

Change in Expression of the Intestinal Polymeric Immunoglobulin Receptor in Acute Liver Necrosis

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ABSTRACT

AIM: Polymeric immunoglobulin receptor (pIgR) transports pIgA unidirectionally to form sIgA, and its effective production is important for the immune stability of the intestinal mucosa. This study aimed at evaluating the expression of pIgR in a mouse model of acute liver necrosis and the relationship with sIgA.

METHODS: We induced acute liver necrosis using D-galactosamine/lipopolysaccharide (GalN/LPS), and assessed the results using immunohistochemistry, Western blotting, real-time quantitative PCR, and radioimmunoassay.

RESULTS: Expression of pIgR mRNA was significantly decreased in acute liver necrosis models ($P < 0.05$), as was the level of pIgR protein ($P < 0.05$), compared to the control group. The intestinal mucus sIgA content was also significantly reduced ($P < 0.05$), and was positively correlated with the expression of pIgR protein in acute liver necrosis models ($r = 0.965$, $P < 0.001$).

CONCLUSIONS: Our findings first demonstrated pIgR expression decreased in the small intestine mucosa in mice with acute liver necrosis, which correlated with the intestinal mucus sIgA content. pIgR decrease in the mucosa of the small intestine may play an important role in reduced sIgA content and the development of intestinal mucosal immune disorder in acute liver necrosis.

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Key words: Mucosal immunity; Acute liver failure; Secretory immunoglobulin

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INTRODUCTION

Secretory immunoglobulin A (sIgA) is an essential part of the intestinal immune system, and comprises a secretory component (SC) connected to two IgA molecules through a non-covalent bond and a J-chain^[1]. The SC is the extracellular part of the polymeric immunoglobulin receptor (pIgR) that is responsible for transportation of pIgA and formation of sIgA^[2]. pIgR is transported unidirectionally and cannot be recycled, and its effective production is thus important for the immune stability of the intestinal mucosa^[3].

Accumulated evidence has shown that disorders of the intestinal mucosal barrier, including intestinal immune dysfunction, occur subsequent to acute liver necrosis^[4,5].

Although pIgR plays an essential role in the intestinal mucosal immune barrier, its expression in the small intestine mucosa in mice with acute liver necrosis has rarely been reported. This study aims at investigating its expression in the intestinal mucosa in a model of acute liver necrosis, through detection of pIgR mRNA and protein expression and the relationship with sIgA in the small intestine mucosa.

MATERIALS AND METHODS

Animals

Six-week-old male BALB/c mice (provided by Laboratory Animal Center in China Medical University, China) were housed at a constant room temperature and constant humidity with free access to food and water, and subjected to a 12-h light/dark cycle. Food was withdrawn overnight prior to experiments. All animal experimental procedures were approved by the Ethics Committee of China Medical University before the commencement of the study.

Reagents

D-galactosamine (GalN) and lipopolysaccharide (LPS, *E. coli* O127: B8) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Goat anti-mouse pIgR antibodies were purchased from R&D (Minneapolis, MN, USA). Rat anti-mouse β -actin monoclonal antibodies were

obtained from Abcam (Cambridge, United Kingdom). Horseradish peroxidase-labeled rabbit anti-goat secondary antibodies and horseradish peroxidase-labeled rabbit anti-mouse secondary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Animal groups and acute liver necrosis model

Sixty mice were randomized into four groups ($n=15$ for each): one group was treated with normal saline (NS control); one group was treated with lipopolysaccharide (LPS)/D-galactosamine (GalN) (LPS/GalN group); one group was treated with LPS (LPS group); and one group was treated with GalN (GalN control). GalN (800 mg/kg body weight, Sigma, USA) and LPS (10 $\mu\text{g}/\text{kg}$ body weight, Sigma, USA) were injected intraperitoneally to induce acute liver necrosis as previously described^[6,7]. Mice in the groups were euthanized 9h after administration of GalN/LPS, intestinal mucus was collected as described previously^[8,9], and a 15 cm-long strip of intestinal tissue near the ileocecal was taken. PBS rinsed the intestine, cut the intestinal tissue longitudinally, scraped the intestinal mucus into the centrifuge tube, mixed it with equal volume of normal saline, and centrifuged for 30 min at 6000 rpm/min. The supernatant was then used to detect sIgA content, and the intestinal tissue was used to detect pIgR expression. The study was approved by the Ethics Committee of China Medical University.

Blood biochemistry assay

Serum alanine transaminase (ALT) levels were determined using an automatic analyzer (Hitachi 7250; Hitachi, Japan).

Immunohistochemical staining for pIgR in small intestinal tissues

Wax sections were routinely dewaxed and dehydrated, and incubated with 3% hydrogen peroxide for 10 min to eradicate endogenous peroxidase. Following antigen retrieval in a high-pressure microwave oven, the sections were blocked with rabbit serum for 20 min at room temperature, and diluted with goat anti-mouse pIgR primary antibody (1:100), followed by a procedure performed according to the instructions for the immunohistochemistry kit. Sections were finally stained with 3,3'-diaminobenzidine, restained with hematoxylin, and sealed with neutral balsam. Cells with yellow membranes and nuclei under the microscope were considered to be positive for pIgR.

Western blotting for pIgR protein expression in the small intestinal mucosa

Small intestinal mucosa tissues were treated as described previously^[10]. The tissues were placed in a homogenizer and mixed with cell lysis buffer and protease inhibitor for homogenization. After 20 min, the tissues were centrifuged and the supernatants were collected for protein content quantification using an ultraviolet spectrophotometer DU800 (Beckman). Protein (50 μg) was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to membranes. Primary (goat anti-mouse pIgR monoclonal antibody, 1:2000) and secondary antibodies (rabbit anti-goat pIgR monoclonal antibody, 1:2000) were added. Specific pIgR bands were located using the chemiluminescence technique. The membranes were washed, incubated with primary (goat anti-mouse pIgR monoclonal antibody, 1:2000) and secondary antibodies (rabbit anti-goat pIgR monoclonal antibody, 1:2000), and subjected to protein hybridization. The β -actin protein band was used as an internal reference.

Detection of pIgR mRNA expression using real-time PCR

Total RNA was extracted from the mouse small intestine using Trizol,

treated with DNase I, purified, and reverse-transcribed to cDNA. Reactions were carried out at 37°C for 15 min and 85°C for 5 s. Expression of the pIgR target gene was detected in intestinal tissues in different groups based on SYBR-Green I fluorescence, relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression. Primers included pIgR-F:5'-TATAGCTGTGTGGGTGGCCAGA-3', pIgR-R:5'-GTCTGCCTGTAGCTGATTGA-3', GAPDH-F:5'-TGTGTCCGTCGTGGATCTGA-3', GAPDH-R:5'-TTGCTGTTGAAGTCGCAGGAG-3'. PCR was performed at 5°C for 30 s, followed by 45 cycles of 95°C for 5 s, 57°C for 20 s, and 72°C 30 s. Extension was performed at 72°C for 2 min.

sIgA content of the intestinal mucus using radioimmunoassay and linear correlation analysis of sIgA and pIgR expression

Intestinal mucus supernatants were adjusted to room temperature. A non-specific standard tube, a zero-standard tube, six standard tubes, and a T tube were designated. I^{125} -sIgA was added to the T tube, I^{125} -sIgA and non-specific binding agent to the non-specific standard tube, I^{125} -sIgA and sIgA antibodies to the zero-standard tube, and I^{125} -sIgA, sIgA antibody, and standard products at different concentrations to the six standard tubes. I^{125} -sIgA, and sIgA antibody (100 μL samples) were added to additional sample tubes, followed by homogenization and storage at 37°C for 1.5 h. The secondary antibody and polyethylene glycol were subsequently added to the tubes, followed by homogenization and storage at 37°C for 0.5 h. They were centrifuged at 3500 rpm for 15 min and the supernatants were removed. The tubes were finally placed in a liquid scintillation counter for 60 s to quantify non-specific binding. The linear correlation between intestinal mucus sIgA content and pIgR expression was analyzed.

Statistical analysis

Statistical analyses were conducted using SPSS 11.0. Data were presented as mean \pm SD ($\bar{x}\pm S$). Measurement data were compared using one-way ANOVA. A value of $P<0.05$ was considered to represent a statistically significant difference.

RESULTS

Serum ALT levels

Compared to the NS control (20.6 \pm 10.4 U/L), the ALT serum level was only slightly elevated in the LPS control (41.6 \pm 13.3 U/L) and the GalN control (68.7 \pm 16.5 U/L) ($P>0.05$), but it increased significantly in acute liver necrosis models (6134.8 \pm 319.0 U/L) ($P<0.05$) (Table 1).

Table 1 Compared to NS control, ALT serum levels were only slightly elevated in the LPS control and GalN control, but increased significantly in the acute liver necrosis group ($P<0.05$)

Group	Number of mice	ALT (U/L)
NS	15	20.6 \pm 10.4
GalN/LPS	15	6134.8 \pm 319.0*
LPS	15	41.0 \pm 13.3
GalN	15	68.7 \pm 16.5

Serum ALT levels in mice. All values are expressed as mean \pm SE. *: $P<0.01$ vs NS control.

Immunohistochemical staining for pIgR protein

pIgR staining of the cytoplasm and/or the membranes of intestinal epithelial cells was significantly weaker in acute liver necrosis models (Figure 1B) compared to the NS control (Figure 1A), LPS control (Figure 1C), and GalN control (Figure 1D).

Semi-quantitative analysis of pIgR protein expression by western blotting

Two specific protein bands of about 120 and 80 kDa were detected using western blotting. The ratio of the absorbance of the sum of the two bands to that of β -actin protein was regarded as the relative expression level of pIgR protein. The relative expression of pIgR protein in the LPS/GalN group was 1.27 ± 0.13 , which was significantly lower than the values of 2.47 ± 0.16 in the NS control, 2.09 ± 0.15 in the LPS group, and 2.34 ± 0.19 in the GalN group ($P < 0.05$) (Figure 2, $n=3$).

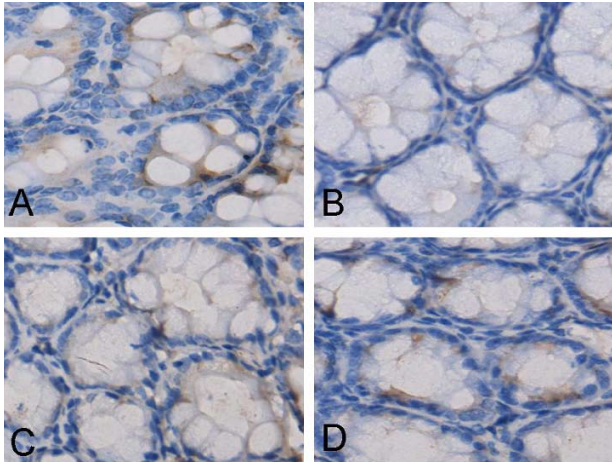


Figure 1 Intestinal pIgR staining determined by immunohistochemistry (400 \times). A: NS control; B: LPS/GalN group: pIgR staining of the cytoplasm and/or the membranes of intestinal epithelial cells was remarkably weak or absent; C: LPS control: pIgR staining of the cytoplasm and/or the membranes of intestinal epithelial cells was slightly weak; D: GalN control: pIgR staining of the cytoplasm and/or the membranes of intestinal epithelial cells was slightly weak.

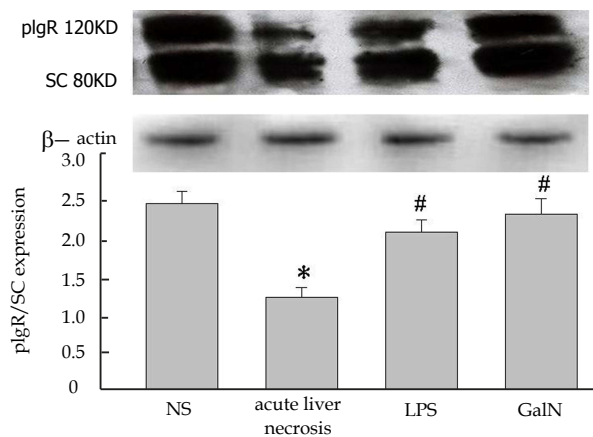


Figure 2 Intestinal pIgR expression determined by Western blotting. pIgR expression in the acute liver necrosis group was significantly lower, while the LPS group and GalN group pIgR expression was only slightly reduced. (* $P < 0.05$, compared with the NS control; # $P < 0.05$, compared with the acute liver necrosis group).

pIgR mRNA expression in intestinal tissues using real-time PCR

Real-time PCR quantitative analyses showed that there were marked decreases in the relative content of intestinal pIgR mRNA in acute liver necrosis models (0.49 ± 0.11) compared to the NS control (1.00 ± 0.03), LPS control (0.90 ± 0.08), and GalN control (0.96 ± 0.05) ($P < 0.01$) (Figure 3, $n=3$).

sIgA content in the intestinal mucus

Intestinal mucus sIgA in the LPS/GalN group was 132 ± 29 ng/g, which was significantly lower than the values of 232 ± 24 ng/g in the NS control, 201 ± 17 ng/g in the LPS control, and 218 ± 21 ng/g in the GalN

control ($P < 0.05$) (Figure 4).

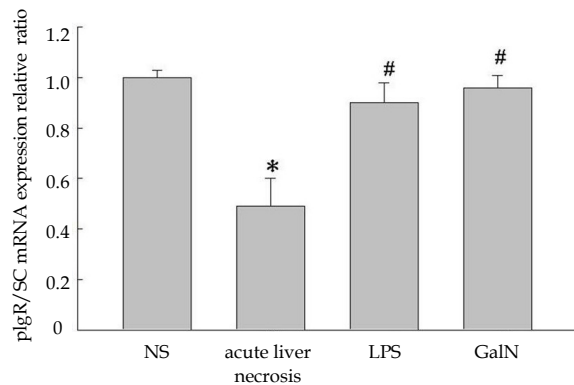


Figure 3 Real-time PCR method for detection of intestinal pIgR mRNA expression. pIgR mRNA expression in acute liver necrosis was significantly decreased, while pIgR mRNA expression in the LPS group or GalN group only slightly reduced compared to the NS control. (* $P < 0.05$, compared with the NS control; # $P < 0.05$, compared with the acute liver necrosis group).

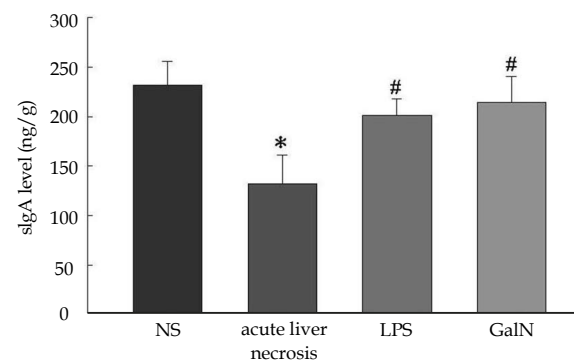


Figure 4 Radioimmunoassay method for detection of intestinal mucus sIgA levels. Compared with the NS control, the content of intestinal mucus sIgA in the acute liver necrosis group was significantly lower, while the content of intestinal mucus sIgA in the LPS group and the GalN group only slightly reduced (* $P < 0.05$, compared with the NS control; # $P < 0.05$, compared with the acute liver necrosis group).

Correlation between sIgA content in the intestinal mucus and pIgR protein expression

Linear correlation analysis of intestinal mucus sIgA and intestinal tissue pIgR was performed for three mice in each group. There was a significant positive linear correlation between intestinal mucus sIgA and intestinal tissue expression of pIgR, with a correlation coefficient of $r=0.965$ ($P < 0.001$) (Figure 5).

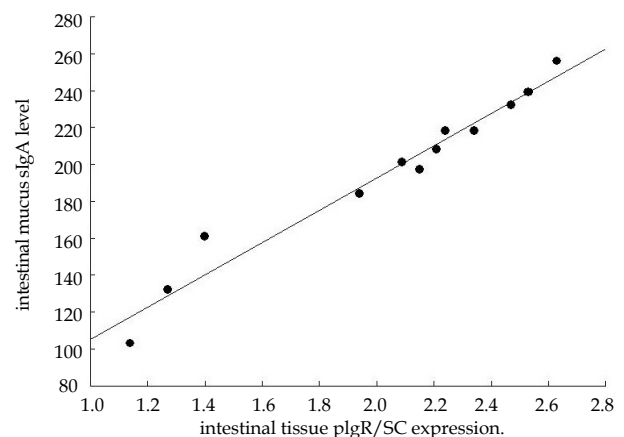


Figure 5 Correlation analysis between intestinal mucus sIgA and intestinal pIgR protein expression. There was a positive linear correlation between

intestinal mucus sIgA content and pIgR protein expression in the small intestine ($r=0.965$, $P<0.001$).

DISCUSSION

The protective antigens secreted by the small intestine comprise a complicated but effective immune system that presents an initial barrier to prevent the massive invasion of microorganisms, thus avoiding intestinal damage as a result of bacteria and toxins^[11]. sIgA is an iconic product in the intestinal mucosal immune system that can bind to bacteria^[12], viruses^[13-15], and other poisonous molecules, and stop them from adhering to the intestinal mucosal membrane. Thus sIgA plays an important anti-infection role^[16,17]. Reductions in sIgA weaken intestinal immune function, induce intestinal bacterial disturbance, and can even lead to intestinal duct-derived systemic infection caused by intestinal bacterial translocation^[18,19].

Recent studies have suggested that patients with chronic liver diseases sustain impairment to immune systems, which worsens over time. These defects in their host defense lead to risks of bacterial infections and increased morbidity^[20]. And bacterial translocation, the key mechanism in the pathogenesis of (spontaneous bacterial peritonitis, SBP), is only possible because of the concurrent failure of defensive mechanisms in cirrhosis^[21]. But the state of intestinal mucosa immunity in acute liver necrosis is not very clear. In agreement with previous reports^[5, 22], we found that injection of GalN/LPS induced increases in serum ALT and the development of severe hepatocyte necrosis. Our study also demonstrated that sIgA decreased in acute liver necrosis models, indicating that impairment to intestinal mucosal immunity was found in acute liver necrosis.

The sIgA content of the intestinal mucus has been reported to correlate with pIgR protein expression in the mucosa of the small intestine^[23]. pIgR is a type I cross-membrane glycoprotein with a molecular weight of about 120 kDa that is transcribed by pIgR mRNA. It is a polymeric IgA and IgM receptor that mediates transfer of immunoglobulin from the basal membrane to the surface of the epithelium^[24]. The extracellular apical domain of sIgR is dehydrated to free SC (molecular weight 80 kDa), or SC is combined with two IgAs and a J-chain to form sIgA (molecular weight 410 kDa), which is secreted into the intestinal cavity^[25].

Is decreased sIgA in the small intestine in acute liver necrosis models caused by reduced pIgR expression? We observed pIgR protein expression in acute liver necrosis models. Our study revealed significantly lower levels of 120KD (pIgR) and 80KD (SC) protein expression in the intestinal mucosa in acute liver necrosis models, and a significant positive linear correlation with mucus sIgA content in the small intestine.

The sIgA content of the intestinal fluid was positively correlated with the expression of pIgR protein in the small intestinal mucosa, indicating that the downward-regulation of pIgR in the mucosa of the small intestine may play an important role in reduced sIgA content and the development of intestinal mucosal immune disorder in acute liver necrosis.

pIgR production and secretion by the intestinal epithelial cells is regulated by multiple factors^[26,27]. Some researchers have suggested that increased serum inflammatory factors damage intestinal mucosal epithelial cells and injure the intestinal microcirculation, induce ischemia and anoxia of the intestinal mucosa, lead to epithelial cell dysfunction, and activate a chain reaction leading to the production of more inflammatory factors, protease and active oxygen, thus aggravating

intestinal inflammation and microcirculation disorders^[28,29]. Intestinal cells or their environment are thus threatened, causing exhaustion of stored pIgR in the intestinal barrier, and decreased production and secretion. Further studies are needed to verify these relationships, and to explore the generation pathway of sIgA in intestinal fluid.

In summary, sIgA decreased in acute liver necrosis models, and downward-regulation of pIgR in the mucosa of the small intestine may play an important role in reduced sIgA content and the development of intestinal mucosal immune disorder in acute liver necrosis.

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