Medium-chain triglycerides enhance secretory IgA expression in rat intestine after administration of endotoxin

Hiroshi Kono, Hideki Fujii, Masami Asakawa, Akira Maki, Hitotake Amemiya, Yu Hirai, Masanori Matsuda, and Masayuki Yamamoto. Medium-chain triglycerides enhance secretory IgA expression in rat intestine after administration of endotoxin. *Am J Physiol Gastrointest Liver Physiol* 286: G1081–G1089, 2004; 10.1152/ajpgi.00457.2003.—The purpose of this study was to determine whether medium-chain triglycerides (MCTs) modulate the inflammatory immune response to LPS and enhance the expression of secretory IgA in the rat intestine. Rats were given either corn oil or MCTs by gavage daily for 1 wk, and LPS or saline vehicle was administered via the tail vein. They were then killed, and serum and sections from the gut were collected for further analysis. Western blot analysis for secretory IgA revealed that MCTs significantly enhanced its expression in the ileum compared with corn oil in rats administered saline. After LPS challenge, expression of secretory IgA was decreased in the corn oil group but not in the MCTs group. The mRNA expression of IL-6 was assessed by real-time RT-PCR, because IL-6 regulates secretory IgA in the intestine. The expression was significantly greater in the MCTs group than in the corn oil group after LPS injection. Increases in expression of proinflammatory cytokines or chemokines such as TNF-α, IL-18, macrophage inflammatory protein-2, and monocyte chemoattractant protein-1 in the ileum were significantly blunted by MCTs. In addition, the mRNA expression of the Th2 IgA-stimulating cytokine IL-10 in the ileum and Peyer’s patches was significantly greater in the MCTs than the corn oil group. In contrast, the mRNA expression of the Th1 IgA-inhibiting cytokine interferon-γ was blunted by MCTs. As a result, intestinal injury was significantly reduced. Therefore, MCTs protect the gut by modulating the immune response to LPS and enhancing secretory IgA expression.

saturated fat; inflammatory cytokines; endotoxiaemia; chemokines; Peyer’s patch

The gut mucosa has been called the “motor” responsible for multiple organ failure in critical illnesses (18). The gut mucosa is the site in which various acute phase proteins (23, 36, 37), gut hormones (38), and cytokines (22) are produced that affect the mucosa as well as the function and integrity of remote organs and tissues. Thus the role of the intestinal mucosa in inflammatory and metabolic responses to sepsis and endotoxiaemia has become more apparent during the past decade.

We reported that a daily supplement of medium-chain triglycerides prevented an increase in gut permeability and intestinal injury after endotoxin administration (11, 12). We speculated that medium-chain triglycerides help protect the gut during endotoxiaemia or sepsis. Proinflammatory cytokines such as TNF-α are involved in triggering a vicious cycle in infectious insults via the redox-sensitive transcriptional factor, NF-κB (14). These cytokines upregulate chemoattracting factors, which subsequently increase the number of leukocyte into the host tissue. Thus inflammatory cytokines and chemokines are involved in organ injury. Alternatively, secretory IgA is synthesized within the lamina propria through the interaction of T and B cells and then transported by overlying epithelial cells onto the mucosal surface using secretory components. There, it binds to specific antigens on bacteria (16, 31), viruses, and other toxic molecules and prevents them from attaching to the mucosal surface. It also plays a role in eliminating infectious agents that have penetrated epithelial cell layers. Intestinal secretory IgA levels correlate inversely with bacterial overgrowth, bacterial translocation, and changes in intestinal permeability in animal models (4). Thus secretory IgA plays an important role in protecting against infection in the intestinal immune system (20, 21). The purpose of this study was to determine whether medium-chain triglycerides enhance the production of secretory IgA and inhibit expression of inflammatory mediators in the intestine. The production of secretory IgA is controlled by cytokine-producing T cells within the gut-associated lymphoid tissue and possibly by cytokines released from the mucosa. T cells, which are major producers of cytokines, are classified into two distinct subsets, Th1 and Th2, based on the pattern of cytokines they secrete. Th1 cytokines such as IFN-γ and TNF-β downregulate IgA production, whereas Th2 cytokines such as IL-4, IL-5, IL-6, and IL-10 upregulate IgA production (15). A balance between Th1 and Th2 cytokines may be necessary to maintain a normal IgA immune response. Therefore, mRNA expression of IFN-γ and IL-10 in the ileum and Peyer’s patches was also investigated.

Materials and methods

**Animals and treatments.** Male Sprague-Dawley rats (250–300 g body wt; Japan SLC, Shizuoka, Japan) were used in this study. They were housed under barrier-sustained conditions and allowed to recover for at least 5 days after arrival before being used. During this acclimation period, rats were fed Purina rat Chow (cat. no. 5001; Dyets, Bethlehem, PA). The experimental protocol followed the institutional and the National Research Council criteria for the care and use of laboratory animals in research. Furthermore, all rats received humane care in compliance with institutional guidelines.

Rats were given medium-chain triglycerides (saturated fat, tritocanoin 8:0; 5 g/kg, Nihon-Yushi, Tokyo, Japan) or the same dose of corn oil (polysaturated fat) by gavage daily for 1 wk (11), and received food and water ad libitum throughout the study. Previous
supernatant was ultracentrifuged at 100,000 g for 10 min at 4°C. Serum was stored at −80°C until assayed for secretory IgA. Serum secretory IgA was measured by an ELISA using polyclonal goat anti-secretory IgA antibody (ICN, Costa Mesa, CA), and data were corrected for each dilution.

**Pathology and histological measurement.** Sections of gut, liver, and lung were collected 9 h after LPS administration and stained with hematoxylin-eosin to assess inflammation and necrosis. Pathology was evaluated in a blind manner by one of the authors and by an oral pathologist. Furthermore, we were able to assess the treatment status of the animals from which the tissues were harvested.

**Immunohistochemistry of secretory IgA in gut.** Paraffin-embedded sections of jejunum, ileum, and colon were deparaffinized, rehydrated, and stained immunohistochemically with polyclonal goat anti-secretory IgA antibody (ICN) by sequential incubation with polyclonal antibodies (StressGen Biotechnologies, Victoria, BC, Canada) in PBS (pH 7.4) containing 10 g/l Tween 20 and 10 g/l BSA (13). Peroxidase-linked secondary antibody and diaminobenzidine (peroxidase) were used to detect specific binding. The slides were rinsed twice with PBS-1 g/l Tween 20 between each incubation and sections were counterstained with hematoxylin as described elsewhere (5). Sections from the same rats were collected and immediately placed on ice. Cold nonreducing Laemmli buffer (50 mM Tris-HCl, pH 6.8, containing 20% g/l SDS and 0.1% v/v glycerol) containing 12.5 mM benzamidine and 21 mM leupeptin (Sigma) was added to the bile (9:1 vol buffer/bile). The resulting mixture was stored at −80°C.

**Preparation of samples and immunoblot analysis of IgA.** To collect bile as a positive control (2, 8, 28), the bile duct was cannulated with a small length of polyethylene-10 tubing, and bile samples were collected and immediately placed on ice. Cold nonreducing Laemmli buffer (62.5 mM Tris-HCL, pH 6.8, containing 20% g/l SDS and 0.1% v/v glycerol) containing 12.5 mM benzamidine and 21 mM leupeptin (Sigma) was added to the bile (9:1 vol buffer/bile). The resulting mixture was stored at −80°C.

**Blood sampling and measurement of serum secretory IgA levels.** In separate experiments, rats were given medium-chain triglycerides (5 g·kg⁻¹·d⁻¹) or the same dose of corn oil for 1 wk. Then, either LPS (10 mg/kg) or a saline vehicle was administered via the tail vein. Blood samples were collected from the aorta 9 h later and centrifuged at 1,200 g for 10 min at 4°C. Serum was stored at −80°C until assayed for secretory IgA. Serum secretory IgA was measured by an ELISA using polyclonal goat anti-secretory IgA antibody (ICN, Costa Mesa, CA), and data were corrected for each dilution.

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**Tissue sample collection and tissue processing.** Tissue samples of the ileum, jejunum, and colon were collected from rats 9 h after saline or LPS administration. Intestinal tissue was washed free of its contents using cold PBS, blotted on filter paper, and weighed. A crude particulate fraction of the intestine was prepared according to the method of Perez et al. (26) with some modifications. Briefly, the organ was homogenized by using a homogenizer (Caf-ramo, Wiarton, ON, Canada). Ten volumes of cold buffer (pH 7.4) were used containing in mM: 10 TES (Sigma), 1 MgCl₂, 12.5 mM benzamidine, and 21 mM leupeptin (Sigma) was added to the bile (9:1 vol buffer/bile). The resulting mixture was stored at −80°C.

**Western blot analysis of secretory IgA in the gut.** Intestinal tissues were collected 9 h after LPS or saline administration for expression of secretory IgA. Samples of bile and intestinal crude particulate fractions were diluted in nonreducing Laemmli buffer. Extracted proteins (20 μg) were separated by 100 g/l SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were blocked with Tris-buffered saline-Tween 20 containing 50 g/l skim milk and probed with polyclonal goat anti-secretary IgA antibodies (ICN) or β-actin (Sigma) followed by horseradish peroxidase-conjugated secondary antibody when appropriate. They were then incubated with an enhanced chemiluminescent substrate (ECL reagent; Amersham Life Science, Arlington Heights, IL) and exposed to X-Omation film (Eastman Kodak, Rochester, NY). Densitometric analysis of the images was performed on a Macintosh computer using NIH image 1.54 analysis software.

**Real-time RT-PCR for mRNA expression of IL-6, TNF-α, and IL-18 in mucosa from the ileum; IFN-γ, and IL-10 in the ileum; and Peyer’s patches.** mRNA was quantified by real-time RT-PCR (TaqMan; PE Applied Biosystems, Foster City, CA). Real-time RT-PCR was performed by using a GeneAmp 5700 Sequence Detection System (PE Applied Biosystems). Predeveloped TaqMan assay reagents (PE Applied Biosystems) were used for sequencing specific primers for IFN-γ (GenBank accession no. AF010466), IL-10 (GenBank accession no. X60675; L02926), IL-6 (GenBank accession no. M26744), TNF-α (GenBank accession no. NM 012676) and IL-18 (GenBank accession no. NM 012677) as described elsewhere.

**Four hours after LPS or saline injection, sections of the ileum and Peyer’s patches near the terminal ileum were collected by using a scalpel, and sections of mucosa from the ileum were collected to measure the mRNA expression of IL-6, TNF-α, and IL-10. Nine hours after LPS or saline administration, sections of mucosa from the ileum were collected to measure the mRNA expression of IFN-γ or IL-10.**

**Reverse transcription of total RNA (2 μg) was performed in a final volume of 100 μl containing 1X TaqMan RT buffer, 5.5 mM MgCl₂, 500 μM of each dNTP, 2.5 μM random hexamers, 0.4 × 10⁵ U/l RNase inhibitor, and 1.25 × 10⁹ U/l multiscribe RT. cDNA samples (2 μl) were used for quantitative RT-PCR (a 10-min step at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C in the presence of specific forward and reverse primers on a TaqMan Universal PCR Master Mix; PE Applied Biosystems). mRNA levels were calculated by using the comparative parameter threshold cycle (Ct) method (6) and normalized to rRNA. To confirm the amplification specificity, the PCR products were subjected to a melting-curve analysis.

**Semiquantitative RT-PCR for the mRNA expression of macrophage inflammatory protein-2 and monocoyte chemoattractant protein-1 in the ileum.** Macrophage inflammatory protein (MIP-2) and monocoyte chemoattractant protein (MCP-1) are chemotactic factors predominately produced by macrophages. They increase the number of macrophages/monocytes and neutrophils recruited to the inflammatory focus and cause tissue injury. Because predeveloped primer and probe for MIP-2 and MCP-1 were not commercially available, the mRNA expression was assessed by semiquantitative RT-PCR. Intestinal tissue samples were collected from animals killed 4 or 9 h after LPS or saline injections. Total RNA was isolated from 25-mg pieces of liver tissue using a RNA purification kit (Qiagen, Hilden, Germany). Reverse transcription of total RNA (2 μg) was performed in a final volume of 100 μl containing 1X TaqMan RT buffer, 5.5 mM MgCl₂, 500 μM of each dNTP, 2.5 μM random hexamers, 0.4 × 10⁵ U/l RNase inhibitor, and 1.25 × 10⁹ U/l multiscribe RT. cDNA samples (2 μl) were used for quantitative RT-PCR (a 10-min step at 95°C, followed by 40 cycles of 15 s at 95°C, and 1 min at 60°C in the presence of specific forward and reverse primers on a TaqMan Universal PCR Master Mix; PE Applied Biosystems). mRNA levels were calculated by using the comparative parameter threshold cycle (Ct) method (6) and normalized to rRNA. To confirm the amplification specificity, the PCR products were subjected to a melting-curve analysis.

**Reverse transcription of total RNA was performed by using the method described above.** PCR primers for MIP-2 (10), MCP-1 (33), and GAPDH contained the following sequences: MIP-2 sense (5'-CAGACGCTTACCGAGTACAGGAC-3') and antisense (5'-TCTGATTTTCTGTCCTC-3'); MCP-1 sense (5'-TCCACACTATGCGAGCTGTC-3') and antisense (5'-TGACACAGCTTACTTATTGGG-3'); and GAPDH sense (5'-TGAAGGTCGGTACACAGGATTTGG-3') and antisense (5'-CATGTTGGCCATGAGTCACAC-3').

**Aliquots (5 μl) of synthesized cDNA were added to 45 μl of PCR mix containing 5 μl of 10× PCR buffer, 1 μl of each deoxynucleotide nucleotide.
Table 1. Effect of LPS and MCT on mortality

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Values are % mortality of 8 rats given 5 mg/kg of corn oil (CO) or medium-chain triglycerides (MCT). Mortality after LPS administration was determined as described in MATERIALS AND METHODS. *P < 0.05 compared with rats given a saline vehicle; †P < 0.01 compared with rats given CO with LPS by Fisher’s exact test.

(1 mM each), 0.5 μl of sense and antisense primers (0.15 mM), and 0.25 μl of DNA polymerase (Gene Amp PCR kit; Perkin Elmer Cetus, Norwalk, CT).

The amplified PCR products were electrophoresed at 100 volts through a 2% agarose gel (BRM, Sigma) for ~30 min. The gels were stained with 0.5 mg/ml ethidium bromide Tris-borate-EDTA buffer (ICN) and photographed with Type 55 Polaroid positive/negative film. Densitometric analysis of the captured image was performed on a Macintosh computer using NIH image 1.54 analysis software. The area under the curve was normalized for GAPDH content.

Statistics. Data are expressed as the means ± SE. ANOVA with Bonferroni’s post hoc test or the Student’s t-test was used to determine significance when appropriate. A P value < 0.05 was considered significant.

RESULTS

Effect of lipid type and LPS on mortality. All animals survived 24 h after saline administration in both groups (Table 1). However, all animals given corn oil appeared moribund 9 h after LPS injection and died within 24 h after LPS administration (Table 1). Mortality was absent in rats given medium-chain triglycerides.

Effect of lipid type and LPS on the gut. Complications were not observed in rats given either medium-chain triglycerides or corn oil for 1 wk. Furthermore, there were no significant differences in body weight between the two groups. Morphological changes were not observed in the gut sections from rats treated with corn oil for 1 wk after saline injection (Fig. 1). In contrast, medium-chain triglycerides significantly increased the length of villi (normal rats, 755 ± 92 μm; rats given corn oil, 762 ± 82 μm; and rats given medium-chain triglycerides, 802 ± 88 μm, P ≤ 0.05). Furthermore, the number of goblet cells in the gut also increased, which was consistent with the results of a previous study (12). After LPS administration, the ileum from rats given corn oil showed hemorrhagic changes in the submucosal layer and necrosis of the epithelial cells. However, these changes were almost completely prevented by medium-chain triglycerides. Furthermore, liver (12) and lung injury was also prevented by medium-chain triglycerides (data not shown).

Effect of lipid type and LPS on serum secretory IgA levels. Serum secretory IgA was not significantly different between the two groups 9 h after saline administration (Fig. 2). After LPS injection, values were decreased by ~40% in rats given corn oil. However, levels increased ~1.5-fold in rats given medium-chain triglycerides.

Effect of lipid type and LPS on intestinal secretory IgA levels. Secretory IgA expression was assessed in the gut by immunohistochemistry (Fig. 3). In rats administered saline, the expression was minimal in each intestinal section. Although LPS administration increased the expression in each gut sec-
tion in both treatment groups, it was greatest in the ileum. Furthermore, elevated expression was observed in all intestinal sections from rats given medium-chain triglycerides (Fig. 3).

To confirm these results, Western blot analyses of secretory IgA and β-actin were performed on intestinal samples (Fig. 4, A and B). The expression was greatest in the ileum, followed by the colon, in untreated normal rats (Fig. 4B). Further analysis was carried out by using samples from the ileum. After saline injection, the expression did not change in rats given corn oil. However, in rats given medium-chain triglycerides, it was significantly increased ~1.2-fold compared with the corn oil group. Furthermore, after LPS administration, the expression decreased significantly by ~60% in rats given corn oil but not in those given medium-chain triglycerides.

mRNA expression of IL-6, TNF-α, and IL-18 in the mucosa of the ileum. The mRNA expression of IL-6, TNF-α, and IL-18 was assessed in the ileum from rats treated with corn oil or medium-chain triglycerides (Fig. 5). Nine hours after saline administration, the mRNA expression of IL-6 was greater in rats given medium-chain triglycerides than in those given corn oil. Although LPS administration significantly increased the mRNA expression of IL-6 in both groups, it was significantly greater in rats given medium-chain triglycerides compared with those given corn oil.

After saline administration, the mRNA expression of TNF-α and IL-18 was not significantly different between the two groups. However, the expression of both cytokines increased approximately twofold in rats given corn oil after LPS administration. The expression was significantly blunted in the medium-chain triglyceride group.
Effect of lipid type and LPS on MIP-2 and MCP-1 mRNA expression in the ileum. After saline administration, the mRNA expression of MIP-2 was significantly greater in the ileum of rats given corn oil compared with those given medium-chain triglycerides (Fig. 6). After LPS administration, the expression significantly increased in rats given corn oil, whereas the increase was significantly blunted in rats treated with medium-chain triglycerides.

After saline administration, the mRNA expression of MCP-1 was not detected in either group. In contrast, LPS increased significantly the expression of MCP-1 in rats given corn oil, and the increase was significantly blunted in the medium-chain triglyceride group. The same results were observed in samples from rats administered LPS after 9 h (data not shown).

mRNA expression of IFN-γ and IL-10 in the ileum and Peyer’s patches. The mRNA expression of Th1 IgA-inhibiting cytokine IFN-γ and Th2 IgA-stimulating cytokine IL-10 was assessed in the ileum and Peyer’s patches from rats treated with corn oil or medium-chain triglycerides (Fig. 7). Nine hours after saline administration, the mRNA expression of IFN-γ was significantly less in rats given medium-chain triglycerides compared with those given corn oil. In contrast, LPS administration significantly increased expression of IFN-γ in rats given corn oil, and this increase was blunted by ~30% by medium-chain triglycerides.

Medium-chain triglycerides significantly increased the mRNA expression of IL-10 in the ileum but not in the Peyer’s patches of rats administered saline. After LPS administration, the expression did not change significantly in rats given corn oil. However, it increased significantly ~1.5- to 2-fold in rats given medium-chain triglycerides.

DISCUSSION

Effects of medium-chain triglycerides on the intestinal inflammatory immune response in endotoxemia. Macrophages are an important part of the immunological and inflammatory
responses. A large population of these cells resides in the normal intestinal mucosa. In inflammatory bowel disease, the mucosal macrophage population increases and is derived from circulating monocytes. They are phenotypically different from the resident population and play a major role in mediating the mucosal inflammation (19). They predominantly secrete proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). In this study, increases in the mRNA expression of TNF-α and IL-18 were blunted by medium-chain triglycerides (Fig. 5). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29).

In this study, increases in the mRNA expression of TNF-α and IL-18 were blunted by medium-chain triglycerides (Fig. 5). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29). Among the inflammatory mediators expressed by proinflammatory cytokines, such as TNF-α and IL-18, which are involved in intestinal injury caused by inflammation (24). IL-18 was a primary mediator of the inflammation associated with dextran sulfate sodium-induced colitis in mice (29).

Among the cytokines produced in the intestinal mucosa during inflammation, IL-6 is important because of its multiple biological effects in the intestine as well as other organs and tissues. IL-6 is an integral part of the inflammatory response to sepsis and endotoxemia. Under different conditions, IL-6 may exert pro- or anti-inflammatory effects. In conditions of "uncontrolled" inflammation, high IL-6 levels contribute to morbidity and mortality (3). In a previous study, systemic IL-6 levels gradually increased up to 6 h after a lethal dose of LPS, and this level correlated with pathophysiology and mortality (12). Furthermore, levels increased significantly in rats given corn oil and correlated with organ injury and mortality (12). Medium-chain triglycerides, however, prevented these events. In addition to the biological roles of systemic levels of IL-6, it has important biological effects on the intestinal mucosa (27). Mucosal levels of IL-6 regulate enterocyte acute-phase protein synthesis (23), protein synthesis in the mucosa (35), and intestinal secretory IgA production (1). IL-6 is also an important regulator of secretory IgA production by B cells in the Peyer’s patches. In this study, the mRNA expression of IL-6 was enhanced by medium-chain triglycerides before and after LPS administration (Fig. 5). Furthermore, intestinal and serum secretory IgA increased significantly in rats given medium-chain triglycerides compared with those given corn oil after LPS administration (Figs. 2–4). Thus medium-chain triglycerides increase the expression of intestinal IL-6, which possibly correlates with increases in expression of secretory IgA in the gut.

The role of intestinal secretory IgA and mucosal immunology during endotoxemia. IgA, but not IgG, antibodies against fecal endotoxins increased significantly in patients with alco-
hol-induced liver disease (25). IgA antibodies against fecal endotoxin correlated closely with the plasma concentrations of alanine aminotransferase, γ-glutamyl transferase, and C-reactive protein in patients with alcoholic liver disease. Because medium-chain triglycerides increased serum and intestinal secretory IgA (Figs. 2–4) and prevented intestinal (Fig. 1) and liver injury (12) after LPS administration, elevated production of secretory IgA by medium-chain triglycerides contributes to the inactivation of endotoxin. Also, secretory IgA located in body tissue was shown to suppress the inflammatory process, thereby reducing its damaging effects on multiple organs (25).

Peyer’s patches are the principal sites responsible for sensitization of secretory IgA. In humans, most of the Peyer’s patches are located in the distal small intestine, which contains microflora similar to the colon due to backwash from the colon through the ileocecal valve (25). The Peyer’s patches were also predominantly located in the distal ileum in rats in this study (data not shown). mRNA expression of Th2 IgA-stimulating anti-inflammatory cytokines such as IL-10 was significantly induced in this region by glutamine after LPS administration (7). These cytokines stimulate IgA production in vitro by cells of the gut-associated lymphoid tissue. Thus intestinal secretory IgA production is associated with the expression of Th2 IgA-stimulating cytokines (17). The mRNA expression of IL-10 was significantly increased in the ileum and Peyer’s patches in rats given medium-chain triglycerides compared with those given corn oil after LPS administration (Fig. 7). Furthermore, the mRNA expression of the Th1 IgA-inhibiting cytokine INF-γ also decreased in rats given medium-chain triglycerides. Therefore, the Peyer’s patches play an important role as an intestinal lymphoid organ in endotoxemia. Medium-chain triglycerides also affect the expression of secretory IgA, which possibly reduces the inflammatory immune response against endotoxin in the gut.

![Image](http://ajpgi.physiology.org/)

Fig. 7. The mRNA expressions of IFN-γ (A, C) and IL-10 (B, D) in the ileum (C, D) and Peyer’s patches (A, B). Tissue samples were collected 4 h after LPS or saline vehicle administration and the mRNA expression of cytokines was measured by RT-PCR as detailed in MATERIALS AND METHODS. The mRNA levels were calculated by using the comparative Ct method and normalized to ribosomal RNA. Data represent the means ± SE (n = 6). *P < 0.01 compared with rats given corn oil with a saline vehicle; #P < 0.05 compared with rats given corn oil with LPS by ANOVA with Bonferroni’s post hoc test.
Clinical implications. It is proposed that medium-chain triglycerides prevent organ injuries and mortality by increasing expression of intestinal secretion of IgA and modulating the triglycerides prevent organ injuries and mortality by increasing immunonutrition using medium-chain triglycerides. Because only one type of medium-chain triglyceride was used, further studies should be performed to determine whether other types of medium-chain triglycerides could be used for clinical purposes. Furthermore, in this study, we concluded that dietary medium-chain triglycerides improved intestinal injury by inhibition of the expression of inflammatory cytokines and chemokines and enhancing intestinal secretory IgA. However, other laboratories have reported that medium-chain triglycerides prevented intestinal atrophy in septic rat (9) and affected the fatty acid composition of the brush-border membrane (34). Thus the direct cytoprotective effects of medium-chain triglycerides on the intestinal epithelial cells could not be proved in this study. Accordingly, further investigation is needed to clarify this issue.

REFERENCES

5. Eldridge SR, Tilbury LF, Goldsworthy TL, and Butterworth BE. Clinical implications.
7. Fukatsu K, Kudsk KA, Zarzaur BL, Wu Y, Hanna MK, and DeWitt RC. TPN decreases IL-4 and IL-10 mRNA expression in lipopolysaccha-
ride stimulated intestinal lamina propria cells but glutamine supplemen-
9. Iba T, Yagi Y, Kidokoro A, Ohno Y, Kaneshiro Y, and Akiyama T. Total parenteral nutrition supplemented with medium-chain triacyl-
13. Kramer DR, Sutherland RM, Bao S, and Husband AJ. Cytokine-
23. Parlesak A, Schafer C, and Bode C. IgA against gut-derived endotoxins: does it contribute to suppression of hepatic inflammation in alcohol-
25. Pritts T, Hungness E, Wang Q, Robb B, Hershko D, and Hasselgren PO. Mucosal and enterocyte IL-6 production during sepsis and endotox-
27. Sivakumar PV, Westrich GM, Kanaly S, Garka K, Born TL, Derry JM, and Viney JL. Interleukin 18 is a primary mediator of the inflamma-

