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Research

# Augmenter of liver regeneration attenuates acute rejection after rat liver transplantation

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**KEYWORDS:**
Augmenter of liver regeneration;
Kupffer cell;
Liver transplantation;
Immune regulation

**Abstract**

**BACKGROUND:** The role of augmenter of liver regeneration (ALR) on liver transplantation immune regulation remains unknown.

**METHODS:** Male Lewis and Brown-Norway (BN) rats were assigned to allograft group (Lewis-to-BN liver transplantation), isograft group (BN-to-BN), and ALR group (Lewis-to-BN, ALR, 100 μg/kg/d, intramuscular injection postoperatively). Rats were sacrificed at indicated times for assessment of cytokines production, T-cell (TC) activation and apoptosis. Kupffer cells (KCs) and TCs were isolated from grafts to assess cytokine expression. Effect of ALR and KCs on TCs was monitored by co-culture of $^{3}$H-thymidine TCs.

**RESULTS:** (1) Treatment with ALR significantly decreased interleukin-2 and interferon-γ expression, promoted TC apoptosis, and prolonged the survival of allografts; (2) KCs in ALR group and isograft group that had significantly increased interleukin-10 and decreased tumor necrosis factor-α expression were able to inhibit TC proliferation and induce their apoptosis relative to KCs in the allograft group; (3) ALR and KCs directly inhibited TC proliferation and activation and induced TC apoptosis.

**CONCLUSIONS:** ALR could inhibit TC proliferation and function both in vivo and in vitro and attenuate acute rejection after liver transplantation.
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Acute rejection is the major cause of early graft loss and risk factor for recipient survival after transplantation. Although immunosuppressive drugs could significantly abrogate acute rejection and prolong recipient survival, they place recipients at increased risk of opportunistic infection and malignancy because of the lack of immunologic specificity.[1] Therefore, inducement of donor-specific transplant tolerance has been suggested as one of the potential solutions to prevent immune rejection.

Augmenter of liver regeneration (ALR) extracted from liver tissue of newborn rats[2] could promote hepatocyte proliferation and liver regeneration after partial hepatectomy. ALR has been extensively detected in tissues and organs, and it could inhibit hepatic natural killer (NK) cell activity by downregulation of cytokine production, including

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interleukin (IL)-2, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).[3,4] It is well known that these cytokines play an important role in T-cell (TC) proliferation and activation,[5] facilitating the recruitment and activation of leukocytes.[6] TC proliferation and activation is the central event of the rejection process, and it can damage the graft directly and injury the graft by cytokine production indirectly.[7] Some study also indicates that ALR could promote the hepatocyte proliferation induced by Kupffer cells (KCs).[8] However, there are no reports to date about its effect on liver transplantation immune regulation.

KCs are the resident macrophages of the liver. Their role in immune response has been highlighted in earlier studies,[9] yet their role in liver transplantation tolerance has been rarely reported.[10] KCs could express co-stimulator molecules (CD80 and CD86) and have strong antigen presentation capability.[10,11] They interact with the recipient TCs that migrate into the graft and play an important role in immune response. Our previous work has demonstrated that graft KCs play a crucial role in liver transplant tolerance.[12]

Accordingly, the purpose of this study was to investigate whether ALR has an immunosuppressive effect and could attenuate the acute rejection of allografts after liver transplantation in rats.

## Methods

### Animals and liver transplantation

Adult male Lewis and Brown-Norway (BN) rats (200 to 250 g weight and 8 to 10 weeks old) were housed in a specific pathogen-free animal facility and randomly assigned to 3 groups. Orthotropic liver transplantation was performed using Kamada's method. For the allograft group, Lewis-to-BN liver transplantation was performed. For the isograft group, BN-to-BN liver transplantation was performed. For the ALR group, Lewis-to-BN liver transplantation was performed, and recipients were treated with recombinant human ALR (100 μg/kg/d, intramuscular injection postoperatively; gratuitous gift from Professor Wang, Institute of Radiation Medicine, Academy of Military Medical Sciences). No immunosuppressant drugs were given to recipients in this study. Each group had 18 recipients, and recipients were sacrificed and graft tissue samples were collected at days 3, 5, and 7 post-transplantation, respectively. Six recipients in each group were set aside for survival observation. All sacrificed rats were subjected to necropsy. All experimental protocols described in this study complied with the Ethics Review Committee for Animal Experimentation of Chongqing Medical University.

### Morphologic changes of liver graft

Morphologic changes were observed under light microscope (Olympus BX51; Olympus, Tokyo, Japan). Acute allograft rejection was defined according to rejection activity index (RAI, Banff score; 0 to 2, no rejection; 3, borderline; 4 to 5, mild rejection; 6 to 7, moderated rejection; 8 to 9, severe rejection).[13]

### Immunohistochemical staining for IL-2, IFN-γ, IL-10, and TNF-α expression in liver graft

The expression of IL-2, IFN-γ, IL-10, and TNF-α in liver grafts was determined using a goat–anti-rabbit streptavidin peroxidase kit (Santa Cruz Biotechnology, Santa Cruz, CA), respectively. Briefly, 4-μm paraffin sections were spread on slides and fixed in acetone; endogenous peroxidase activity was quenched with .03% $H_2O_2$. Sections were incubated with rabbit anti-rat IL-2, IFN-γ, IL-10, and TNF-α monoclonal antibodies (Santa Cruz) and Biotin-labeled goat anti-rabbit secondary antibody (Santa Cruz), respectively. It was followed by the streptavidin–horseradish peroxidase complex. Bound antibody was detected with 3,3′-diaminobenzidine (Santa Cruz). Cells with brown granules appearing in the cytoplasm or cell membrane were considered positive expression. The expression intensity was determined with HPIAS-1000 image analysis system under the light microscopy (×400).

### Flow cytometry for TC activation and apoptosis in liver graft

TCs were isolated[14] from liver grafts and identified with anti-CD4 antibodies. Flow cytometry was used to quantify $CD4^+CD25^+$ cells and apoptosis cells following a common protocol.

### Western blot for IL-2 and IFN-γ protein expression in TCs of liver graft

Isolated TCs were lysed in the lysis buffer containing 1.0% Nonidet P-40, 150 mmol NaCl, 10 mmol Tris-Cl (pH 7.5), and 1 mmol EDTA. Extracted protein was separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membrane was incubated with rabbit anti-rat IL-2 and IFN-γ antibodies (diluted 1:500; Santa Cruz) and fluorescein isothiocyanate–conjugated goat anti-rabbit immunoglobulin G (diluted 1:250, Santa Cruz) for 1 h. This was developed with diaminobenzidine reagent, visualized with the gel imaging system (Doc Gel 2000; Bio-Rad, Hercules, CA), and finally analyzed by Bio-Image Analysis System.

### Real-time fluorescent quantitative polymerase chain reaction for IL-2 and IFN-γ messenger RNA expression in TCs of liver graft

Fluorescent quantitative polymerase chain reaction (FQ-PCR) was used to measure intra-graft messenger RNA (mRNA) expression of IL-2 and IFN-γ. Total RNA was

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