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Dietary medium-chain triglycerides promote oral allergic sensitization and orally induced anaphylaxis to peanut protein in mice

Jianing Li, MS*, Yu Wang, BS*, Lihua Tang, PhD*, Willem JS de Villiers, MD PhD*, Donald Cohen, PhD[§], Jerold Woodward, PhD[§], Fred D Finkelman, MD[‡], and Erik RM Eckhardt, PhD*,[§]

*University of Kentucky, Graduate Center for Nutritional Sciences and Internal Medicine Department, Division of Digestive Diseases and Nutrition, Lexington, KY, USA

[§] University of Kentucky, Department of Immunology, Microbiology and Molecular Genetics

[‡] Cincinnati Veterans Affairs Medical Center, Department of Medicine, Cincinnati, OH, USA; University of Cincinnati College of Medicine, Department of Medicine, Division of Immunology, Allergy and Rheumatology, Cincinnati, OH; and Cincinnati Children's Hospital Medical Center, Division of Immunobiology, Cincinnati, OH.

Abstract

BACKGROUND—The prevalence of peanut allergies is rising. Peanuts and many other allergen sources contain significant amounts of triglycerides, which affect absorption of antigens but have unknown effects on sensitization and anaphylaxis. We recently reported that dietary medium-chain triglycerides (MCT), which bypass mesenteric lymph and directly enter portal blood, reduce intestinal antigen absorption into blood compared to long-chain triglycerides (LCT), which stimulate mesenteric lymph flow and are absorbed in chylomicrons via mesenteric lymph.

OBJECTIVE—Test how dietary MCT affect food allergy.

METHODS—C3H/HeJ mice were fed peanut butter protein in MCT, LCT (peanut oil), or LCT plus an inhibitor of chylomicron formation (Pluronic L81; “PL81”). Peanut-specific antibodies in plasma, responses of the mice to antigen challenges, and intestinal epithelial cytokine expression were subsequently measured.

RESULTS—MCT suppressed antigen absorption into blood, but stimulated absorption into Peyer's patches. A single gavage of peanut protein with MCT as well as prolonged feeding in MCT-based diets caused spontaneous allergic sensitization. MCT-sensitized mice experienced IgG-dependent anaphylaxis upon systemic challenge and IgE-dependent anaphylaxis upon oral challenge. MCT feeding stimulated jejunal-epithelial TSLP, IL-25 and IL-33 expression compared to LCT, and promoted Th2 cytokine responses in splenocytes. Moreover, oral challenges of sensitized mice with antigen in MCT significantly aggravated anaphylaxis compared to challenges with LCT. Importantly, effects of MCT could be mimicked by adding PL81 to LCT, and in vitro assays indicated that chylomicrons prevent basophil activation.

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Corresponding author: Erik Eckhardt, Ph.D. Graduate Center for Nutritional Sciences 588 Charles T. Wethington building 900 S. Limestone Lexington, KY 40536-0200 erik.eckhardt@uky.edu 859 218 1741.

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CONCLUSION—Dietary MCT promote allergic sensitization and anaphylaxis by affecting antigen absorption and availability and by stimulating Th2 responses.

Keywords

Peanut allergy; triglycerides; TSLP; adjuvant; chylomicrons; intestinal epithelium; Th2 responses

Introduction

Peanut allergy affects about 2% of the Western population, and its prevalence is rising¹⁻⁴. The condition is rarely outgrown and there is no cure. To stem the rise of food allergies, it is important to unravel mechanisms that lead to allergic sensitization. Peanuts and many allergenic foods contain significant amounts of triglycerides (“fat”) or are most likely ingested with a fat-rich meal, especially in Western societies, which are also more affected by food allergies. However, little is known about the effect of dietary fat in allergic sensitization or immune responses to dietary proteins.

Recent work has demonstrated that the intestinal epithelium plays a key role in immune responses to dietary antigens. Intestinal epithelial cells control access of luminal antigenic material to the lamina propria and beyond, and it has been suggested that increased intestinal permeability could be a risk factor for allergic sensitization^{2, 5, 6}. On the other hand, properly controlled intestinal absorption of small amounts of dietary antigen may protect against food allergies by promoting oral tolerance⁷. However, the mechanisms involved in soluble antigen absorption are poorly understood.

Fatty acids, released in significant amounts from dietary triglycerides in the upper gastrointestinal tract, have potent detergent properties and may induce transient mucosal damage and gut leakiness⁸ which could enable translocation of dietary antigen. The type of dietary fat might determine antigen absorption. We recently observed that dietary long-chain triglycerides (LCT), which contain fatty acids that have more than 12 C-atoms and are absorbed via mesenteric lymph as part of chylomicron particles, promoted the absorption of the dietary antigen ovalbumin (OVA) into lymph and blood⁹. In contrast, dietary medium-chain triglycerides (MCT; fatty acids with 12 or fewer C-atoms), which are absorbed via portal blood, promoted less antigen absorption⁹. This would suggest that MCT should differ from LCT in their effects on allergic sensitization and anaphylaxis.

Fatty acids of different chain length may also differ in pharmacological properties, especially in their effects on the intestinal epithelium with which they interact in large numbers. Intestinal epithelia not only control antigen absorption, but also secrete factors that significantly affect nearby immune cells. For example, the intestinal epithelial cytokine TSLP (Thymic Stromal Lymphopoietin) promotes the induction of Th2- responses through multiple mechanisms¹⁰. Interestingly, TSLP has been implicated in allergic diseases, including experimental food allergy¹¹. Other Th2-biasing cytokines, such as IL-25 (IL-17E) and IL-33, are also expressed in the intestinal epithelium, and support Th2-mediated expulsion of parasitic worms^{12, 13}. Fatty acids are known to affect intestinal epithelial cytokine expression^{14, 15}, although it is unclear how epithelial cytokines relevant to food allergy are regulated.

Based on these putative immune-modulating effects of dietary fat, we evaluated how dietary fats affect oral sensitization in naïve mice and immune responses to oral antigen challenges in sensitized mice. We decided to compare LCT with MCT, based on their different effects on OVA absorption. As model system for oral sensitization we slightly modified a recently reported, adjuvant-free model¹⁶. This model and a classical systemic sensitization model

were also used to test the effect of triglycerides during oral antigen challenges. To acutely block chylomicron formation during LCT feeding, a small amount of the chylomicron secretion inhibitor PL81 was added in some experiments^{9, 17, 18}.

Interestingly, when we replaced LCT in peanut butter with MCT or gavaged OVA with MCT, we observed marked allergic sensitization, which was associated with a significant induction of intestinal epithelial Th2 cytokine expression in the jejunum, a reduction of antigen absorption into blood, and an increase in antigen absorption into Peyer's patches. LCT did not induce sensitization unless PL81 was added. Moreover, our data suggest that chylomicron formation during antigen ingestion may promote oral tolerance and may protect against allergic sensitization.

Methods

Animals

Experiments with peanut protein were performed with male C3H/HeJ mice. Experiments with OVA used male C3H/HeJ mice, female BALB/c mice and DO11.10 mice (BALB/c background) of both genders, and female Sprague-Dawley (SD) rats. Approximately 70% of CD4 T-cells of the transgenic DO11.10 strain express a T-cell receptor for an OVA peptide (residues 323-339)¹⁹. The mice, ordered at 5 weeks of age from The Jackson Laboratory, were housed three per cage in a room of a specific pathogen-free animal facility with a 12h light / dark cycle, and were used at 6 weeks of age. Rats were purchased from Taconic. Unless indicated otherwise, the animals received standard rodent diets and filtered tap water ad libitum. All animals were handled in strict accordance with good animal practice as defined by the relevant national and local animal welfare bodies, and all animal work was approved by the Institutional Animal Care and Use Committee of the University of Kentucky (Animal Welfare Assurance Number of the University of Kentucky A3336-01; U.K. IACUC protocol 2008-0306).

Gavage suspensions and diets

Gavages—Jif-brand peanut butter or grade V OVA (Sigma Aldrich Corp.) were used for gavage studies. Peanut butter was diluted with two volumes of MCT oil (distilled coconut oil, Nestle Nutrition Corp.) and passed through a sieve with a 70 μ m pore size to prevent clogging of the feeding tube. The filtrate was centrifuged (4 minutes; 16,800xg) and the supernatant replaced with triglycerides or water to reconstitute the initial volume of peanut butter. Triglycerides consisted of MCT oil or LCT (food grade peanut oil; “Hollywood” brand). In some cases, PL81 was added at 3% by volume to LCT to block chylomicron secretion into lymph^{9, 17, 18}. We found this quantity to completely block chylomicron formation without toxic side effects^{9, 18}. PL81's effectiveness is illustrated in Fig 1B.

Diets—All diets were from Harlan Teklad Corp. Their standard rodent diet, with casein as protein source and soybean oil as fat source, was considered the LCT diet. The MCT diet differed only in fat source (partially hydrogenated coconut oil instead of soybean oil). In OVA studies, 10% of casein was replaced with egg-white solids (Deb-El brand). In peanut feeding studies, 10% of casein was replaced with defatted peanut flour (Byrd Mill brand) and soybean oil was replaced with peanut oil.

Allergic sensitization

By acute feeding: Mice were fasted for 4h, then gavaged once with 0.3 ml of antigen suspensions in various vehicles (~80 mg peanut protein or 60 mg OVA). An 18G 38mm polypropylene feeding tube with rounded tip (Harvard Apparatus Corp.) was used to avoid injury. Mice were returned to standard diets and blood was drawn 18 to 21 days later for

antibody measurements. In some instances (as indicated), mice received a second oral sensitization one week after the first. *By chronic feeding:* Mice were fed MCT- or LCT-based peanut or control diets for 4 weeks, with weekly blood sampling. *By systemic sensitization:* Mice received two intraperitoneal (I.P.) injections with 10 µg OVA in 0.2 ml alum (Accurate Chemical and Scientific Corp.), with one week between injection. One week later, blood was tested for anti-OVA IgE. Rats were sensitized by a single subcutaneous injection with 0.1 mg OVA and 1 mg alum in saline and their blood was obtained two weeks later after euthanasia. Serum was also obtained from non-sensitized animals.

ELISA for antigen-specific IgE and IgG

To detect antigen-specific IgE and IgG, we developed an ELISA, which involved coating 96 well BD Falcon ELISA plates with 20 µg peanut butter protein or 20 µg OVA in carbonate buffer (pH 9.6). Washed plates were treated with blocking solution ("NAP blocker", GBiosciences Corp.), and mouse serum, diluted 1:500 in blocking solution for IgG measurements and 1:10 for IgE measurements, was added for 1 h at room temperature. Unbound antibody was washed with Tris-Buffered Saline containing Tween 20 (TBST). Alkaline-phosphatase conjugated rabbit anti-mouse IgG (1:5000; A1902, Sigma-Aldrich.) or goat anti-mouse IgE (1:800; SouthernBiotech Corp.) were added in blocking solution, and incubated for 60 min or longer at room temperature in complete darkness (in case of IgE sometimes several hours). After washing unbound antibody, AP substrate (femto-ELISA-AP Substrate; GBiosciences) was added and the absorption at 450 nm (A450) was read after addition of 50 µl stop solution (3 M NaOH).

In vitro splenocyte stimulation assays

Spleens were aseptically removed, gently minced, and passed through a 70 µm mesh into culture medium (Dulbecco's Modified Eagle's Medium:F12 (1:1; Lonza Corp.) with 10% heat-inactivated fetal calf serum (Gibco Corp.) and antibiotics (Gibco)). Cell viability was assessed with Trypan Blue staining. Splenocytes were seeded in 96-well plates at 1×10^6 live cells/cm² and kept in an incubator maintaining 100% humidity, 37°C, and 5% atmospheric CO₂. Cells were pulsed with homogenized peanut butter (50 µg protein / ml) or 1 µg / ml OVA peptide 323-339 (Invivogen Corp) or vehicle (control), and medium obtained 4 days after the pulse was analyzed for cytokine content (ELISA kits from eBiosciences).

In vitro basophil stimulation assays

RBL-2H3 cells, a rat leukemia line with basophil and mast-cell characteristics, were obtained from American Type Culture Collection and grown till confluency in 96 well tissue culture plates in Eagle's Medium with 10% heat-inactivated serum and antibiotics. They were exposed to serum from OVA-sensitized rats (1:1 dilution) or naïve rats for two hours, washed twice with PBS containing 0.1% BSA, then incubated with 0.1 ml of serial dilutions of OVA in PBS/BSA, for 1h. Included with the OVA was an emulsion with chylomicron-like composition and physiological properties²⁰ (Intralipid 20%; Baxter Healthcare) at 1:1000 or 1:500 dilution. One hour after the incubation, 30 µl medium was mixed with 30 µl 4-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma Aldrich; 1 mg / ml in citrate buffer, pH 5) and the coloring reaction was stopped after 1h with carbonate buffer (pH 10.5). p-Nitrophenol was subsequently quantified by measuring the optical density at 405 nm. OD405 values of OVA-free solutions were subtracted to correct for light absorption by emulsion particles. The emulsions were non-toxic at the indicated dilutions as reflected by lack of stimulation of spontaneous hexaminidase release and the cells' appearance after incubation was identical to controls (not shown).

In vivo antigen challenges

For systemic challenges, mice received 30 mg peanut butter protein I.P. in 0.3 ml sterile PBS¹⁶. Body temperature was measured telemetrically immediately before and every 5 minutes after the challenge, using subcutaneously placed micro transponders (Bio Medic Data Systems Corp.), and mast cell histamine release was estimated by measuring mouse mast-cell protease 1 (mmcp-1) serum levels 90 minutes after the challenge¹⁶ (eBioscience ELISA). Signs of anaphylaxis were estimated with a semi-quantitative scoring, with “0” being assigned to mice with no symptoms, “1” to mice which were stationary but moved when provoked, “2” to mice which remained immobile even when provoked, and “3” to mice lying on their side.

In some experiments, mice were injected ip 16h before the challenge with rat-monoclonal blocking antibodies against IgE (clone EM-95; 0.1 mg/mouse) or Fc γ RII/III (clone 2.4G2; 0.5 mg/mouse), to determine whether anaphylaxis was IgE- or IgG- dependent. These antibodies have been described elsewhere^{21, 22}.

For oral challenges with peanut protein, 80 mg peanut butter protein was resuspended in 0.3 ml vehicle. For oral challenges with OVA, 50 mg OVA was suspended in 0.15 ml water and mixed with 0.15 ml triglycerides.

Effect of triglycerides on epithelial Th2 cytokine expression

To study acute effects of dietary fat on intestinal epithelial cytokine expression, fasted mice were gavaged with 0.3 ml MCT or LCT (with or without PL81) and jejuna were obtained 5 h later. To study chronic effects, mice were fed 3 wks with MCT or LCT diets and intestines were obtained from non-fasted mice in the morning. Epithelia were isolated by treating washed sections with 1 mM dithiothreitol and 30 mM Ethylenediaminetetraacetic acid as described elsewhere²³. Epithelial RNA was isolated with an EZRNA kit (Omega-Biotech Corp.) and reverse-transcribed into cDNA with a Q-script kit (Quansys Corp.). The cDNA was analyzed for abundance of TSLP, IL-25 and IL-33 mRNA relative to GAPDH with primer pairs ACG GAT GGG GCT AAC TTA CAA / AGT CCT CGA TTT GCT CGA ACT (TSLP), ACA GGG ACT TGA ATC GGG TC / TGG TAA AGT GGG ACG GAG TTG (IL-25), ATT TCC CCG GCA AAG TTC AG / AAC GGA GTC TCA TGC AGT AGA (IL-33) and CCA GGT TGT CTC CTG CGA CTT / CCT GTT GCT GTA GCC GTA TTC A (GAPDH) using a Bio-Rad CFX-96 realtime quantitative polymerase chain reaction machine.

Effect of triglycerides on Th2 responses

To test the effect of MCT on Th2 responses to dietary antigen, DO11.10 mice were fed egg protein-containing MCT or LCT diets (or corresponding egg-free diets) for one week and spleen cells were challenged *ex vivo* with OVA peptide or not. Cytokines in the culture supernatants were quantified by ELISA (eBioscience).

Effect of triglycerides on antigen absorption

Peanut butter protein was labeled with ¹²⁵I according to a slightly modified iodine monochloride procedure²⁴. Prior to protein labeling, the peanut butter was delipidated with hexane - isopropanol (2:1), resuspended in phosphate-buffered saline (PBS), dialyzed against PBS, and concentrated with a 10 kDa ultra filter. Fasted C3H/HeJ mice were gavaged with 80 mg peanut butter protein spiked with radiolabeled protein, suspended in 0.3 ml triglycerides. Plasma ¹²⁵I levels 30 minutes after gavage were measured in a gamma counter. Absorption was expressed as percentage of gavaged material. Absorption of OVA was studied using DQ-OVA (Invitrogen), which only emits fluorescence when degraded in lysosomes. For this, fasted BALB/c mice received gavages of 1 mg DQ-OVA in water,

MCT, LCT, or LCT + PL81, and were then deprived of food for at least another hour. The next day, single cell suspensions from mesenteric lymph nodes (MLN), Peyer's patches and spleen were stained with Alexa 647 anti-CD11c (Biolegend Corp.) and analyzed by flow cytometry (FACScalibur, Becton Dickinson corp.).

Statistics

Results were analyzed with Graphpad Prism version 5 and are displayed as average $\pm$ S.E.M. ANOVAs were followed by between-group post-hoc analyses (Newman-Keuls). Anaphylaxis scores were compared with Mann-Whitney U tests. Temperature data were analyzed by comparing maximum temperature drop or area under the curve. Columns in graphs that do not share letter labels differ significantly from each other ($P < 0.05$). All figures show representative results of at least two repeats per experiment.

Results

MCT and LCT differentially affect antigen absorption and dissemination

MCT were previously found to decrease absorption of dietary OVA into blood compared to LCT⁹. To test whether this also applies to peanut protein, radiolabeled peanut protein was fed to fasted mice together with MCT, LCT, or LCT + PL81, and blood was collected 30 min later. As shown in Fig 1A, gavage with MCT resulted in significantly reduced antigen absorption compared with LCT. However, addition of PL81 to LCT (which trapped chylomicrons within jejunal epithelial cells; Fig 1B) reduced absorption to levels seen with MCT (which does not cause chylomicron release).

To further test the effect of postprandial chylomicron formation on antigen absorption, we measured DQ-OVA uptake by antigen presenting cells one day after DQ-OVA gavage in the presence of different triglycerides. Surprisingly little signal was found in the MLN of either group (<1% positive cells positive), with slightly stronger signal in the spleen (approximately 3%). However, there were no significant differences between groups for any of these sites (not shown). In contrast, a pronounced difference was observed in the percentage of DQ-OVA positive cells in the Peyer's patches among groups, with significantly more DQ-OVA-positive cells after gavage with MCT and LCT + PL81 than after gavage with water or LCT (Fig 1C). Thus, prevention or inhibition of chylomicron formation suppressed antigen absorption into the circulation while enhancing antigen delivery to Peyer's patches.

Dietary MCT promote allergic sensitization

Because MCT and LCT differed in their effects on antigen absorption, we next evaluated their effects on immune responses to dietary antigens. Strikingly, antigen-naïve male C3H/HeJ mice gavaged once with peanut protein (80 mg) in MCT produced significantly more IgE and IgG against peanut protein than mice gavaged with the same amount of protein in LCT or water (Fig 2A, B). Usage of another LCT, soy oil, yielded the same results (Fig 2C). The protective effect of LCT was abrogated by adding PL81 (Fig 2A, B). In addition, splenocytes from mice gavaged with peanut protein in MCT produced more IL-13 when stimulated *in vitro* (Fig 2D). The failure to induce sensitization with LCT may involve induction of oral tolerance by LCT, since a single gavage of peanut protein in LCT prevented subsequent sensitization by antigen in MCT (Fig 2E). Interestingly, the pro-allergenic effect of MCT was not limited to peanut protein, since a single gavage of OVA (60 mg) in MCT or LCT + PL81 also resulted in increased IgE anti-OVA antibody production compared to gavage in water or LCT (Fig 2F).

Collectively, these data suggest that dietary MCT promotes allergic sensitization to food antigens and that chylomicrons may prevent sensitization.

Dietary MCT promote intestinal epithelial Th2 cytokine expression and Th2 bias

Recent reports indicated that intestinal epithelial cells can contribute to allergic sensitization by releasing cytokines that promote Th2 responses¹¹. To determine whether the sensitizing effect of MCT was associated with increased TSLP expression, we measured intestinal epithelial TSLP mRNA in jejunal epithelia 5h after gavage with MCT or LCT. As shown in Fig 3A, mice gavaged with MCT had significantly higher TSLP mRNA in jejunal epithelial cells than mice gavaged with LCT, unless PL81 was added (Fig 3B).

We next tested whether chronic MCT feeding also stimulates intestinal epithelial TSLP mRNA expression. Indeed, C3H/HeJ mice fed three weeks with the MCT diet had significantly higher levels of TSLP mRNA in their duodenal and jejunal epithelia than control mice (Fig 4A). TSLP, which is more highly expressed in the distal gastrointestinal tract²⁵, was not upregulated by MCT in sites distal from fat absorption. There was also a stimulatory effect of MCT on IL-25 and IL-33 mRNA expression (Fig 4B). However, TSLP protein levels were below the level of detection by ELISA (not shown).

The sustained effect of MCT on epithelial cytokine expression prompted us to test whether addition of peanut protein to MCT diets would cause sensitization during sustained dietary exposure. Indeed, C3H/HeJ mice receiving peanut protein in the context of MCT had detectable amounts of anti-peanut IgE in their serum after 4 weeks (Fig 4C).

Thus, dietary MCT promote allergic sensitization, which partially involves upregulation of expression of Th2-biasing cytokines in the epithelium of the jejunum. To investigate whether dietary MCT causes a general Th2 bias, we fed DO11.10 mice with MCT- or LCT-diets with or without egg-white protein for 1 week and then stimulated splenocytes *ex vivo* with OVA peptide. In the absence of OVA peptide, none of the splenocyte preparations produced detectable IL-4 or IFN- γ (Fig 5). In contrast, stimulation with OVA peptide resulted in significant production of these cytokines, due to the large numbers of OVA-reactive CD4 T-cells in DO11.10 mice. However, splenocytes from mice fed MCT diets with OVA produced more IL-4 and less IFN- γ than the LCT/OVA diet, suggesting that dietary MCT had indeed promoted Th2 responses to the antigen.

IgG-dependent anaphylaxis upon systemic antigen challenge

We next tested whether sensitized mice would develop anaphylaxis upon antigen re-exposure. Mice (C3H/HeJ) were gavaged once with peanut protein in MCT, LCT, or LCT plus PL81, then challenged I.P. 18 days later with 30 mg peanut protein in 0.2 ml PBS. As shown in Figure 6A and B, mice sensitized with peanut protein in MCT or LCT+PL81 released much more mmcp-1 into the bloodstream and showed a greater drop in body temperature than mice sensitized by gavage with peanut protein in LCT. Semi-quantitative clinical scoring of anaphylaxis (based on mouse mobility) in a separate experiment yielded negligible scores in mice sensitized with peanut protein in water or LCT, while mice sensitized with MCT or LCT plus PL81 had significantly increased scores upon challenge (Fig 6C). However, diarrhea or significant stool softening was not evident in any group. To determine whether anaphylaxis was IgE- or IgG-dependent, mice sensitized by gavage of peanut protein in MCT were treated 16h before the systemic challenges with rat monoclonal antibodies that block IgE- (EM-95) or IgG- mediated pathways of anaphylaxis (2.4G2), or both²⁶. As shown in Fig 6D, anaphylaxis was prevented when the mice were pre-treated with anti-Fc γ R2/3, but not anti-IgE, suggesting that anaphylaxis was IgG-dependent. The

mild drop in body temperature in naïve mice may relate to complement activation by peanut protein²⁷.

IgE- dependent anaphylaxis upon oral antigen challenge

Next, we tested whether mice sensitized by gavage of peanut protein in MCT develop anaphylaxis when re-exposed to antigen via the oral route. To this end, mice were gavaged with peanut protein in MCT on day 1 and 8 (double sensitization), and injected 40 days later with IgE-blocking antibodies or vehicle. One day later, the mice received a third gavage with peanut protein in MCT, and body temperature was monitored. As shown in Fig 7A, oral antigen challenged mice showed a significant drop in body-temperature, provided that no blocking antibody had been injected. In contrast, mice pre-treated with blocking antibodies against IgE did not respond to the oral antigen challenge, suggesting that orally-induced anaphylaxis was IgE- dependent. It should be emphasized that sensitization in this experiment was enhanced by performing two gavages with MCT, separated by one week. In contrast, gavage with peanut protein in LCT prior to gavage with MCT led to tolerance (Figure 2D).

Orally induced anaphylaxis is exacerbated when chylomicron formation is inhibited

To test how MCT might affect responses to oral antigen challenge, mice sensitized by two gavages with peanut protein in MCT were orally challenged 40 days later with peanut protein in water, LCT (peanut oil), MCT, or LCT + PL81 (Fig 7B). In agreement with the experiment in Fig 7A, mice challenged with peanut protein in MCT showed a significant drop in body temperature (Fig 7B). On the other hand, mice challenged with peanut protein in water or LCT showed no significant response. However, when PL81 was added to LCT, the response was similar to that with MCT (Fig 7B, C). Chylomicron formation may have protected against anaphylaxis, most likely by inhibiting the ability of absorbed antigen to gain access to mast cells or basophils. In vitro studies indeed revealed that basophils decorated with anti OVA IgE responded significantly less when OVA was added in the presence of chylomicron-like particles (Fig 7D). Moreover, in an OVA-based model of food allergy, it was observed that allergic responses were significantly more severe when challenges occurred with MCT or LCT + PL81 than with LCT alone (Fig 7E). In particular, the response of mice challenged with MCT was very robust.

Discussion

In addition to confirming and expanding our previous observation that more antigen is absorbed when ingested with LCT than with MCT⁹, we have made six novel and important observations: 1) antigen delivery to Peyer's patches is significantly enhanced by MCT; 2) both acute and chronic MCT feeding promote allergic sensitization to concomitantly ingested antigens, as shown by increased antibody production and anaphylaxis following antigen re-exposure; 3) ingestion of antigen with MCT promotes the expression of the Th2-biasing cytokines TSLP, IL-25 and IL-33 by upper gastrointestinal tract epithelial cells; 4) MCT-based diets induce a Th2 bias in the host (probably a result of point 3); 5) MCT promote the ability of ingested antigen to induce anaphylaxis in sensitized mice; and 6) almost all MCT effects are mimicked by mixing LCT with an inhibitor of epithelial cell chylomicron secretion, suggesting that chylomicrons inhibit antigen access to mast cells, basophils and dendritic cells that present antigen in a stimulatory manner. Each of these points and its relevance is discussed below.

Antigen absorption

We previously demonstrated that chylomicron formation promotes intestinal antigen absorption into the bloodstream⁹. We now additionally demonstrate that failure to secrete

chylomicrons causes retention of LCT (and presumably, associated antigens) within the intestinal mucosa and lamina propria, where they could more readily interact with relevant immune cells. A similar mechanism may also explain how feeding MCT instead of LCT increases antigen within Peyer's patches. The role of Peyer's patches in food allergy is unclear, although they are reported to be associated with oral tolerance^{28, 29}. We found, however, that DQ-OVA was present in a large fraction of Peyer's patch dendritic cells, when gavaged with MCT or LCT plus PL81. In contrast, surprisingly few DQOVA positive cells were found in the MLN, even when DQ-OVA was gavaged with LCT. The apparent conflict between the latter observation and our previous finding of increased OVA in the MLN of OVA/LCT-gavaged mice may be explained by the possibility that chylomicrons traffic through the MLN but prevent the uptake or processing of associated antigens by MLN dendritic cells.

Sensitization by MCT-rich diets and the role of chylomicrons—Our findings demonstrate that MCT-based diets promote allergic sensitization to simultaneously ingested antigens in an acute and a chronic feeding model. MCT-containing oils are regularly prescribed to patients with fat malabsorption or intestinal inflammation, and are added to some infant formulas and commercially available peanut butter (Eckhardt, Li, unpublished observations). The amounts of MCT required to promote peanut allergy remains to be determined, however. MCT, unlike LCT, do not form chylomicrons, which might protect from allergic sensitization. Chylomicron induction of macrophage cytokine production^{30, 31}, for example, may prevent Th2-biasing phenotypes in antigen-presenting cells (APC) and may, as suggested by our data, promote oral tolerance (Fig 3C). This is perhaps because of the high chylomicron content of retinol³², which promotes oral tolerance by stimulating regulatory T-cell development³³⁻³⁵.

Effect of MCT on Th2 cytokine expression in epithelial cells—The first cells to interact with dietary fatty acids are intestinal epithelial cells. These play a role in food allergies because of their barrier function, and of their active participation in immune responses to microbial and dietary antigens. One novel epithelial cytokine, TSLP, was recently shown to be important for the induction of experimental food allergy¹¹. Interestingly, our studies showed that mice fed MCT via acute gavage or via dietary enrichment increase intestinal-epithelial expression of three Th2-biasing cytokines: TSLP, IL-25 and IL-33. This effect is greatest in the jejunum, which is the principal site of fat absorption. This may explain why MCT feeding also promoted antigen-driven IL-4 production and decreased IFN- γ production by isolated splenocytes.

Chylomicrons and anaphylaxis—In addition to their protective role in the sensitization phase of peanut and OVA allergy, chylomicrons were also protective in the effector phase of food allergy. Both in the peanut protein model and in a classical OVA model, gavage of the antigens with LCT did not cause anaphylaxis, unless PL81 was added. In contrast, gavage of antigens with MCT caused clinically significant anaphylaxis. This suggests that chylomicrons prevent the access of ingested antigens to mast cell- and basophil-associated IgE. This hypothesis is supported by in vitro basophil activation tests in which the presence of chylomicrons greatly reduced the effect of OVA on basophils (Fig 7D). This inhibitory effect should more than compensate for the increase in antigen absorption that is associated with chylomicron formation.

We recognize that these in vitro studies have some limitations. We used Intralipid as a substitute for chylomicrons, which are similar in size and lipid content but lack Apolipoprotein B48. However, intralipid particles acquire other apolipoproteins from serum²⁰ and have metabolic clearance rates similar to those for chylomicrons³⁶. Additionally, we have not determined whether inhibition of basophil activation results from

OVA sequestration by chylomicrons⁹ or from a direct inhibitory effect of chylomicrons on basophils. Our findings nevertheless suggest that postprandial lipid transport via the lamina propria in the upper GI tract, where most mast cells reside, or through the bloodstream, has important effects on the effector phases of food allergies.

Ingestion of antigen with water, rather than a fat, also failed to induce anaphylaxis. We currently cannot distinguish the possibility that antigen ingested with water is poorly absorbed from the possibilities that antigen ingested with water becomes associated with chylomicrons or is otherwise protected from access to mast cell and basophil IgE. Regardless of the mechanism involved, our finding is still potentially relevant for optimization of oral desensitization, as currently attempted in small clinical trials³⁷. Feeding antigen with LCT might prevent anaphylaxis during oral treatment while boosting its effectiveness by promoting oral tolerance (Fig 2D).

Concluding remarks—Our study suggests that dietary MCT may have a previously unappreciated effect on immune responses to dietary antigens, both on sensitization and anaphylaxis, by affecting antigen absorption and by promoting a Th2 bias. Although it is premature to vilify MCT, they nevertheless could be a novel dietary risk factor for allergies. In this respect, it is important to note that considerable MCT is present in breast- and infant formula. On the other hand, the Th2-biasing properties of MCT could be exploited to treat or prevent “Th1/Th17 diseases”, such as Crohn's disease and diabetes. Interestingly, most of the effects of MCT could be mimicked by adding an inhibitor of chylomicron formation to LCT, which suggests that postprandial chylomicron formation plays an important role in immune responses to dietary antigens. This intriguing observation suggests that subtle genetic defects in the production, secretion, transport and clearance of chylomicrons may be a risk factor for food allergy development.

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Abbreviations

APC	Antigen-Presenting Cells
IgG/IgE	Immunoglobulin E/G
I.P.	intraperitoneal
LCT	Long Chain Triglycerides
MCT	Medium Chain Triglycerides
MMCP-1	mouse mast cell protease 1
OVA	ovalbumin
PL81	Pluronic L81
TSLP	Thymic Stromal Lymphopoietin

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Key Messages

- Compared with ubiquitous long-chain triglycerides (LCT), medium-chain triglycerides (MCT) promoted allergic sensitization to concomitantly ingested antigens while reducing antigen absorption into blood, increasing antigen absorption into Peyer's Patches, stimulating intestinal epithelial Th2 cytokine expression, and seemingly a Th2 bias.
- MCT also significantly promoted orally induced allergic responses.
- The effects of MCT could be mimicked by addition of an inhibitor of chylomicron secretion to LCT.

Figure 1. Effect triglycerides on antigen absorption. (A) Plasma ^{125}I 30 mins after gavage of radiolabeled peanut protein with indicated vehicles (n=6). (B) PL81 blocks LCT absorption as reflected by increased buoyancy due to lipid entrapment. Tissue was obtained 5h after gavage. (C and D) CD11c+ and DQ-OVA+ cells in Peyer's patches 16h after DQ-OVA gavage in indicated vehicles (n=4).

Figure 2.

MCT causes allergic sensitization. Anti-peanut antibodies (A,-C) 18 days after gavage with peanut protein (80 mg) in indicated vehicles (n=6). LCT in A,B was peanut oil, in C soybean oil. (D) Splenocyte IL-13 release (n=4). (E) Gavage of peanut protein in LCT (OT) one week before gavage with MCT induces oral tolerance (n=6). (F) Anti-OVA IgE 18 days after gavage with OVA (60 mg) in indicated vehicles (n=6).

Figure 3. TSLP mRNA in epithelial cells of the jejunum 5h after gavage of 0.2 ml MCT, LCT, or saline (A) or 0.2 ml LCT, LCT + PL81, or saline + PL81 (B). N=6.

Figure 4.

Effect MCT feeding on intestinal epithelial Th2 cytokine expression. (A) TSLP mRNA in epithelia from indicated sections 3 weeks after feeding (n=5). (B) IL-25 and IL-33 mRNA in jejunal epithelial cells 3 weeks after feeding (n=5). (C) IgE against peanut protein during 4 weeks feeding with diets enriched with peanut flour (n=6).

Figure 5.

Promotion of Th2 responses by dietary MCT. DO11.10 mice were fed MCT or LCT- based diets containing egg white (“MCT+egg”, “LCT+egg”) or without egg white (“MCT”, “LCT”) egg white (n=5 per group). One week later, splenocytes were stimulated *ex vivo* with OVA peptide and indicated cytokines were measured with ELISA.

Figure 6.

IgG-dependent anaphylaxis upon systemic challenge. Mice were gavaged once with peanut butter protein in indicated vehicles, then challenged by i.p. injection 18 days later (n=6). Parameters included plasma mmcp-1 90 minutes after the challenge (A), body temperature (B), and clinical score (C). Pretreatment with blocking antibodies against IgG receptors (2.4G2), but not against IgE (EM-95), blunted anaphylaxis (D).

Figure 7.

IgE-dependent orally induced anaphylaxis is aggravated by MCT. (A) Body temperature of mice (n=6-7) challenged with peanut protein in MCT with or without pre-treatment with IgE blocking antibody. Sensitization occurred by gavage on days 1 and 8, challenges were performed on day 48. In (B), Mice (n=6) were sensitized as in (A), but the oral challenge was performed by gavage of peanut protein in different vehicles. (C) shows plasma mmcp-1 levels in mice in panel (B). (D) shows inhibition of basophil activation by including Intralipid (two different dilutions) with OVA added to sensitized basophils (n=4 per group). In (E), BALB/c mice (n=9 per group) were sensitized I.P. with OVA in alum, then challenged with 60 mg OVA by gavage in the indicated vehicle. All experiments were repeated at least twice with similar outcome, except B and C, which were performed once.