflects late-stage apoptosis, Q3-2 is a double-negative area, and Q4-2 reflects early apoptosis. The Q2-2 and Q4-2 quadrants in **Figure 9** above reflect the apoptotic rate of LPDCs (Q2-2 and Q4-2 quadrants represent total apoptotic rates. From left to right and from top to bottom, normal control group, LPS + 1.5 h, 6 h, 12 h, 24 h, 36 h groups).

small intestine. High inflammatory response resulted in the damage to the immune cell function, excessive apoptosis, short survival cycle, and excessive activation of anti-inflammatory reaction in sepsis [29]. These events are consistent with the changes in the expression of IL-12 and IL-10, which may indicate whether or not the intestinal immune response enter immunosuppression and the Th1/Th2 reaction is unbalanced at 6 h [52].

A study shows that sepsis leads to apoptosis and reduces the depletion of seplenic interdigitating and follicular DCs [53]. The apoptotic level of LPDCs was increased in each group after the LPS injection (**Figures 9** and **10**). This phenomenon leads to the reduction in the number of LPDCs mainly in the late stage of sepsis. We also found that the peak period of autophagy of LPDCs in sepsis model mice appears before the peak of apoptosis. With the increase in the number of LPDCs, the ability of autophagy was improved. It further sped up the apoptosis of LPDCs. With the development of sepsis, the apoptosis of LPDCs dramatically decreased the number of LPDCs, which resulted in the evident decrease in the autophagy of LPDCs at 24 h (**Figure 8**). Accordingly, the apoptosis of LPDCs inhibited the occurrence of autophagy, which eventually led to the death of mice. Our results demonstrate that the autophagy and apoptosis of the LPDCs were intricately linked with the change in immune suppression [8]. Inhibition of the LPDC autophagy results in the increased sensitivity of LPDCs to apoptotic stimuli, while the increased autophagy of LPDCs improves the cell survival by inhibiting apoptosis. The balance between apoptosis and autophagy may influence cell homeostasis during sepsis development. The interaction between autophagy and apoptosis and the effect on immune cell responses in sepsis may reveal new therapeutic approaches for the detection and treatment of sepsis. Because its unpredictable side effects may undermine the potential benefits of apoptosis inhibition, we speculate that it is possible to restore apoptosis-autophagy balance by inducing autophagy. Autophagy, which suppresses the apoptosis of immune cells, can re-engage in the host immune function and can avoid the side effects of completely inhibiting apoptosis.

Some studies indicate that there are large migrations of early peripheral blood DCs to the intestinal tract in sepsis [14]. We speculate that the change trend of the number of DCs in peripheral blood is the opposite of that of LPDCs. After intraperitoneal injection of LPS, the number of LPDCs in the sepsis mice was significantly increased at 1.5 h of sepsis and

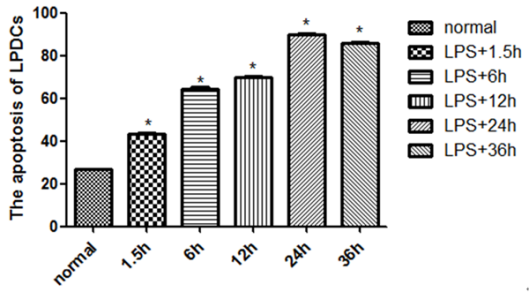

Figure 10. Apoptosis of the LPDCs from the mouse small intestine of each group. Note: *indicates a significant difference compared with the normal group (P<0.05).

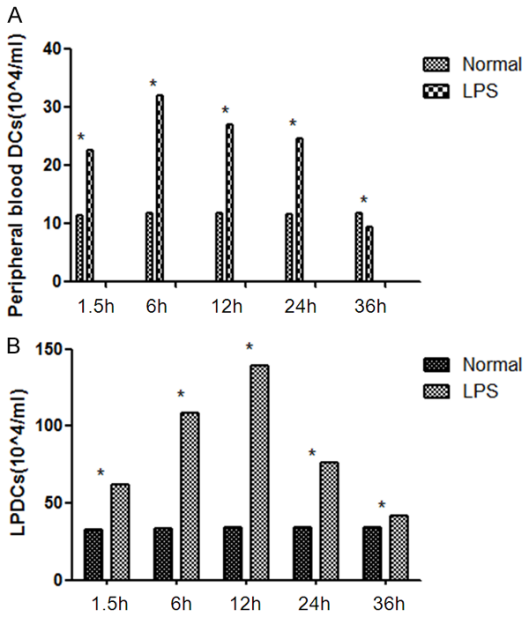

Figure 11. Effect of LPS-injection on the number of dendritic cells in peripheral blood of each group (A) and The number of LPDCs from the mouse small intestine in each group of mice (B). Note: *compared with the normal group (P<0.05).

began to decline after 12 h (**Figure 11**). Pro-inflammatory response induced by LPS was dominant within 6 h, DCs activated the immune response. The immature DCs in the peripheral blood migrated to the intestinal tract and gradually matured. The immature LPDCs swallowed the antigen. The intake of antigen (including *in vitro* processing) or LPS stimulation resulted in the differentiation of these cells into mature LPDCs [54]. The mature LPDCs expressed higher levels of MHC-II and secreted high level of IL-12 (**Figures 5** and **7**). We conclude that there depletion in numbers of peripheral blood DCs leads to the increase in the number of LPDCs. Meanwhile, only a small number of LPDCs was on apoptosis. The autophagy of LPDCs was significantly enhanced. It plays a protective role on the body within 6 h. The mice gradually entered the sepsis immune paralysis period after 6 h, which leads to DCs in the peripheral blood depleted. So DCs that are transferred to the gut are reduced. The number of LPDCs decreased autophagy ability. In addition, the mature LPDCs were also made less autophagy. And the apoptosis of LPDCs decreased, leading to the protective effect of autophagy reduced in sepsis, which in turn leads to sepsis body into immunosuppressive state quickly and further becomes the key cause for the death of the sepsis mice. Therefore, anti-immunosuppressive therapy such as the supplement the number of LPDCs is important in the treatment of sepsis. As a major target that can regulate the immune system, DCs has gradually become one of the latest immunotherapy tools for sepsis. Since the attack of sepsis on the body greatly affects the DCs and causes immunosuppression, the immunotherapy for DCs in order to change this state is imminent. Preventing excessive maturation of DCs may prevent depletion of DCs, replenish DCs, and reduce DC apoptosis in sepsis, and is considered to be one of the most important sepsis DCs immunotherapy mechanisms. Since sepsis is a series of severe and complex syndromes, the combination of various methods using DCs as a target, carrier, or effector will treat sepsis more effectively.

In summary, we speculated that autophagy of DCs retards the progression of the disease, resulting in the loss of the deadly sepals, including the excessive secretion of tissue inflammation and inflammation-associated cytokines. The peak period of autophagy of LPDCs in sepsis model mice is earlier than the peak period of peripheral blood inflammatory mediators. Our study also validates that autophagy of the LPDCs promotes apoptosis during the high inflammation stage of sepsis. By contrast, apoptosis of the LPDCs inhibited autophagy during the immunosuppression stage. Thus, autophagy and apoptosis play a dichotomous role in this model during the different sepsis period, which affects the immune dynamics. Hence, autophagic and apoptotic responses of the LPDCs during different periods of sepsis will be critical to the development of novel strat-

egies to fight this deadly disease. We appreciate that the current study did not focus on the mechanism of the autophagy and apoptosis of LPDCs. The relationship between autophagy and apoptosis may be related to the family protein of bcl-2 and mitochondria [55]. These will be main focuses in our future studies.

Conclusions

(1) The level of autophagy in small intestine LPDCs is correlated with the immune response ability at different stages of sepsis. LPDCs autophagy plays an important role in immune protection of sepsis. (2) During the development of sepsis, the autophagy and apoptosis of murine small intestine and murine lamina propria dendritic cells (LPDCs) are reciprocally regulated. (3) The change in autophagy and apoptosis of LPDCs are related to immunosuppression of sepsis.

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Disclosure of conflict of interest

None.

Address correspondence to: Wan-Jiang Zhang and Dr. Fang Wu, Department of Pathophysiology, Shihezi University School of Medicine/The Key Laboratory of Xinjiang Endemic and Ethnic Diseases/ Collaborative Innovation Center of High Incidence of Zoonoses Communicable Disease Prevention in The Western Region, Shihezi 832002, Xinjiang, China. E-mail: zwj1117@126.com (WJZ); xjwufang@126.com (FW)

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