Activating rat intestinal mucosal mast cells by fat absorption

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The gut plays a unique role in the digestion and absorption of nutrients (e.g., fat) as well as host defense, which requires the close interaction and coordination of both gut epithelial cells and the immune cells situated in the lamina propria (3). Intestinal mast cells, the highly specialized secretory cells, exert important functions in innate and adaptive immune responses either through inducing epithelial secretion or by directing subsequent immune responses via release of mast cell mediators. These novel findings indicate that intestinal MMC are activated and degranulate to release MMC into lamina propria after lipid feeding. These novel findings as in the serosa and in rectal and gastric muscularis (10). Once activated by stimulators, either by immunoglobulin (Ig) E-dependent or IgE-independent agonists (e.g., microbe), mast cells degranulate and release the preformed mediators such as histamine, proteases, or release de novo synthesized mediators including lipid mediators prostaglandin D2 (PGD2) and leukotrienes as well as cytokines like interleukins (ILs) and chemokines (1, 11).

The regulation of the intestinal immune system by dietary food intake is not well understood. Several studies have suggested a close link between the intestinal immune cells with dietary fat absorption (9, 15, 31, 45). In particular, it was reported that intestinal lymphocyte flux was stimulated by administration of olive oil into stomach (31). In addition, intestinal macrophages, dendritic cells, or intraepithelial lymphocytes were found to be modulated by intraduodenal or in vitro exposure to long-chain fatty acids (LCFAs) (9, 15, 45). However, little is known about the effects of luminal fat intake on intestinal mast cell function. Dietary fats are digested and absorbed by the enterocytes and packaged into chylomicrons (43). Both chylomicrons, containing LCFAs, and lymphocytes are transported almost exclusively in mesenteric lymph because of their size (43). Thus the GI lymphatic system not only functions to prevent protein and fluid accumulation in the intestinal interstitium but is also intimately coupled with other aspects of GI function. In this study, by using the conscious lymph fistula rat model, a unique and highly sensitive tool for studying the secretions of cells associated with the intestinal mucosa (23), we determined the relationship between intestinal MMC with fat absorption.

MATERIALS AND METHODS

Liposyn II 20% was from Hospira (Lake Forest, IL). Trilinolein (no. T-250) was purchased from Nu-Chek-Prep (Elysian, MN), and tricaprylin (no. T-9001) was from Sigma-Aldrich (St. Louis, MO). Phospholipids (L-81) (no. 9003–11-6) was from BASF (Parsippany, NJ). Histamine ELISA kit (no. 409010) was from Neogene (Lexington, KY), and the rat mast cell protease II (RMCPII) monoclonal antibody (no. RS-MF4) and ELISA kit (no. RS-RM2) were purchased from Morelun Scientific (Aberdeen, Scotland). PGD2 ELISA kit (no. 512021) or PGD2 ELISA kit (no. 512041) were purchased from Cayman Chemical (Ann Arbor, MI); IL-6 assay kit (OptEIA rat II-L6) (no. 550319) was from BD Biosciences (San Diego, CA). Triglyceride assay kit (no. TR213) was from Randox Laboratories (Crumlin, London, UK). Alexa Fluor-488 conjugated secondary donkey anti-mouse antibody (no. A-11008) was from Invitrogen (Life Technologies) (Carlsbad, CA).

Animals. Male adult Sprague-Dawley rats, weighing 300–350 g (Harlan, Indianapolis, IN), were used. Animals were allowed to acclimate to our animal facility for 2 wk before the experiment. During this period, the animals were fed regular rodent chow and housed in a room with a 12-h:12-h light/dark cycle. Both the temperature and the humidity of the room were maintained.

The gut plays a unique role in the digestion and absorption of nutrients (e.g., fat) as well as host defense, which requires the close interaction and coordination of both gut epithelial cells and the immune cells situated in the lamina propria (3). Intestinal mast cells, the highly specialized secretory cells, exert important functions in innate and adaptive immune responses either through inducing epithelial secretion or by directing subsequent immune responses via release of mast cell mediators. These novel findings indicate that intestinal MMC are activated and degranulate to release MMC into lamina propria after lipid feeding. These novel findings as in the serosa and in rectal and gastric muscularis (10). Once activated by stimulators, either by immunoglobulin (Ig) E-dependent or IgE-independent agonists (e.g., microbe), mast cells degranulate and release the preformed mediators such as histamine, proteases, or release de novo synthesized mediators including lipid mediators prostaglandin D2 (PGD2) and leukotrienes as well as cytokines like interleukins (ILs) and chemokines (1, 11).

THE GUT PLAYS A UNIQUE ROLE in the digestion and absorption of nutrients (e.g., fat) as well as host defense, which requires the close interaction and coordination of both gut epithelial cells and the immune cells situated in the lamina propria (3). Intestinal mast cells, the highly specialized secretory cells, exert important functions in innate and adaptive immune responses either through inducing epithelial secretion or by directing subsequent immune responses via release of mast cell mediators (1, 3, 11). Intestinal mast cells have also been demonstrated to play important roles in gastrointestinal (GI) disorders such as food allergy, irritable bowel syndrome and inflammatory bowel diseases (IBD) (1, 3, 6, 8, 11, 25). According to the protease content, two mast cell subtypes have been found in human GI tract (1) as well as in rodent intestine (10), in which mucosal mast cells (MMC) are located predominantly in the lamina propria and connective tissue mast cells are located mainly in the proximal, nonmucosal GI regions as well...
Lymph and duodenal cannulation and lymph collection. The lymph fistula rat model was established as described (4, 29). All procedures were approved by the University of Cincinnati Internal Animal Care and Use Committee and compliant with the NIH Guide for the Care and Use of Laboratory Animals. Briefly, animals were fasted overnight before surgery. Under halothane anesthesia, the superior mesenteric lymph duct was cannulated with soft vinyl chloride tubing according to the method previously described (4, 29). A duodenal silicone infusion tube (1.6 mm OD) was inserted about 2 cm into the duodenum via a fundal incision of the stomach and was fixed by a purse-string suture. Postoperatively, the animals were intraduodenally (i.d.) infused with 5% glucose in saline (145 mM NaCl, 4 mM KCl, and 0.28 M glucose). The animals were secured in Bollman restraining cages and recovered overnight in temperature regulated box (maintained at 28°C). The animals were i.d. infused continuously at 3 ml/h of a saline solution before the experiment. Lymph was collected in cornical centrifuge tube on ice for 1 h before lipid infusion serving as fasting lymph. The nutrient infusion was then given as a single bolus through the duodenal infusion cannula. During bolus infusion, the continuous i.d. infusion of saline was temporarily stopped for 30 min to avoid overextending the small intestine, followed by continuous infusion of saline at 3 ml/h. Lymph was collected continuously at 30-min intervals during the first hour after bolus infusion and then hourly for the remaining 5 h of the experiment. The collected lymph was aliquoted and kept at −20°C for later assays.

Nutrient infusion. Two groups of animals were intraduodenally infused with lipid, consisting of 2.215 ml of Liposyn II (20%) plus 0.785 ml of saline or saline only as control. Liposyn II 20 consists of 50:50 blend of safflower and soybean oil with a caloric content of 2 kcal/ml (19); or

Measurement of lymphatic PGD2 and IL-6. Lymphatic PGD2 level was measured using PGD2 EIA kit (no. 512021, Cayman Chemical) and later by the PGD2 express EIA kit (no. 512041) due to discontinuation. The latter kit tends to provide a higher maximal detection dose of PGD2 (15,000 pg/ml vs. 10,000 pg/ml) with the EIA kit (no. 512021) according to the manufacturer’s manual. Briefly, 50 μl of lymph was mixed with 50 μl of enzyme AChE tracer (or Express AChE tracer) and further incubated with PGD2 monoclonal antibody (or Express EIA monoclonal antibody) at room temperature for 2 h. After five rinses with 300 μl of washing buffer, 200 μl of Ellman’s Reagent was added to each well of the plate, allowing the plate to incubate at room temperature for 1 h; then the plate was read in a microplate reader using a 420-nm filter. PGD2 levels were obtained by interpolation from standard curve constructed according to the manufacturer’s instruction.

Lymphatic IL-6 concentrations were measured by OptEIA rat IL-6 kit according to supplier’s instruction. Briefly, 100 μl of lymph was incubated with anti-rat IL-6 capture antibody for 2 h at room temperature; after five rinses with 300 μl PBS/Tween 20 (0.05% vol/vol), the biotinylated anti-rat IL-6 detection antibody was added and incubated for 1 h, followed by subsequent rinses (5×) with washing buffer and incubation with streptavidin-horseradish peroxidase conjugate. Then the plates were incubated with 100 μl of substrate solution and were read at 450 nm. IL-6 concentrations were calculated against the standard curve.

Chemical assays of TG and protein in lymph. Lymphatic triacylglycerol levels were measured by the triglycerides assay kit. This enzymatic assay measured the glycerol released from the hydrolysis of triglycerides. Briefly, 5 μl of lymph was added to 200 μl of reagent. After 20 min of incubation at 37°C, optical density was read at 500 nm. TG concentration was calculated from the standard solution provided by Randox. Protein concentration in lymph was assayed by the Bradford technique (no. 500–0002; Bio-Rad Laboratories, Hercules, CA) using BSA as standard. Standards or experimental samples were assayed. Protein concentrations and concentrations of the samples were calculated from the standard curve.

Immunohistochemistry. Rats, fasted overnight (18 h) but allowed ad libitum access to water, were gavaged with either 3 ml of saline or 3 ml of Liposyn II 20% (4.4 kcal); after 1 h, rats were killed by decapitation and were bled. The jejunum was removed and used for the assay of immunohistochemistry according to the procedure modified that described from Ref. 20. The jejunum samples (<1 cm), trimmed of the connective tissues, were immersed in 4% paraformaldehyde at 4°C overnight. After being washed with PBS (3 × 10 min), the tissues were stored in PBS containing 30% sucrose and 0.1% sodium azide at 4°C for 24 h. Cryoprotected samples were prepared by rapidly freezing the tissues with Tissue-Tek optimum cutting temperature (OCT) 4583 compound (no. 4583, Diagnostics Division, Elkhart, IN), cooled in liquid nitrogen, and stored at −80°C until frozen sectioning. Fresh frozen sections (10 μm) of the circular jejunum samples were air dried and prefixed with 70% acetone/30% methanol at −20°C for 10 min and then washed in PBS (3 × 10 min) to remove OCT compound. The sections were further incubated with 5% normal donkey serum or 0.3% Triton X-100 in PBS at room temperature for 30 min to suppress nonspecific binding of antibodies and then incubated with the primary monoclonal mouse anti-RMCP II antibody (Moreud) diluted 1:100 in PBS with 0.5% normal donkey serum and 0.05% Triton X-100 overnight at 4°C. The specificity of the primary antibody was tested and confirmed by Western blot analysis using rat small intestine tissues (18) as well as lymph samples showing a single ~29-kDa band of RMCPII on the blot (figure not shown). In addition, negative controls were obtained by omitting the
primary antibody from the staining protocol. The next day, after being washed 3 × 10 min in PBS, the sections were incubated with donkey anti-mouse IgG Alexa Fluor-488 conjugate diluted 1:200 in PBS for 1 h at room temperature. After being washed in PBS, the sections were mounted with FLURO-GEL mounting solution (no. 17985–10; Electron Microscopy Sciences, Hatfield, PA). Immunofluorescence detection was carried out with a Leica TCS 4D confocal microscope equipped with argon-krypton laser and appropriate optics and filter modules for Alexa Fluor-488 detection, and images were captured by coupled device digital camera system. Positively stained cells were counted in three to five sections per animal. Seven to ten well-oriented villus-crypt units were examined per section (41). MMC was considered degranulated if it was diffusely surrounded by numerous fluoro- rescently stained granules or if it showed decreased fluorescent density in each mast cell (24, 39, 46). The percent of degranulated MMC was counted.

Statistical analysis. Values are expressed as means ± SE. Data were analyzed by two-way repeated-measures ANOVA followed by Tukey’s post hoc test for multiple comparisons using Prism v.5 (GraphPad Software, San Diego, CA). The analyses examined the difference between groups as well as among different time points within the groups. Differences were considered statistically significant at P < 0.05.

RESULTS

Effect of lipid infusion on lymph flow and protein flux. Lymph flow rates for the experimental groups are shown in Fig. 1A. The fasting lymph flow as well as the total lymph flow during 0.5 h to 6 h after infusion of Liposyn II was not significantly altered (18.2 ± 1.0 ml vs. 18.5 ± 1.6 ml in saline group, P > 0.05), except for a temporary drop (2.0 ± 0.2 ml/h, n = 8, P < 0.01) at 1 h after Liposyn infusion compared with that after saline infusion (2.8 ± 0.2, n = 6) (Fig. 1A). On the other hand, Liposyn II caused a dramatic increase in lymphatic protein output (the product of protein concentration and lymph flow rate), peaking at 2 h, and lasted until 4 h postlipid infusion (Fig. 1B). Over the entire 6-h period, the total protein flux in lymph increased by 47% in the rats receiving Liposyn II (108.7 ± 10.6 mg, P < 0.01, n = 8) vs. saline (74.2 ± 14.5 mg, n = 6). Our results indicate that bolus infusion of Liposyn II increases lymphatic protein flux, which is consistent with the previous report that intraluminal perfusion with bile-oleic acid in cat significantly increases intestinal lysosomal protein flux (12).

Release of histamine and RMCP II into lymph after lipid infusion. It is reported that the postprandial increase in intestinal lymphatic protein flux is due at least in part to an increased intestinal capillary permeability (12). Histamine is a vasoactive factor capable of increasing vascular permeability (32). Therefore, we measured lymphatic histamine concentrations during Liposyn II infusion. As shown in Fig. 1C, a significant onefold increase of histamine level (39.2 ± 1.7 ng/ml vs. 16.5 ± 1.4 ng/ml in saline group, P < 0.01) was observed at 1 h after Liposyn infusion, indicating the release of histamine into lymph after lipid infusion.

Release of histamine is a hallmark of mast cell activation. To test whether intestinal mast cells are activated by lipid infusion, RMCP II, a specific protease marker for the rat intestinal MMC degranulation (10), was measured by ELISA. As shown in Fig. 1D, the RMCP II concentration in mesenteric lymph significantly increased after lipid infusion, starting at 0.5 h and reaching the peak value by ∼20-fold (839.9 ± 96.4 ng/ml vs. 41.1 ± 8.4 ng/ml in saline group, P < 0.01) at 1 h and then gradually reduced to fasting levels by 4 h. The time for

Fig. 1. Lymph flow rate (A), protein output (B), lymphatic histamine (C), and rat mucosal mast cells (MMC) protease II (RMCP II) concentrations (D) after intraduodenal (i.d.) infusion of saline (n = 6) or Liposyn II 20% (4.4 kcal) (n = 8). Data are expressed as means ± SE, *P < 0.05, **P < 0.01 vs. saline.
RMCPII to peak in lymph coincides with that of histamine release, all at 1 h postinfusion. The lymphatic RMCPII output (the product of RMCPII concentration and lymph flow rate) was also significantly increased in Liposyn II (peak level of 1,052.6 \(\pm\) 114.7 ng/h at 1 h postinfusion, \(n = 8\), \(P < 0.01\)) compared with that in saline group (154.9 \(\pm\) 10.6 ng/h, \(n = 6\)). The total RMCPII output within 3 h postinfusion was significantly increased in lipid group (2,832.1 \(\pm\) 440.0 ng, \(P < 0.01\)) compared with saline controls (483.1 \(\pm\) 22.9 ng). Our data indicate that during fat absorption intestinal MMC is activated, resulting in the release of RMCPII into lymph.

**Immunofluorescence staining of intestinal MMC.** To further confirm the degranulation of intestinal MMC after lipid infusion, immunohistochemistry was carried out to specifically detect intestinal MMCs with monoclonal anti-RMCPII antibody and the Alexa Fluor-488 (green)-conjugated secondary antibody. As shown in Fig. 2, A and B, cells specifically stained with green fluorescence, representing MMC, are sparsely distributed along the intestinal lamina propria in both saline- and lipid-gavaged rat jejunum. In contrast, when sections were incubated with secondary antibody only, no fluorescence was observed (Fig. 2C), indicating minimal nonspecific staining of the secondary antibody. Numbers of MMC in control rats were of five to eight cells per villus-crypt units in the jejunum, similar to the reported data (41). There was no significant difference in the number of MMC between the saline- and Liposyn II-gavaged jejunum. However, the rate of degranulated MMCs, characterized as the cells diffusely surrounded by numerous fluorescently stained granules or decreased fluorescence density (24, 39, 46) (Fig. 2B), was significantly increased in the jejunum of the animals gavaged with lipid (43.9 \(\pm\) 3.1%) compared with saline (11.5 \(\pm\) 6.4%, \(P < 0.05\)). We demonstrated for the first time the fluorescence image of the degranulation of intestinal MMC in response to lipid feeding.

**Lipid induces the release of RMCPII into circulation.** The accumulation of RMCPII in peripheral blood is used as a specific marker of intestinal MMC activation (34). To determine whether lipid infusion will increase RMCPII level in peripheral blood, we measured serum RMCPII concentrations after i.d. infusion of Liposyn II or saline control. As shown in Fig. 3, the fasting serum RMCPII levels averaged 3.9 \(\pm\) 0.7 ng/ml in the saline group and 7.5 \(\pm\) 1.1 ng/ml in the Liposyn II group at 1 h postinfusion, which was significantly higher than the control (3.9 \(\pm\) 0.7 ng/ml). The serum RMCPII concentration in the saline group remained stable throughout the 6-h period, whereas in the Liposyn II group, a significant increase was observed at 2 h (6.8 \(\pm\) 1.1 ng/ml) and 4 h (8.5 \(\pm\) 1.4 ng/ml) postinfusion, indicating that lipid infusion increases the release of RMCPII into circulation.
ng/ml; after lipid infusion, serum RMCPII concentration increased by approximately onefold, peaking at 1 h and returning to the fasting levels by 4 h, showing a similar pattern as the lymphatic RMCPII. Our study demonstrates for the first time an elevation of RMCPII in peripheral blood in response to fat absorption. The comparison of the lymphatic and serum RMCPII showed ~10-fold higher fasting and ~100-fold higher lipid-induced peak lymph RMCPII level than serum RMCPII concentrations, demonstrating that lymphatic samplings are a much more sensitive means of studying secretions by the intestinal cells in vivo.

**Release of PGD2 and IL-6 into lymph.** In addition to the preformed mediators such as histamine and RMCPII, activated mast cells release de novo synthesized mediators such as lipid mediator PGD2, the major prostaglandin produced by MMC (16, 22). As shown in Fig. 4A, Liposyn II significantly increased the lymphatic PGD2 concentration by approximately onefold measured with PGD2 EIA kit (no. 512021, Cayman Chemicals). Unlike the rapid release of histamine and RMCPII, the increase of PGD2 started at 2 h, peaked at 3 h (815.1 ± 144.4 vs. 405.0 ± 48.7 for saline group, n = 6, P < 0.05), and lasted as long as 8 h after lipid infusion, indicating the long-lasting release pattern of the lipid mediator. Interestingly, the lymphatic TG content, which also increased after Liposyn II infusion, showed a similar time course of PGD2, starting at 0.5 h postlipid infusion, reaching peak at 5 h (1,953.7 ± 365.4 vs. 148.5 ± 13.8 mg/dl in saline group, n = 6, P < 0.01) and lasting until 12 h postlipid infusion (Fig. 4B), suggesting a possible link between the transport of TG and the release of lipid mediator PGD2 in lymph.

Activated mast cells release cytokines including IL-6 (1). The lymphatic IL-6 concentrations in fasting lymph and the entire 6-h lymph in saline group were all below the detection range (<78 pg/ml). However, after lipid infusion, lymphatic IL-6 increased to the level above that range as from 182.4 ± 27.8 to 222.3 ± 3.6 pg/ml (n = 6) during 1 h to 5 h postinfusion (Fig. 4C), demonstrating that lipid induces a rapid release of IL-6 into lymph.

**Comparison of the release patterns of RMCPII and PGD2.** To further understand how repeated lipid infusion affects the release patterns of both the preformed mediator (RMCPII) and the newly de novo synthesized mediator (PGD2), we i.d. infused two doses (4.4 KCal/3 ml for each dose) of Liposyn II separated by a 4-h interval because the release of RMCPII declined by 4 h. As shown in Fig. 5A, RMCPII release significantly increased after the first challenge of lipid, peaking at 1 h, but failed to respond to the second dose of lipid. In contrast, lymphatic PGD2 (determined by PGD2 Express EIA kit, no. 512041; Cayman Chemicals) increased by 1.6-fold after the first dose of lipid and lasted during the 4 h when the second dose of lipid was applied. Following the second lipid meal, it resulted in a further increase in lymphatic PGD2, starting at 2 h after the second dose of Liposyn (1,953.5 ± 315.9 pg/ml at 6 h vs. 841.1 ± 83.1 pg/ml at 4 h, P < 0.01). It peaked to a much higher response (~18-fold) at 3 h after the second dose of lipid relative to the PGD2 level when starting to infuse the second dose of lipid (841.1 ± 83.1 pg/ml) (Fig. 5B), suggesting a potentiating effect of consecutive doses of Liposyn II on the PGD2 production. Our data indicate very different responses between RMCPII and PGD2. Challenge with the first lipid meal resulted in the complete discharge of preformed RMCPII, and the second lipid meal failed to elicit further response. In contrast, the feeding of a second lipid meal resulted in a further increase in PGD2 release into lymph.

**Effect of the amount of fat and the carbon chain length of fatty acids on MMC activation.** To further determine whether the amount of lipid ingested affects MMC activation, increasing doses (0.55, 1.1, 2.2, and 4.4 kcal) of Liposyn II were i.d. infused. All four doses raised the levels of lymphatic RMCPII above that of the saline control at 1 and 2 h postlipid infusion; the RMCPII secretion showed a profound dose-dependent
response (Fig. 6A). Multiple-comparison analysis detected significant differences among the saline and lipid dose (2.2 and 4.4 kcal) groups (Fig. 6A). In addition, cumulative RMCP II secretion, calculated as area under the curve over the 3-h lymph collection period, increased in response to the lipid load (Fig. 6B) in a dose-dependent manner.

To determine whether different carbon chain length of fatty acids modulates MMC activation, the effects of long-chain TG, trilinolein (C18:2, n-6 TG), and medium-chain TG, tricaprylin (C8:0), on lymphatic release of RMCP II and PGD2 were compared. We studied linoleic acid (C18:2, n-6) because it is the major (~65.8%) fatty acid in Liposyn II. As shown in Fig. 6C, bolus i.d. infusion of 120 µmol trilinolein emulsion plus vehicle significantly increased lymphatic RMCP II level, whereas infusion of 120 µmol tricaprylin (C8) emulsion did not alter lymphatic RMCP II concentration. Similarly, the lymphatic PGD2 concentration increased by 64.5% (P < 0.05 over fasting level, n = 6) in trilinolein-infused rats but not in tricaprylin-infused rats (data not shown), indicating that only LCFA but not medium-chain fatty acid (MCFA), is effective in activating the MMC. As expected, lymphatic TG content increased dramatically in the trilinolein group, whereas no significant increase in lymphatic TG content was observed in tricaprylin-infused rats except for a marginal increase at 1 h (237.9 ± 19.6 vs. 124.1 ± 22.2 mg/dl in vehicle group, n = 6, P < 0.01) (Fig. 6D).

Fig. 6. Effect of the amount of Liposyn II (A and B) and the type of fatty acids (C and D) on lymphatic RMCP II release. A: response to four infused lipid doses (0.55, 1.1, 2.2, and 4.4 kcal). B: correspondent areas under the curve. Values (fold amounts above saline) are means ± SE. *P < 0.05, **P < 0.01 vs. 1/4 dose (1.1 kcal, n = 6), ###P < .01, full dose (4.4 kcal, n = 8) vs. half dose (2.2 kcal, n = 8) Liposyn II. C and D: infusion of trilinolein or tricaprylin on lymphatic RMCP II concentrations (C) and triacylglycerol (TG) contents (D). Values are means ± SE; n = 6. *P < 0.05, **P < 0.01 vs. lipid emulsion (control).
microns (44). The inhibitory effect of L-81 on the TG transport was confirmed by the prevention of TG transport in lymph after infusion of Liposyn II plus L-81 (Fig. 7A). However, the lymphatic RMCP II concentrations (Fig. 7B) as well as RMCP II output (data not shown) still respond to Liposyn infusion in the presence of L-81, but the response was reduced by 55% relative to the peak value in the lipid-only group (Fig. 7B), suggesting that the activation of intestinal MMC is partially dependent on the formation and secretion of chylomicrons.

**DISCUSSION**

Using conscious lymph fistula rats, we demonstrated for the first time that intestinal MMC are activated by fat absorption to release a series of mediators including the preformed mediators (protease RMCP II and histamine) and the de novo synthesized mediator such as PGD2 into lymph. Our study further demonstrated that the extent of MMC activation depends on the amount of fat infused intraduodenally, and only LCFA is effective but not the MCFA on MMC activation. Our findings showed for the first time a link between activation of intestinal MMC to the physiological process of lipid absorption.

The RMCP II concentrations in lymph, with average of ~10-fold higher than fasting serum and ~100-fold higher than postlipid serum RMCP II levels, demonstrate the utilization of the lymph fistula rat as a more sensitive way of studying the molecules secreted by the cells in the GI tract. Another advantage of the conscious lymph fistula model is the fact that the mesenteric lymph collected in our experiment has not entered the circulation, has not been metabolized by the liver, and thus directly reflects the secretion of intestinal mast cells. Therefore, we believe that the conscious lymph fistula rat model is an excellent model for monitoring the secretion of the inflammatory factors by the intestinal immune cells in the lamina propria.

Liposyn II 20% is a safflower oil (10%)- and soybean oil (10%)-based emulsion that generates a mixture of mostly unsaturated fatty acids with linoleic acid as the major component (65.8%). Our result showing that n-6 TG trilinolein demonstrated a significant increase following the gastric administration of olive oil, but there is no corresponding increase after the administration of MCFAs (30). In the present study, we have shown that the release of RMCP II into lymph increases only after intraduodenal infusion of trilinolein but not after tricaprylin, demonstrating that LCFA is effective at inducing intestinal MMC activation.

Intestinal handling of fatty acids is highly dependent on fatty acid chain length (38). It is known that MCFAs and short-chain fatty acids (SCFA), due to their relatively higher aqueous solubility than LCFA s, are less dependent on luminal micellar solubilization for rapid uptake by enterocytes than are LCFA s (38). In addition, MCFAs and SCFA s are poor substrates for mucosal activation and esterification by the monoacylglycerol pathway of TG synthesis and are preferentially transported in the unesterified form in the portal vein, whereas LCFA s are preferentially packaged into chylomicrons and transported via the lymph (48). It has been reported that the functions of intraepithelial lymphocytes as well as dendritic cells were differentially modulated when exposed in vitro to LCFA s (C14:0-C24:0) than exposed to MCFAs (C6:0-C12:0) (15, 45).

Study on the lymphocyte flux in rat intestinal lymph have also demonstrated a significant increase following the gastric administration of olive oil, but there is no corresponding increase after the administration of MCFAs (30). In the present study, we have shown that the release of RMCP II into lymph increases only after intraduodenal infusion of trilinolein but not after tricaprylin, demonstrating that LCFA is effective at inducing intestinal MMC activation.

From the LCFA and MCFA data presented in this study, it would seem that the formation and secretion of chylomicron is potentially linked to the activation of MMC. To test this possibility, we used the well-established inhibitor of chylomicron formation, L-81 (14). As expected, the presence of L-81 in the intestinal lumen completely abolished the lymphatic transport of chylomicrons (TG content) (Fig. 7A). However, the blocking of the formation and transport of chylomicron by L-81 did not abolish but significantly (55% reduction) reduced the peak RMCP II secretion (Fig. 7B). This observation suggests that chylomicron formation may be partially responsible for MMC activation, but there are some other factors responsible for the activation of MMC by fat absorption of LCFA s. MMC, as we observed (Fig. 2, A and B), are distributed in the intestinal lamina propria without apical surfaces exposed to the gut lumen. Therefore, it is less likely that the MMC can

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**Fig. 7.** Effect of pluronic L-81 (L-81) on lymphatic TG contents (A) and RMCP II concentrations (B). Means ± SE, n = 6. *P < 0.05, **P < 0.01 vs. saline; ##P < 0.01, L-81+Liposyn II vs. Liposyn II.
directly sense the intraluminal fatty acids to induce its activation, but rather through the signals from intestinal epithelial cells. It has been reported that the production of growth-regulated oncogene/cytokine-induced neutrophil chemoattractant-1 and IL-6 by the cultured rat intestinal epithelial cells were enhanced by the exposure to LCFAs like oleic, linoleic, or arachidonic acids but not to MCFA octanoic acid (50). The underlying mechanism of how MMCs are activated by fat absorption is not clear and certainly warrants further studies. It should be noted that the fat absorption-induced MMC activation observed in our study may only reflect one aspect of the activation of the whole intestinal immune system in response to fat absorption. Considering the important role played by the MMC in the enteric neuro-endocrine-immune network (21), MMC, through releasing various MMC mediators including cytokines and chemokines, may play a key role in orchestrating the subsequent immune responses of the other immune cells (e.g., macrophage, dendritic cells) (9, 45) to fat absorption.

Our observations raise another important question: does the activation of MMC play a physiological role in the absorption and lymphatic transport of lipid? During fat absorption, chylomicrons are packaged and secreted by the enterocytes into the intercellular space by exocytosis (43). To enable the transit of the chylomicrons from the intercellular space to the lamina propria, it requires the potential distention of the intercellular space and the possible breakage of the basement membrane (43). There is considerable evidence showing that intestinal paracellular permeability is affected by mast cell mediators including interferon-γ, TNF-α, IL-1β, IL-4, IL-13, and tryptase (21). In vitro studies also show that RMCPII directly increases epithelial permeability by decreasing the expression of the tight junction-associated protein occludin and zonula occludens-1 (40). In addition, RMCPII can selectively attack type IV collagen, which is present in intestinal basement membranes (33). Therefore, the role of the MMC activation may involve the perforation of the basement membrane coupled with a loosening of the junctional complex to increase the intestinal permeability for the transport of chylomicrons during normal processing of fat absorption. The increased intestinal permeability during fat absorption has been confirmed in our previous study (26), in which mucosal epithelial integrity measured by the blood-to-lumen clearance of 51Cr-EDTA increased in a dose-dependent manner after intraduodenal perfusion with emulsified lipids (20 mM sodium taurocholate and 10–40 mM oleic acid). The histological evidence of jejunal mucosal injury and restitution was also documented during and after lipid perfusion (26). In addition, increased intestinal capillary permeability was also observed after intraduodenal perfusion of bile-oleic acid solution, resulting in, at least in part, postprandial increase of intestinal lymphatic protein flux (12), which is similar to our observation that the lymphatic protein output is increased during Liposyn II infusion (Fig. 1B).

It has been reported that the integrity of the tight junction is compromised by chronic consumption of high-fat diet (42). It is entirely possible that the disrupted intestinal barrier during chronic consumption of a high-fat diet is attributable to inappropriate and exaggerated intestinal mucosal immune responses that can potentially result in increased entry of pathogens or microorganisms into the body from the intestinal lumen. The observations of the increased MMC infiltration (36) and the excessive production of the proinflammatory factors such as histamine, TNF-α, and ILs (e.g., IL-8, IL-13) in patients with IBD (35, 47) would support an important role of intestinal MMC in the pathogenesis of IBD. Clinical studies also show that high-fat diet is a primary risk factor contributing to the high incidence of IBD, and low-fat diet or enteral nutrition (containing oligosaccharides, amino acids, and SCFA) have a clear beneficial effect in patients with quiescent Crohn’s disease (2, 31, 37). Further defining the relationship between intestinal MMC and fat absorption will yield new and important insights into nutritional and therapeutic management of IBD.

It is interesting that the circulating RMCPII is also increased during lipid infusion, demonstrating that the intestinal MMC mediator can enter into circulation. It is possible that the other MMC mediators like cytokines (IL-6) may also be released in response to fat absorption and enter the circulation; if so, the gut would seem to be potentially an important source of the circulating inflammatory factors in clinical conditions, including diet-induced obesity. Considering the profound dose-dependent effect of the amount of fat on MMC activation (Fig. 6), it is plausible that chronic consumption of a high-fat diet (mainly containing LCFAs) may produce a considerable amount of MMC mediators from gut that further enter into circulation, thereby leading to obesity, a chronic low-grade inflammatory state characterized by increased circulating levels of proinflammatory cytokines (e.g., IL-6) (17). Dietary fat is first exposed to the intestinal mucosa. We therefore hypothesize that chronic high-fat diet, through modulating immune functions in the intestinal mucosa, may produce gut-derived proinflammatory cytokines, which will contribute to the pool of the circulating proinflammatory factors. This hypothesis is supported by the recent findings that high-fat diet induces intestinal inflammation (7), which precedes obesity and insulin resistance (5). In addition, genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice (27).

REFERENCES

MUCOSAL MAST CELLS IN FAT ABSORPTION


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