Gut Lymph Purification in Rats With Acute Lung Injury Caused By Gut Ischemia Reperfusion Injury

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Research

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Abstract

Rationale: It is unclear whether removing the danger-associated molecular patterns (DAMPs) of gut lymph (GL) in the rats of gut ischemia-reperfusion injury (GIRI) model may reduce the distant organ lung injury.

Objective: To determine whether oXiris gut lymph purification (GLP) may remove the DAMPs of GL in the rats' model of acute lung injury (ALI) caused by GIRI.

Methods: The experimental rats were divided into four groups: Sham group, GIRI group, GIRI + gut lymph drainage (GLD) group, and GIRI + GLP group. After successful modeling, the lung tissue samples of rats in each group were taken for hematoxylin-eosin (HE) staining and detection of expression levels of apoptotic indexes. The level of DAMPs was detected in blood and lymph. We observed its microstructure of type II alveolar epithelial cells (AECII), and detected the expression level of apoptosis indexes.

Measurements and Main Results: GIRI-induced destruction of alveolar structure, thickened alveolar walls, inflammatory cell infiltration emerged in the GIRI group, HMGB-1 and IL-6 levels significantly increased, and HSP70 and IL-10 levels reduced in lymph and serum. Compared with GIRI group, the lung tissue damage in GIRI + GLP group significantly improved, the expression level of HMGB-1 and IL-6 in the lymph and serum reduced, and HSP70 and IL-10 increased. The organelle structure of AECII in GIRI + GLP group was significantly improved compared with the GIRI group.

Conclusions: oXiris GLP blocks the key link between DAMPs and mononuclear phagocyte system to inhibit inflammation and cell apoptosis, thereby reducing ALI induced by GIRI.

Introduction

Blood flow redistribution, as a protective reflex in the acute phase reaction of critical illness, results in gut ischemia-reperfusion injury (GIRI).[1] The gut as the "trigger" theory of sepsis and multiple organ dysfunction syndrome (MODS) was proposed in the 1980s.[2] Then Deitch et al. firstly proposed the gut lymph (GL) theory.[3] The theory points out that the intestinal mucosal (IM) barrier is destroyed after GIRI, and the cells that have undergone programmed lytic necrosis release danger-associated molecular patterns (DAMPs) into the intestinal space. Through GL fluid (GLF) of GL system (GLS), DAMPs are absorbed into GLS and diffuses into the systemic blood circulation (SBC), thereby activating the mononuclear phagocyte (MP) system (MPS). This continuous iteration produces a closed loop system that contributes to the cascade of inflammation.[4–6]

In the pathogenesis of acute lung injury (ALI) caused by GIRI, the GL theory is the main pathogenesis. The pulmonary vascular bed is the sentinel organ where DAMPs in the GL enter the SBC and activate MPS to induce an inflammatory waterfall effect.[7, 8] The latest experimental evidence showed that GL, as a carrier of DAMPs, is transported to the lungs and SBC, leading to ALI and MODS.[1] Sufficiently GL drainage (GLD) or GL ligation (GLL) significantly reduced the risk of generator organ injury in rats.[9–14]

These studies suggest that the GLF plays a pivotal role in MODS pathogenesis. Therefore, the gut is thought to be the origin of inflammation and the mediator of MODS.[15–19] However, both GLD and GLL, as "anti-physiological" therapies are unfavorable for application in clinical practice.[20, 21] Therefore, we theoretically constructed a therapeutic measure—GL purification (GLP) system to block the key link between DAMPs and MPS.

GL theory states that the GLP is a therapeutic technology in which the GLF is drained out of the animal or human body via thoracic duct puncture and catheterization, then purified using a GLP device and transfused back into the body. In the GLP, the filtration membrane technology is pivotal. oXiris (Gambro), an adsorbed hemofiltration membrane, is a substitute product based on the hydrogel structure of a propylene and sodium mesylate polymer (AN69), which has been widely applied in recent years to treat sepsis and septic shock.[22, 23] Therefore, we aim to determine whether oXiris-GLP may reduce the damage of distal organs (lung) via removing the DAMPs from the GLF after GIRI-induced ALI.

Materials And Methods

Animals

All experiments involving animals were performed in accordance with the guidance of the Animal Care and Use of Laboratory Animals. The Ethics Committee of Experimental Animals and Use of Laboratory Animals of Zunyi Medical University approved all animal experiments. Healthy adult male Sprague-Dawley rats (specific-pathogen-free grade, weighing 250 ± 50 g) were purchased from Hunan Slack Jingda Experimental Animal Co., Ltd., with the license number SCXK (Xiang) 2019-0004. Rats were maintained at $22 \pm 2^\circ\text{C}$ and humidity (45-55%) on a 12-h light/dark cycle, and housed under controlled conditions: Food and water supplied ad libitum.

Grouping and modeling

The rats were randomly assigned into four groups: (i) Sham group($n = 12$), underwent GL stem puncture and catheterization without clamping the superior mesenteric artery (SMA), and collected GLF for testing, without other interventions; (ii) GIRI[24] group ($n = 12$), based on step (i), clamped SMA for 60 minutes, then loosened for another 120 minutes; (iii) GIRI + GLD group ($n = 12$), the operation method is the same as that of the GIRI group, but the collected GLF has been infused back into the body through the jugular vein; (iv) GIRI + GLP group ($n = 12$), suffered the same operation method as the GIRI + GLD group, but the collected GLF was treated with the oXiris GLP and then infused into the body.

Adsorption column based on oXiris biofilm technology

The fabrication details of the adsorption column: The coats were sawed neatly along a 0.25 mL scale using two 1 mL injectors with the core rod removed. The rubber chips adhering to the coats were washed with saline solution and dried. After combining the broken ends of both coats, the oXiris filter wires were filled in. The joint of the coats of the two syringes were pasted across using a pre-cut 1.5 cm wide

disposable medical transparent dressing for compactness. The oXiris adsorption column was sterilized with ethylene oxide, sealed and stored at room temperature.

Jugular venipuncture catheterization for re-infusion of GLF

Jugular venipuncture catheterization: after establishing the GLD model, the skin from the right sternoclavicular connection from the midpoint of the neck was incised vertically to 1.5 cm to expose the subcutaneous connective tissue. After gradual blunt separation, the jugular vein was visualized and isolated. Subsequently, we induced congestion and swelling by clamping the proximal and distal vein with a noninvasive vascular clamp, then cut a small aperture in the middle of this section, which was slightly smaller than the external diameter of the silica gel catheter that was pre-flushed with heparin saline. Finally, within the vein orifice, the vascular clamp was loosened, the silica gel catheter was sutured and fixed, and the connective tissue and skin were sutured; GLF reperfusion: Fill the tubing of the peristaltic pump with saline in advance and connect the peristaltic pump to the jugular vein of the rats.

Cell separation and extraction

Monocyte separation. Dilute 5 ml of the whole blood of the modeled mouse with 5 ml equal volume of PBS, drop the diluted blood onto the surface of the separation solution, and then centrifugation was carried out at 2000 rpm for 20 minutes. Take the middle buffy coat cells into a clean centrifuge tube and add PBS to wash. After centrifugation, the supernatant was discarded, the cell suspension was added to the prepared culture plate. Add 50 ng/ml PMA to the isolated mononuclear cell culture medium and continue culturing for 48 hours to induce macrophages and DCs.

Lymphocyte separation. After collecting the lymph fluid, centrifuge at 1500 rpm for 3 minutes, discard the supernatant, add medium to resuspend the cells, and add the cell suspension to a culture dish for culture.

Alveolar epithelial cell separation. Take out the rat lung tissue and add it to a buffer solution containing 10% bi-antibody. Inject 0.25% pancreatin for digestion at 37°C for 25 minutes. The lung tissues are slightly cut and complete medium is added to terminate the digestion. Collect all the liquid, filter with a 70uM filter, 1500 rpm, 5 min centrifugation to collect the cells and resuspend them, add them to 500 ug/ml IgG-coated petri dishes, culture and purify for half an hour, collect the purified cell suspension and add it to the prepared culture in the board. Make a mark and place the cells in an incubator for normal culture.

Histological examination

At room temperature, the collected lung tissue was fixed with 4% paraformaldehyde for 24 hours, then embedded in paraffin to make it dehydrated, and then cut into thin slices (thickness of 5 µm). The sections were stained with hematoxylin and 0.5% eosin for 5 and 3 min, respectively, in room temperature (~ 22°C). Observe the tissue section under an optical microscope (CX41, OLYMPUS).

Detection of DAMPs in the GLF and plasma

The DAMPs in the GLF and plasma were detected via enzyme-linked immunosorbent assay (ELISA) per the manufacturer's instructions.

Western Blot (WB)

The sample is lysed with 400 µl lysis buffer (P0013, Beyotime Institute of Biotechnology) containing 100 mmol/L PMSF (ST506, Beyotime Institute of Biotechnology) for 30 minutes, then transfer to the EP tube. Centrifuge for 10 min in a high-speed centrifuge at 2000 r/min. The protein concentration was determined according to the BCA kit. The protein was denatured, loaded, and subjected to sodium dodecylbenzene sulfonate gel electrophoresis (SDS-PAGE) for 2 hours, and then transferred to the membrane with a constant current of 300 mA for 80 min. Incubate the primary antibody solution at 4°C overnight; incubate the secondary antibody solution at room temperature for 2 hours. Drop the ECL luminescent liquid on the film and expose it in the gel imaging system (Chemi Doc™ XRS+, Bo Le Biomedical Products (Shanghai) Co., Ltd.). Use "ImageJ" software to analyze the gray value of each band.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol lysate was added to the sample to extract miRNA. Measure the concentration and purity of RNA (OD₂₆₀/OD₂₈₀) with UV-Visible spectrophotometer. The RNA was used to synthesize cDNA with an RNA reverse transcription kit. Use RT-qPCR instrument for quantification (CFX Connect™ Real Time, Bole Life Medical Products (Shanghai) Co., Ltd.). The reaction system is as follows: RNase Free dH₂O 9.5 µl, cDNA 1 µl, upstream primer 1 µl, downstream primer 1 µl, 2×SYBR Green PCR Master Mix 12.5 µl. The reaction steps are as follows: pre-denaturation 95°C, 10 min; denaturation 95°C, 10 s; annealing 58°C, 30 s; extension 72°C, 30 s; 40 cycles. The primer sequence is provided by General Biosystems (Anhui) Co., Ltd., and the sequence is shown in the table below. β-actin F was used as an internal control, and the relative expression levels of bcl-2, Bax, FAS, and FASL were calculated according to the $2^{-\Delta\Delta C_t}$ method.[25]

The primer sequences were as follows: bcl-2, forward 5'-GCGTCAACAGGGAGATGTCA-3' and reverse 5'-TTCCACAAAGGCATCCCAGC-3'; Bax, forward 5'-GCGATGAACTGGACAACAAC-3' and reverse 5'-GCAAAGTAGAAAAGGGCAACC-3'; Fas, forward 5'-GCCCATTTTGCTGTCAACCG-3' and reverse 5'-GTCTTCAAGTCCACACGAGGT-3'; Fas-L, forward 5'-CACCAACCACAGCCTTAGAGT-3' and reverse 5'-GAGCGGGGGTTCCCTGTTAAG-3'; β-actin, forward 5'-GCCATGTACGTAGCCATCCA-3' and reverse 5'-GAACCGCTCATTGCCGATAG-3'.

Function detection of MPs

The state and function of MPs were detected, and lymphocytes were separated from the collected GLF and cultured with MPs, and then their proliferation, apoptosis and secretion functions were tested. CCK8 measured its proliferation rate, flow cytometry (Novocyte 2060R, Aisen Biology (Hangzhou) Co., Ltd.) measured its apoptosis rate and ELISA measured its inflammatory factor secretion function.

Detection of the dendritic cells' (DCs) antigen-presenting function

The number of DCs was adjusted to 5×10^4 /mL, and 1.5 mL of DCs suspension was added to each hole in the 6-well plate. Next, 150 μ L of GLF was added to each well and cultured in a 5% CO₂ incubator at 37°C for 24 hours. Cells were scraped and suspended with 5 mL of PBS, then centrifuged at 250 g for 10 minutes. The precipitate was resuspended with 5 mL of PBS and centrifuged for another 10 minutes, then the cells in PBS were resuspended, and 1 μ L of OX-6 and 2 μ L of OX-42 were added to avoid light for up-flow detection within 1 hour.

AECII cells were identified by transmission electron microscopy (TEM)

AECII was identified by TEM. AECII was incubated for 48 hours and digested with 0.125% trypsin. Collect the cell suspension and centrifuge at $100 \times g$ at 4°C for 10 min. The supernatant was removed and the cells were fixed to 4% glutaraldehyde for 24 hours at room temperature. The cell pellets were rinsed three times for 10 minutes at 4°C in PBS and fixed at 4°C with 1% osmium tetroxide for 30 minutes, then rinsed three times with PBS and observed by TEM (JEM-1230(80KV), JEOL).

Statistical analysis

Statistical analysis was performed using SPSS software (version 25.0; IBM, Corp.). The data are expressed as mean $\pm$ standard deviation. In order to compare the differences between groups, a one-way analysis of variance and post hoc Dunnett's T test were performed. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Rat GIRI-induced ALI

HE staining was used to detect the pathological changes of alveolar tissue after modeling. The alveolar tissue of the Sham group was normal, and the alveolar structure of the GIRI group was obviously damaged (Fig. 1—A) at 4th, 8th, 24th, and 48th h after modeling. The significant decrease in oxygenation index (OI) value after modeling (Fig. 1—B). The results of ELISA showed that the levels of NO, PLA2 and TNF- α in the serum of GIRI rats increased at all time points, and each index increased higher at 24th h, so 24th h was chosen for follow-up experiments (Fig. 1—C).

GLP treatment alleviated ALI in rats after GIRI

The microscopic results of HE stained lung tissue sections showed that in the Sham group, the alveolar tissue structure was normal. In the GIRI group, the alveolar structure was obviously damaged. Compared with the model or GIRI + GLD group, the lung tissue lesions were significantly improved in the GIRI + GLP group, (Fig. 2—A).

Regarding the level of apoptosis in lung tissue. WB results showed that compared with the Sham group, the expression of apoptosis-related genes Bax, FAS and FASL in the GIRI group were significantly

increased, and the expression of Bcl-2 was significantly reduced. Compared with the GRI group, the expression levels of Bax, FAS and FASL in the GRI + GLD and GRI + GLP groups were significantly reduced, and the expression of Bcl-2 was significantly increased. And compared with the GRI + GLD group, Bax, FAS and FASL decreased more significantly in the GRI + GLP group, and Bcl-2 increased more significantly (Fig. 2–B). The qRT-PCR results were consistent with WB, but there was no significant difference in Bcl-2 in the lung tissues of each group (Fig. 2–C).

GLP treatment of GRI reduced DAMPs and increased anti-inflammatory factors

The levels of DAMPs in GLF and plasma of all rats were detected by ELISA. Compared with the Sham operation group, the level of pro-inflammatory mediators (HMGB-1, IL-6) in the GRI group was significantly increased, while the level of anti-inflammatory mediators (IL-10, HSP70) decreased. In the GRI + GLD group and GRI + GLP group, the levels of HMGB-1 and IL-6 were significantly reduced, and the levels of HSP70 and IL-10 were significantly increased. Compared with the GRI + GLD group, the GRI + GLP group had higher levels of anti-inflammatory factors HSP70 and IL-10 (Fig. 3).

GLP treatment of GRI improved the function of MPs

Flow cytometry will measure the apoptosis rate of MPs. Compared with the GRI group, the apoptosis of monocytes in the GRI + GLD group and the MPs in the GRI + GLP group was significantly inhibited. Compared with the GRI group co-culture group, the apoptosis rate of the GRI + GLD group and the GRI + GLP group co-cultured significantly decreased, and the GRI + GLP group had a more obvious effect than the GRI + GLD group (Fig. 4–A). However, the CCK8 test showed that there was no significant difference in the proliferation activity between the groups of cells (Fig. 4–B).

ELISA was used to detect the levels of IL-6 and IL-10 secreted in the supernatant of MPs in each group. The results show that in the MPs of each group without lymphocyte culture, compared with the Sham operation group, the IL-6 content in the GRI group was significantly increased; compared with other groups, the content of IL-6 in the GRI + GLP group was significantly reduced, while the content of IL-10 was not significantly different. Among the MPs in each group of lymphocyte culture, compared with the Sham group, the IL-6 content in the GRI group was significantly increased, and the IL-10 content did not change significantly; compared with the GRI group, IL-10 increased significantly in the GRI + GLD group and the GRI + GLP group, but there was no significant change in IL-6 (Fig. 4–C).

GLP intervention improved the antigen presentation ability of DCs

The results showed that in the group without lymphocyte treatment, compared with the Sham group, the expression levels of CD40, CD86 and CD80 in the GRI group were significantly up-regulated, while the expression level of MHC-II was significantly down-regulated. Compared with the GRI group, CD40 was significantly down-regulated, and the expression levels of CD86, CD80 and MHC-II were significantly up-

regulated in GRI + GLP group. In the lymphoid treated DC cell group, compared with the Sham group, the expression levels of CD40, CD86 and CD80 in the GRI group were significantly down-regulated. Compared with the GRI group, CD40 was significantly down-regulated in the GRI + GLP group, and the expression levels of CD86, CD80 and MHC-II were significantly up-regulated, indicating that GRI inhibited the presentation of DCs after GRI, whereas GLP promoted it (Fig. 5).

GLP treatment of GRI reduced AECII apoptosis

Identification and Microstructure Observation of AECII. Primary AECII cells in the lung tissues of each group of rats were extracted, and a large amount of SP-A was detected in the cells by immunohistochemistry, which proved that the cells were AECII. The microstructure of cells in each group was observed by transmission electron microscope (Fig. 6–A). The cell structure in the Sham group was normal, while the organelle structure in the GRI group was damaged, a large number of vacuoles appeared, and the microvilli on the cell membrane surface were not obvious. In the GRI + GLD group and GRI + GLP flow group, the structure of organelles was significantly improved, and the number increased significantly, and many plate-shaped bodies with spiral layers were seen. And GRI + GLP is better than GRI + GLD group (Fig. 6–B).

Apoptosis of AECII was detected by flow cytometry. Compared with the Sham group, the apoptosis rate of AECII in the GRI group increased significantly; compared with the GRI group, the apoptosis rate of AECII in the GRI + GLP group reduced significantly. However, the apoptosis rate of AECII in the GRI + GLD group was not significantly different from that in the GRI group (Fig. 6–C).

WB was used to detect the expression levels of Bax, Bcl-2, FAS and FASL in AECII in each group. Compared with the Sham group, the expression of apoptosis-related genes Bax, FAS and FASL in AECII of the GRI group were significantly increased, and the expression of Bcl-2 was significantly decreased. Compared with the GRI group, the expression levels of Bax, FAS and FASL in the GRI + GLD and GRI + GLP flow groups were significantly reduced, but only the expression of Bcl-2 in the GRI + GLP group was significantly increased (Fig. 6–D).

Discussion

Gut ischemia for 30 minutes occurred in the mild injury of the IM epithelium (IME). As the ischemic time increases, intestinal wall cells gradually become necrotic and release endogenous DAMPs.[26] It directly damaged IME cells leading to increased intestinal wall permeability and promoting intestinal DAMPs.[27] The translocated DAMPs flow to remote organs with the SBC and activate their innate immune system to produce a large number of stimulating mediators and cytokines, leading to tissue inflammation.[28]

The DAMPs caused by GRI mainly involves the GL pathway. Traumatic-hemorrhagic shock-induced lung injury, neutrophil activation, endothelial cell activation and injury are caused by gut-derived factors carried in GL instead of portal blood.[29] After GRI, DAMPs translocate into the GLF and then flow to the SRC, and combine with the Toll-like receptor 4 (TLR4) of peripheral MPs to produce a large number of

inflammatory factors that target remote organs. Gut-derived DAMPs reach the lungs and SRC via the "GL pathway", leading to the occurrence of ALI and MODS.[3]

At present, the researches on GL intervention in sepsis mainly focuses on GLD and GLL. GIRI caused damage to distant organs, but both of these interventions need to block the GLS.[19, 30] The GLS, as a supplement to the SRC system, plays an important role in maintaining body fluid balance and nutrient metabolism. However, GLD and GLL are "anti-physiological" treatment methods, which are difficult to apply in clinical practice. 'oXiris-GLP therapeutic system' not only meets the physiological needs of critically ill patients, but interrupts the critical link between DAMPs and MPs. The lymphatic drainage catheter is used to establish an extracorporeal lymphatic circulation pathway to drain GLF out of the body, and then use the GLP system to purify the GL and return it to the body.

Judging from the current results, GLP therapy of GIRI-induced ALI has significant effects on both the macroscopic (lung tissue) and microscopic (AEC_x) of the lungs, and it significantly inhibited lung and cell apoptosis. Both GLD and GLP reduced DAMPs (HMGB-1, IL-6) in GL and peripheral blood, thus reducing the triggering signal cascade by binding to TLR4, inducing activation of nuclear factor kappa B (NF- κ B) pathway, thereby reducing inflammation. GLP significantly increased anti-inflammatory factors (HSP70 and IL-10) and reduced the apoptotic rate of MPs, regardless of whether they were co-cultured with lymphocytes. Compared with GIRI, it inhibited the apoptosis of MPs and the level of IL-10 expression and reduced the level of IL-6 expression to a certain extent. This suggests that GLP plays a certain two-way regulatory role in the body's innate immune response. It mainly plays an immune role by inhibiting the percentage of apoptosis of MPs, and by inhibiting the cytokines HMGB-1, IL-6 and through the ability of DC antigen presentation to play its regulatory role. This experiment provides basic medical evidence for the research and development of the ALI-GLP treatment system, and also lays the foundation for GLP therapy to become a new discipline.

Conclusions

oXiris GLP blocks the interaction of DAMPs of gut-lymph-lung pathway and MPs to inhibit inflammation and cell apoptosis, thereby reducing ALI induced by GIRI.

Declarations

Ethics approval and consent to participate

The requirement for written consent from participants was waived because of the study design of animal experiment.

Consent to publish

All authors have read and approved the manuscript and agree to submit it for consideration for publication in the journal. We confirm that we have read the journal's position on issues involved in

ethical publication and affirm that this report is consistent with those guidelines.

Availability of data and materials

The authors state that all the data and materials are true and available in the study.

Authors' comments

We had full access to all of the data in this study, and we take complete responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure of Conflicts of Interest

All authors have no conflicts of interest to disclose.

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Authorship contributions

WZ had full access to all data in the present study and accepts responsibility for data management and accuracy of the data analyses. Study concept and design: WZ and MMS. Acquisition and interpretation of data: CJ, SCZ, and LLW. Drafting of the manuscript: CJ, SCZ, and WZ. Critical revision of the manuscript for important intellectual content: CJ, MMS, and WZ. Administrative, technical, or material support: CJ, LLM, and BHL. Study supervision: WZ, MMS, and CJ. All authors agree to submission of the final version of this manuscript. WZ is the study guarantor.

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Figures

Figure 1

Pulmonary pathological changes, blood gas indicators and the influence of inflammation after intestinal ischemia-reperfusion in rats. Rats only turned the intestines, did not ligate the superior mesenteric artery (Sham group), and ligated the superior mesenteric artery for 1 hour before reperfusion (GIRI group). (A) Pathological changes were examined by H&E staining and light microscopy. Scale bar, 200 μ m; (B) Respiratory index. (C) ELISA to detect serum NO, PLA2 and TNF- α levels. Data are expressed as the mean $\pm$ standard deviation (n=6 rats per group). *P<0.05 with comparisons shown by lines. GIRI, gut ischemia-reperfusion injury; ELISA, enzyme linked immunosorbent assay; H&E, hematoxylin and eosin; NO, nitric oxide; PLA2, lipoprotein-associated phospholipase A; TNF, tumor necrosis factor.

Figure 2

GLD and oXiris GLP are given to the model of GIRI-induced ALI in rats The pathological changes of the lung tissue of each group of rats were detected by HE. Scale bar, 200 μ m (Figure. 2A). West blotting (Fig. 2B) and PR-qPCR (Fig. 2C) detected the expression levels of lung tissue apoptosis indicators Bax, Bcl-2, FAS and FASL. * p <0.05, compared with Sham group; # p <0.05, compared with GIRI group. GIRI, gut ischemia-reperfusion injury; GLD, gut lymphatic drainage; GLP, gut lymph purification; HE, hematoxylin and eosin.

Figure 3

ELISA detected lymph and serum HMGB-1, IL-6, HSP70 and IL-10 levels * p <0.05, compared with the sham operation group; # p <0.05, compared with the model group. ELISA, enzyme linked immunosorbent assay; HMGB, high mobility group box chromosomal protein; HSP, heat shock proteins; IL, interleukin.

Figure 4

The function of each group of mononuclear macrophages and the effect of co-cultivation with lymphocytes (A) Cell flow cytometry chart; (B) Histogram of apoptosis results; (C) Histogram of proliferation. (D) The content of IL-6 and IL-10. * $p < 0.05$, compared with the sham operation group; # $p < 0.05$, compared with the model group. IL, Interleukin; GIRI, gut ischemia-reperfusion injury; GLD, gut lymphatic drainage; GLP, gut lymph purification.

Figure 5

Flow cytometric detection of the expression level of Dendritic cells' antigen presenting molecules in each group of cells * $p < 0.05$, compared with the sham operation group; # $p < 0.05$, compared with the model group. GIRI, gut ischemia-reperfusion injury; GLD, gut lymphatic drainage; GLP, gut lymph purification.

Figure 6

The effect of GLP on AEC II after GIRI treatment (A) The expression of SP-A in the cells was detected by immunohistochemistry. (B) Observe the microstructure of AEC II by transmission electron microscope. (C) Flow cytometric detection of AEC II apoptosis. (D) Western blot detection of AEC II apoptosis expression level. * $p < 0.05$, compared with the sham operation group; # $p < 0.05$, compared with the model group. GLP, gut lymph purification; GIRI, gut ischemia-reperfusion injury; AEC II, alveolar epithelial type II cell.