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Glutamine modulates CD8αα$^+$ TCRαβ$^+$ intestinal intraepithelial lymphocyte expression in mice with polymicrobial sepsis

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ABSTRACT

Objectives: CD8αα$^+$ T-cell receptor (TCR) αβ$^+$ intestinal intraepithelial lymphocytes (IELs) were found to have a regulatory function in the mucosal immune system. Glutamine (GLN) is an amino acid with immunomodulatory effects. The aim of this study was to investigate the influences of GLN on the proportion of CD8αα$^+$ TCRαβ$^+$ IELs and associated inflammatory mediator gene expression in polymicrobial sepsis.

Methods: Mice were randomly assigned to a normal (NC) group, a sepsis with saline (SS) group, or a sepsis with GLN (SG) group. The NC group was fed a chow diet. Sepsis was induced by cecal ligation and puncture (CLP). The SS group was administered saline, and the SG group was given 0.75 g GLN/kg body weight via a tail vein after CLP. Mice were sacrificed 12 h after CLP, and CD8αα$^+$ TCRαβ$^+$ IELs were isolated for further analysis.

Results: Sepsis resulted in a lower percentage of CD8αα$^+$ TCRαβ$^+$ IELs, and higher messenger (m)RNA expression of complement 5a receptor, interleukin (IL)-2 receptor β, IL-15 receptor α, and interferon-γ by CD8αα$^+$ TCRαβ$^+$ IELs. These immunomodulatory mediator genes decreased, whereas IL-7 receptor and transforming growth factor-β expressions increased in CD8αα$^+$ TCRαβ$^+$ IELs in septic mice with GLN administration. Annexin V/7-AAD staining revealed significantly lower apoptotic rates of CD8αα$^+$ TCRαβ$^+$ IELs in the SG group.

Conclusion: A single dose of GLN administered after the initiation of sepsis increased the percentage of CD8αα$^+$ TCRαβ$^+$ IELs, prevented apoptosis of CD8αα$^+$ TCRαβ$^+$ IELs, and downregulated CD8αα$^+$ TCRαβ$^+$ IEL-expressed inflammatory mediators. These results suggest that GLN influenced the distribution and cytokine secretion of the CD8αα$^+$ TCRαβ$^+$ IEL subset, which may ameliorate sepsis-induced inflammatory reactions and thus mitigate the severity of intestinal epithelial injury.

Introduction

The intestinal epithelial surface is the largest potential entry site in the body for bacteria and other pathogens. However, invasion by microorganism from the gut into a host is prevented by a complex mucosal epithelial immune system. Intestinal intraepithelial lymphocytes (IELs) are a unique population of T cells that reside within the epithelium of the small intestine where they form the first line of immune defense against invading pathogens while preserving the integrity of the mucosal barrier [1]. IELs are comprised of three subsets of CD8+ T cells: approximately half of IELs express a T-cell receptor (TCR) γδ and a CD8αα coreceptor. The other half express a TCRαβ that can be subdivided into equal parts according to the expression of the CD8αβ or CD8αα form of the coreceptor [2]. Although the population of IELs expressing CD8αα$^+$ TCRαβ$^+$ in the small intestine of mice is lower than that expressing CD8αα$^+$ TCRγδ$^+$, CD8αα$^+$ TCRαβ$^+$ IELs were found to have an important immunoregulatory role in the gut [3]. A study performed by

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Saurer et al. [4] found that CD8αα$^+$ TCRαβ$^+$ IELs are activated after infection; however, they do not become cytotoxic nor initiate self-destruction in the gut.

Sepsis is a common cause of death in critically ill patients. Sepsis is initiated by bacteria and their related toxins. When a host is exposed to bacterial toxins, immune cells are activated, and a variety of endogenous mediators are oversecreted. Sepsis is known to result in impaired function of the intestinal mucosal barrier [5]. A previous study using cecal ligation and puncture (CLP) to produce a polymicrobial septic challenge indicated that sepsis induced a concomitant increase in the percentage of CD8αα$^+$ γδ$^+$ cells and a decrease in CD8αα$^+$ αβ$^+$ cells in the small intestinal IEL compartment of the gut [6]. A deficiency of γδT lymphocytes contributes to mortality and immunosuppression in sepsis [6]; however, the regulatory role of CD8αα$^+$ TCRαβ$^+$ IELs in a host's immune response in sepsis remains unclear.

Glutamine (GLN) is the most abundant amino acid in mammalian plasma and tissues. A previous study found that severe GLN deficiencies occur rapidly in critical illness, and the magnitude of GLN deficiency is correlated with mortality in the intensive care setting [7]. A number of trials revealed that GLN supplementation improves infectious morbidity and mortality in critically ill patients [8,9]. GLN is an essential metabolic component for the proliferative response of enterocytes, and is a critical substrate for rapidly proliferating immune cells [10]. GLN is considered a conditionally essential amino acid during certain disease conditions [11]. A study performed by Nose et al. [12] found that GLN-supplemented total parenteral nutrition (TPN) modulated IEL-derived cytokine profiles and thus preserved the intestinal epithelial barrier function. However, no study, to the present, has investigated the effects of GLN on the development and population distribution of CD8αα$^+$ TCRαβ$^+$ IELs in sepsis. We hypothesized that GLN would influence the distribution and cytokine secretion of the CD8αα$^+$ TCRαβ$^+$ IEL subset, which may mitigate sepsis-induced inflammatory reactions. Therefore, we analyzed gene expressions involved in the development and antiapoptosis of total TCRαβ$^+$ cells in IELs. Also, messenger (m) RNA of inflammatory mediators-expressed by CD8αα$^+$ TCRαβ$^+$ IELs was analyzed in a mouse model of polymicrobial sepsis.

Materials and methods

Animals

Male C57BL/6 mice at 8 wk to 12 wk old and weighing 22 g to 25 g at the beginning of the experiment were used in this study. All mice were housed in a temperature-controlled and humidity-controlled room and were allowed free access to a standard chow diet for 1 wk before the study. Care of laboratory animals was in full compliance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996), and protocols were approved by the institutional Animal Care and Use Committee of Taipei Medical University.

Experimental procedures

There were three groups in this study: one normal control group (NC) and two sepsis groups. All mice were fed a chow diet. Mice in the NC group were healthy and underwent no operation. Polymicrobial sepsis was induced by CLP. CLP was performed as previously described [13]. Briefly, mice were anesthetized, a 1-cm incision was made below the diaphragm, and the cecum was isolated. Approximately, 50% of the cecum was ligated just below the ileocecal valve with 3–0 silk. The distal cecum was then punctured in two places with a 23-gauge needle to allow a small amount of fecal material to extrude into the peritoneal cavity, and then it was replaced into the abdomen. The abdominal wound was closed in two layers. Animals were given subcutaneous fluid resuscitation (40 mL/kg body weight) after CLP. All of the above manipulations were performed by the same person to ensure consistency. After the operation, one of the sepsis groups was injected with saline (SS group), and the other group was given 0.75 g GLN/kg body weight (SG group) once via a tail vein 1 h after CLP. GLN was administered as alanyl-glutamine dipeptide (Dipeptiven; Fresenius-Kabi, Homburg, Germany). Mice in the NC group were sacrificed at the time CLP was performed in the sepsis groups. Mice in the SS and SG groups were sacrificed 6, 12, and 24 h after CLP to examine the percentage of the CD8αα$^+$ TCRαβ$^+$ subset of IELs. These time points were chosen because 6 and 12 h are considered the early septic stage, whereas 24 h is the late phase in this model [14]. All mice survived in the NC group (n = 6) and sepsis groups 6 and 12 h after CLP (n = 6 for each group at respective time points). The survival rate for 24 h after CLP did not differ between the two sepsis groups (seven of eight mice in the SG group versus six of seven mice in the SS group). All mice were anesthetized and then sacrificed by cardiac puncture. Blood samples were collected in tubes containing heparin and centrifuged at 3000 g for 10 min at 4°C. After harvesting the blood, 2 mL of sterile saline was intraperitoneally injected into the peritoneal cavity to obtain peritoneal lavage fluid (PLF). Plasma and PLF samples were stored at −80°C for analysis of inflammatory cytokines. Small intestine tissues were collected from septic mice 12 and 24 h after CLP for histologic examination. The small intestine CD8αα$^+$ TCRαβ$^+$ IEL subset was only prepared in septic groups 12 h after CLP for the analysis of inflammatory mediator gene expressions.

Measurements of inflammatory cytokines in plasma and PLF

Concentrations of monocyte chemotactic protein (MCP)-1 in plasma, and interleukin (IL)-1β, MCP-1 and macrophage inflammatory protein (MIP)-3α in PLF 6, 12, and 24 h after CLP were measured using enzyme-linked immunosorbent assay kits according to the manufacturer's instructions (eBioscience, San Diego, CA, USA; R&D Systems, Minneapolis, MN, USA).

Preparation of IELs

IELs were isolated using a previously described procedure with some modifications [15]. The small intestine was trimmed of fat and Peyer's patches. Fecal contents were flushed out with cold calcium- and magnesium-free (CMF) phosphate-buffered saline (PBS) (Ca^{2+}- and Mg^{2+}-free PBS with 1 mM HEPES, 2.5 mM $NaHCO_3$, and 2% fetal bovine serum (FBS); pH 7.3). The small intestine was cut longitudinally, then into 5-mm pieces, and washed twice with cold CMF PBS. Tissues were placed into 20 mL of RPMI1640 medium with 10% FBS and incubated for 30 min at 37°C with gentle shaking. Tissues and medium were then transferred to a 50-mL centrifuge tube and vortexed at the maximum setting for 15 s, and the supernatant was poured off. This step was repeated twice, using fresh medium each time, and cells in the supernatants from each treatment were combined. Supernatants were pooled and filtered through a 40-μm nylon mesh, pelleted at 1600g for 10 min, and resuspended in 40% Percoll (GE Healthcare, Munich, Germany) in RPMI 1640 medium. This cell suspension was overlaid with 70% Percoll in RPMI 1640 and centrifuged at room temperature and 2800 g for 20 min without interruption. IELs were recovered from the 40%/70% interphase and washed twice in RPMI 1640 medium.

Flow-cytometric analysis

To measure the distribution of TCRαβ$^+$ CD8αα$^+$ in IELs, 10^6 IELs were suspended in 100 μL staining buffer. IELs were incubated with purified rat anti-mouse CD16/CD32 (2.4G2; BD Pharmingen, San Diego, CA, USA) at 4°C for 10 min followed by IELs being stained with Pacific blue (PB) anti-mouse CD103 (2E7; Biolegend, San Diego, CA, USA), allophycocyanin anti-mouse TCRβ (H57-597; Biolegend), phycoerythrin (PE) anti-mouse CD8α (53-6.7; BD Pharmingen), and fluorescein isothiocyanate (FITC) anti-mouse CD8β (YTS156.7.7; Biolegend) at 4°C for 15 min. After two washes, cells were analyzed by FACS Calibur flow cytometry (BD FACSCantoII).

Total IEL CD8αα$^+$ TCRαβ$^+$ T-cell isolation

To study gene expressions of CD8αα$^+$ TCRαβ$^+$, total IELs were stained with PE anti-mouse TCRβ (H57-597; Biolegend), PB anti-mouse CD8α (53-6.7; Biolegend), and FITC anti-mouse CD8β in order to eliminate CD8αβ$^+$. IELs were purified on a cell sorter (BD FACSAria III). The CD8αα$^+$ TCRαβ$^+$ subsets were isolated from IELs, and the purity (≥98%) was ensured.

mRNA extraction and a quantitative real-time reverse-transcription polymerase chain reaction analysis

Total RNA was isolated from purified CD8αα$^+$ TCRαβ$^+$ IELs using an RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA pellet was dissolved in RNase-free water. The total RNA solution was stored at −80°C for the subsequent assay. The concentration of RNA was determined and quantified by measuring the absorbance at 260 and 280 nm on a spectrophotometer. Complementary (c)DNA was synthesized from total RNA using a RevertAid™ first-strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania) according to standard protocols. Reverse transcription (RT) was carried out by subsequent incubation for 5 min at 65°C, 60 min at 42°C, and 5 min at 70°C. cDNA was stored at −80°C until used. Specific mRNA genes were amplified by a real-time RT-polymerase chain reaction (PCR)