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Partial Replacement of Dietary (n-6) Fatty Acids with Medium-Chain Triglycerides Decreases the Incidence of Spontaneous Colitis in Interleukin-10–Deficient Mice[1,2]

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Abstract

Enteral nutrition has a primary therapeutic effect in active Crohn's disease. It is unknown which nutrient(s) account for this action, but a role for both the amount and type of dietary fat has been postulated. Some clinical and experimental data suggest that medium-chain triglycerides (MCT) may reduce intestinal inflammation. We aimed to assess the effect of replacing part of the dietary fat with MCT on the incidence and severity of colitis in interleukin (IL)-10(−/−) mice under specific pathogen-free conditions. Twenty-four IL-10(−/−) 4-wk-old mice were randomized to receive a control diet based on sunflower oil [(n-6) fatty acids (FA)] and an experimental isocaloric, isonitrogenous diet with 50% sunflower and 50% coconut oil (MCT diet). When the mice were 12 wk old, they were killed and the colon was examined for the presence of colitis, lymphocyte subpopulations and apoptosis, ex vivo cytokine production in supernatant of colon explants, toll-like receptor (TLR)-2 and TLR-9 mRNA, and FA profile in colonic tissue homogenates. Colitis incidence was lower in the IL-10(−/−) mice fed the MCT diet (1/12) than in the mice fed the control diet (8/12; $P = 0.03$). The histological damage score was also lower in the former ($P < 0.0005$). Feeding the MCT diet resulted in fewer total and apoptotic intraepithelial CD3+ and lamina propria CD3+CD4+ lymphocytes, as well as downregulated production of IL-6 and interferon-γ, and reduced TLR-9 mRNA. We conclude that partial replacement of dietary (n-6) FA with MCT decreases the incidence of colitis in a model of spontaneous intestinal inflammation and provide experimental arguments for a possible primary therapeutic effect of MCT in human Crohn's disease. J. Nutr. 139: 603–610, 2009.

Introduction

The term inflammatory bowel disease includes 2 entities, ulcerative colitis and Crohn's disease, of unknown etiology and chronic relapsing course. Although their etiology is still elusive, it is widely accepted that they occur due to a persistent uncontrolled immune response to luminal antigens (mostly bacterial) in genetically predisposed individuals.

In the last 2 decades, enteral nutrition has been assayed as the primary treatment in active Crohn's disease. Although both early (1–3) and recent (4) meta-analyses agree that enteral nutrition is not better than the standard steroid therapy, the overall remission rate is as high as 60%, supporting the idea that (at least in a subset of patients) enteral nutrition could be an effective primary therapy in Crohn's disease (1).

It is not clear which nutrient(s) present in enteral nutrition accounts for its primary antiinflammatory effect, but a role for both the amount and type of dietary fat has been postulated (5). Diets with very low fat content appear to be particularly useful (4), whereas high amounts of (n-6) fatty acids (FA)[8] have been in general associated to a poorer response (5,6). Moreover, changes in dietary habits toward an higher intake of (n-6) FA and other types of fat have been associated with an increased risk for Crohn's disease in Asian countries (7,8). On the other hand, controversial results have been obtained with fish oil derivatives [(n-3) FA] (9,10) and monounsaturated fat (11,12).

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[8] Abbreviations used: FA, fatty acids; IEL, intraepithelial lymphocytes; IFN, interferon; IL, interleukin; IQR, interquartile range; ISOL, in situ oligoligation; LPL, lamina propria lymphocytes; LT, leukotrienes; MCT, medium-chain triglycerides; TLR, toll-like receptor; SPF, specific pathogen-free; TNBS, tri-nitrobenzene-sulfonic acid; TNF, tumor necrosis factor.

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Medium-chain triglycerides (MCT) are traditionally considered an easy-to-oxidize fat source without immunomodulatory properties (13). However, data from studies using MCT-containing intravenous fat emulsions suggest that this lipid source can influence the immune response (e.g. increasing surface markers of adhesion in blood granulocytes and monocytes) (14). Some clinical data from patients with Crohn's disease suggest that replacing part of the dietary fat with MCT may help in inducing clinical remission (15–18). Furthermore, previous work in our laboratory showed that feeding MCT in rats with tri-nitrobenzene-sulfonic acid (TNBS) colitis decreased the production of proinflammatory eicosanoids (19).

In this study, our aim was to assess the effect of replacing part of the dietary fat with MCT on the incidence and severity of colitis in a model of interleukin (IL)-10–deficient mice under specific pathogen-free (SPF) conditions. IL-10(−/−) mice develop spontaneous colitis with deep ulcers, mononuclear cell infiltration, and cryptitis that resembles human Crohn's disease (20). To gain insight into the mechanisms of such an effect (in the event that it occurs), we also evaluated: *1*) changes in the colonic cytokine profile, and subpopulations of intraepithelial (IEL) and lamina propria lymphocytes (LPL) as effectors of the acquired immunity; *2*) the percentage of apoptotic IEL and LPL, because Crohn's disease has been related to an lymphocyte apoptosis deficiency (21); and *3*) toll-like receptor (TLR)-2 and 9 mRNA as markers of the epithelial barrier function (innate immunity) (22).

TABLE 1 Composition of experimental MCT and control diets[1]

	MCT diet	Control diet
Nutrients	*g/100 g*	
Protein[2]	20	20
Carbohydrates[3]	60	60
Fat	8	8
Fiber[4]	4	4
Vitamins and minerals[5]	6	6
FA	*g/100 g total fat*	
8:0	—	—
10:0	0.5	—
12:0	28.1	—
14:0	15.3	—
16:0	14.0	19.8
16:1(n-7)	0.1	0.2
18:0	3.8	2.8
18:1(n-9)	12.7	24.1
18:2(n-6)	22.6	46.7
18:3(n-6)	0.7	0.9
18:3(n-3)	1.3	1.6
20:3(n-9)	0.6	
20:3(n-6)	0.3	0.8
20:4(n-6)	—	—
20:5(n-3)	—	—
24:0	0.1	—
Total SFA	62	22.6
Total MUFA[6]	12.8	24.3
Total PUFA	24.9	50

[1] Diets were specially manufactured for this study by Harlam Ibérica (Barcelona, Spain).
[2] Based on casein (1.5% L-cystine added).
[3] Based on corn starch (65%), maltodextrin (20%), and sucrose (15%).
[4] As cellulose.
[5] AIN-93G-VX (TD 94047) and AIN-93-MX (TD 94046), plus choline bitartrate (2.5 g/kg) (23).
[6] Monounsaturated FA.

Materials and Methods

Experimental design. Twenty-four IL-10(−/−) mice of the C57BL/6J genetic background (Charles River) were randomized at 4 wk of age to receive 1 of 2 isocaloric and isonitrogenous diets: a control diet using sunflower oil as fat source, which is rich in linoleic acid [18:2(n-6)], and an experimental diet containing 50% of fat as sunflower oil and 50% as coconut oil, which mainly contains MCT (MCT diet) (**Table 1**). Both diets were supplied as pellets. The control diet was based on the AIN-93G standard formulation (23) (Table 1). One set of mice was used for the experiment so that no bias due to seasonality or changes in the stabling conditions could occur.

Mice were kept under SPF conditions in an isolator (Harlam Ibérica) at a constant temperature (22°C) with a 12-h-light:12-h-dark cycle. Five Balb/c mice acted as sentinels, undergoing sequential microbiological monitoring. Mice consumed ad libitum irradiated diets (with gamma electromagnetic radiation) and autoclaved drinking water. The experiments were conducted according to the EUROGUIDE on the Accommodation and Care of Animals Used for Experimental and Other Scientific Purposes (24) and approved by the Experimental Animal Ethics Committee of the Health Sciences Research Institute "Germans Trias i Pujol."

When the mice were 12 wk old, they were killed by cervical dislocation under anesthesia with isofluorane and blood samples and the colon were harvested. Body weight and colon weight:length ratio were recorded. A mouse was classified as colitic if it showed at least 3 of the following features: *1*) weigh loss >5% over the 8-wk experiment; *2*) anemia (hemoglobin <12 g/L); *3*) blood in the feces (as assessed visually); and *4*) a colonic histological damage score >3 (see below).

After harvesting, colons were washed as described below and split into 3 longitudinal sections. One of them was used for IEL studies (see below), the second one for blind histological examination by an experienced pathologist and apoptosis assessment by the peroxidase in situ oligoligation (ISOL) method (see below). The third longitudinal section was further split into 3 sections: one was used for explant culture and cytokine measurements in the supernatant, the second one for mRNA quantification of TLR, and the third one for FA assay.

Histological examination. The colonic section devoted to histological examination was embedded in paraffin and 3 slices from the proximal, middle, and distal colon were stained with hematoxylin-eosin and examined under light microscopy. Intestinal inflammation was scored from 0 to 12, as the result of the sum of the 6 following criteria (25): mucosal ulceration (0–2), epithelial hyperplasia (0–2), mononuclear cell (0–2) and neutrophil infiltration (0–2), crypt atrophy (0–2), and necrosis (0–2).

IEL and LPL isolation. IEL and LPL were isolated as described (26) with slight modifications. In brief, colons were cleared of fecal contents and incubated for 10 min with Ca^{2+}/Mg^{2+}-free Hank's balanced salts solution medium/10% fetal bovine serum, HEPES, antibiotics, and dithiothreitol 1 mmol/L at pH 7.2. Mesenteric tissue and Peyer's patches were excised and discarded. Dissected colonic sections were then incubated in the previous solution plus 1 mmol/L EDTA at 4°C for 20 min with gentle stirring to separate the epithelial compartment in the supernatant. IEL were layered by centrifuging the supernatant on a 40/80% discontinuous Percoll gradient. Deepithelized colonic tissue was resuspended in RPMI 1640 medium with collagenase V (1 g/L), hyaluronidase (1 g/L), DNAase (1 g/L) (Sigma), and antibiotics overnight at 37°C. After several washings and filtering the specimen through a 200 mesh nylon filter, LPL were purified on a 40/100% discontinuous Percoll gradient.

Viability of IEL and LPL was assessed using microscopic examination of methylene blue-stained samples.

IEL and LPL phenotype and apoptosis assessment by flow cytometry. Cells were washed in PBS and then incubated with APC-labeled anti-CD3, fluorescein isothiocyanate-labeled anti-CD8, and

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fluorescein isothiocyanate-labeled anti-CD4 (BD Pharmigen) for 10 min at 4°C. Then, cells were incubated using an Annexin V-PE/7-ADD apoptosis detecting kit (BD Pharmigen) and analyzed in a flow cytometer (FACScalibur, Becton-Dickinson). Apoptosis of CD4+ and CD8+ cells was analyzed in separate tubes.

Assessment of apoptosis by peroxidase ISOL. Three paraffin-embedded slices from a longitudinal colonic section were used to assess apoptosis using a Peroxidase ISOL Apoptosis Detection kit ApopTag (Chemicon International). This method used a T4 DNA ligase and diaminobenzadine as peroxidase substrate, which causes brownish staining in apoptotic cells. Hematoxylin counterstaining was performed. The amount of apoptotic lymphocytes was expressed in relation to the total number of CD3+ cells found in an adjacent slice by standard immunohistochemistry techniques.

Determination of cytokine levels. A specimen from the mid-colon were explanted in RPMI 1640 medium with antibiotics and the 48-h supernatant was obtained. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ concentrations were measured in the supernatant using SearchLight mouse inflammatory cytokine array (Pierce). Each well prespotted with cytokine-specific antibodies was incubated with 50 μL 1:5 diluted supernatant. Biotinylated secondary antibodies were added, followed by streptavidin-horseradish peroxidase. This reacts with specific substrate to produce a chemiluminescent signal that was detected with a cooled CCD camera (BioImaging Systems). The signal produced was proportional to the amount of each cytokine in the original standard or sample.

Colonic TLR mRNA measurement. Total RNA from colonic samples was isolated by using the RNeasy Mini kit (Qiagen). cDNA was synthesized from RNA by a Reverse Transcription system using random primers (Promega) at 45 cycles/60°C in 5 mmol/L $MgCl_2$ annealing buffer. PCR amplification was performed with DNA Master Sybr Green I (Roche). The primer sequences used for amplification in real-time PCR were: forward, 5′-AAGAGGAAGCCCAAGAAAGC-3′ and reverse, 5′-GATGGAATCGATGATGTTG-3′ for TLR-2; forward, 5′-CTGAGCACCCCTGCTTCTA-3′ and reverse, 5′-CCACAGAGACGATACTGTTG-3′ for TLR-9; and forward, 5′-TGGAATCCTGTGG CATCCATGAAAC-3′ and reverse, 5′-TAAAACGCAGCTCAGTAACAGTCCG-3′ for β-actin.

Polymerase amplification was performed in PCR buffer (3 mmol/L of $MgCl_2$, 50 mmol/L KCl, Tris-HCl at pH 8.3 and 5% glycerol) containing 200 μmol/L dATP, dCTP, and dGTP; 400 μmol/L dUTP; 500 mmol/L of each primer; water and DNA Master Sybr Green I. The denaturation conditions consisted of 10 min/95°C followed by 45 cycles of 5 s/95°C, 10 s/50°C and 20 s/72°C and then by standard melting curve analysis. Samples were cooled to 40°C for 30 s. Quality control was assessed in standardized PCR conditions, including negative controls. mRNA quantification for each TLR was calculated by extrapolation from a standard curve obtained from the same tissue and normalized against the housekeeping mRNA (β-actin).

Determination of colonic FA profile by GC-MS. This was done to ascertain if the study diets have any influence on colonic FA composition. Colonic samples were mixed with internal standard solution (C17:0, Sigma), homogenized, and directly transesterified as described by Lepage and Roy (27). FAME were identified using proton transfer chemical ionization MS coupled to GC (GC 8000 series + MD 800 Thermo Finnigan; Fisons). This almost exclusively yields the $[M+H]^+$ quasimolecular ion for most esters (28). This methodology decreases molecular fragmentation and thus improves yield and sensitivity when working with small samples.

FAME were separated in a GLC (GC 8000 series; Fisons) equipped with the highly specific polar capillary column BPX70 (SGE). The GLC was equilibrated daily using a commercial mixture of FAME (Sigma-Aldrich Química) from C8:0 to C24:0 within a concentration range of 80—200 ng/μL. Samples were injected on splitless mode under the following conditions: 5 min solvent delay, 250°C injector and detector temperatures, with helium (2 Bar) as carrier gas. The oven temperature started at 100°C following a ramp of 20°C/min until it reached 250°C. MS analysis was performed under positive chemical ionization mode with methane as a reactive gas.

Statistical analysis. Results are expressed as frequency or median and interquartile range (IQR) unless otherwise noted. Proportions were compared using Fisher's exact test. Mann-Whitney U tests were used to compare quantitative variables. $P < 0.05$ was considered significant. Statistical analysis was carried out with SPSS 12.0 package.

Results

Colitis incidence and morphological findings. The incidence of colitis was significantly less in IL-10(−/−) mice fed the MCT diet than in those receiving the control diet (**Table 2**). The colonic weight:length ratio, a measure of the degree of colonic inflammation, was significantly greater in the control group (Table 2). This was associated with a progressive weight loss compared with the MCT group, which was significant from wk 10 onwards (**Fig. 1**). The hemoglobin concentration was lower in controls (mean ± SEM) (111 ± 18 g/L) than in the MCT group (142 ± 10 g/L) ($P < 0.05$).

The histological damage score was significantly lower in the MCT group compared with control mice (Table 2). Microscopic examination (**Fig. 2**) showed extensive neutrophil and mononuclear cell infiltration as well as abundant lymphoid aggregates in the lamina propria. Epithelial damage mainly consisted of goblet cell depletion and small ulcerations. Both crypt abscesses and hyperplasia also were evident.

IEL and LPL phenotypes. Feeding the MCT diet significantly decreased the percentage of CD3+ IEL and LPL compared with the control group (**Table 3**). Such a decrease was mainly due to a decrease in the CD3+CD4+ subpopulation, so that the CD3+CD4+:CD3+CD8+ ratio was lower in the MCT diet-fed mice in the IEL but not in the LPL.

IEL apoptosis. Feeding mice the MCT diet resulted in significantly more apoptotic cells in each IEL subpopulation (**Table 4**). In the case of LPL, only CD3+CD4+ cells differed between the groups (Table 4).

When IEL apoptosis was evaluated using the ISOL method, the number of apoptotic CD3+ IEL was also significantly higher in mice receiving the MCT diet [median: 1.9 (1.5–2.3) $\times 10^{-6}$ apoptotic cells/CD3+] than in those receiving the control diet [median: 0.6 (0.4–0.7) $\times 10^{-6}$ apoptotic cells/CD3+; P = 0.022]. Assessment of apoptosis of LPL by the ISOL method yielded results similar to those from flow cytometry (data not shown).

Cytokine profile. Production of the proinflammatory Th1 cytokines IL-6 and IFNγ in 48-h cultures of colonic explants was

TABLE 2 Incidence of colitis and colonic morphological variables in IL-10 (−/−) mice fed control or MCT diets for 8 wk[1]

	MCT	Control
Incidence of colitis, *n (%)*	1 (8)	8 (67)*
Colonic weight:length ratio, *mg/cm*	18.0 (17.5–22.0)	23.0 (19.0–28.0)*
Histological score	0 (0–1.5)	3.5 (2.0–5.0)*

[1] Values are medians (IQR), n = 12 or n (%). *Different from MCT, $P < 0.05$.

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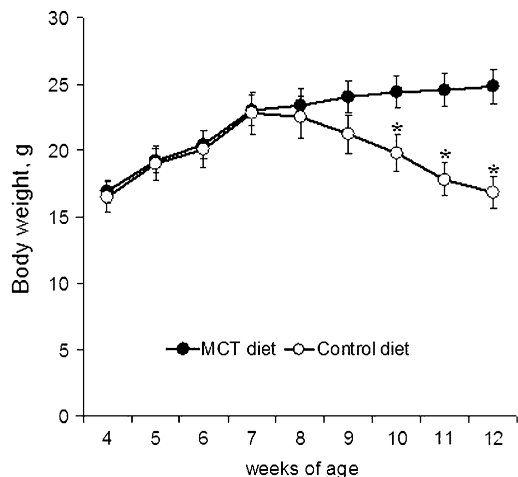

FIGURE 1 Weight evolution in IL-10 (−/−) mice fed control or MCT diets for 8 wk (from 4 to 12 wk of age). Values are means ± SEM. *Different from MCT, $P < 0.05$ (Mann-Whitney U test).

significantly higher in IL-10(−/−) mice fed the control diet than in those fed the MCT diet (**Table 5**).

TLR mRNA. In mice fed the MCT diet, TLR-9 mRNA was significantly lower [0.03 (0–0.03) times β-actin expression] than in those fed the control diet [0.40 (0.10–1.20) times β-actin expression; $P = 0.005$]. TLR-2 mRNA did not differ between mice fed the control diet [0.23 (0.15–0.44) times β-actin expression] and the MCT diet [0.60 (0.40–1.10) times β-actin expression].

Colonic FA profile. Replacing part of the dietary (n-6) FA with MCT resulted in an increase in the percentage of medium-chain FA in the colon of IL-10(−/−)mice (**Table 6**). Paradoxically, this dietary manipulation also increased the proportion of linoleic acid [18:2(n-6)] in the colon.

Discussion

IL-10(−/−) mice spontaneously develop chronic intestinal inflammation with deep ulcers, mononuclear cell infiltration, and cryptitis that resembles human Crohn's disease (20). Such damage is usually less extensive and severe in mice housed in SPF than in conventional conditions. Also, the C57BL/6J background leads to a more attenuated colonic inflammation than other strains of IL-10(−/−) mice. We have chosen this model for the present study instead of more aggressive models (e.g. TNBS enteritis or colitis), because previous data suggested that colonic damage that is too severe might not be sensitive to dietary manipulation (19,29)

The main finding of this study is that partial replacement of (n-6) FA, the predominant FA in standard rodent diets, with MCT results in a marked decrease in the incidence of colitis in IL-10(−/−) mice. Also, as a group, mice fed the MCT diet showed decreased macroscopic and histological colonic damage. This was associated with a wide array of immunological changes. However, these findings do not necessarily mean that colitic mice in the experimental group had a less severe colonic inflammation, because only 1 animal of this group developed colitis.

We also investigated the immunological effects of MCT on intestinal inflammation to the phenotypic profile and prevalence of apoptosis of IEL, the ex vivo colonic production of a wide array of cytokines, and TLR mRNA. However, as in the case of the histological score, such an analysis could be biased by the fact that only 1 animal in the MCT group developed colitis.

In agreement with the reported increase in CD3+ CD8+ IEL in the rectal mucosa of Crohn's disease patients (30) and enhanced cytolytic activity of these cells (31), IL10(−/−) mice treated with the control diet had the highest proportion of CD3+ IEL, most of them also expressing the CD8 phenotype. This was associated with a high incidence of colitis. Indeed, cell transfer experiments to immunodeficient recombinase-activating gene-2(−/−) mice suggest that the genesis of colitis in IL-10(−/−) mice is mediated by these cells (32). The administration of the MCT diet resulted in a dramatic decrease in CD3+ IEL.

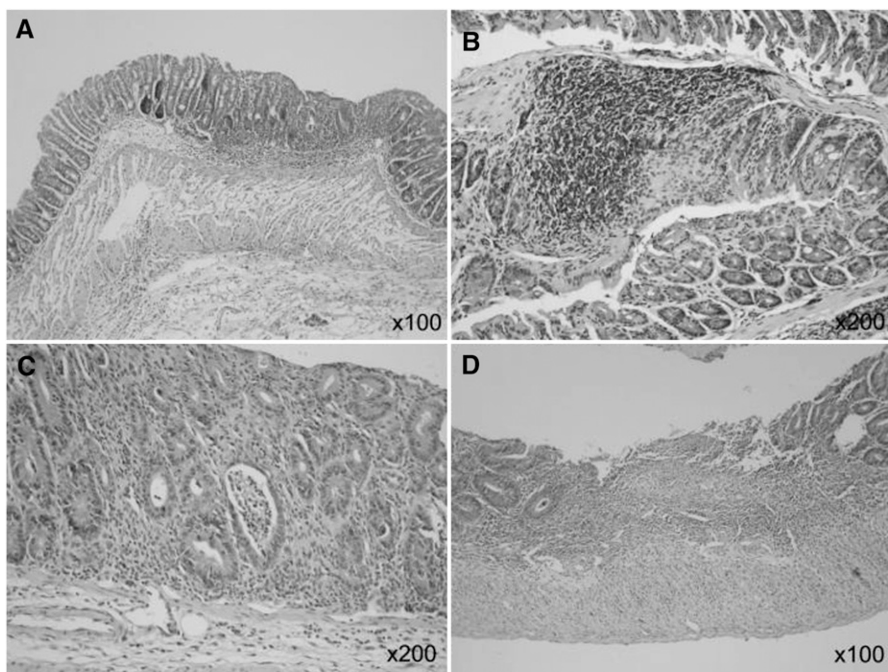

FIGURE 2 Main microscopic features in IL-10(−/−) mice. Areas of mucosal inflammation with infiltrate of the lamina propria, goblet cell depletion, and transmural edema (*A*). Lymphoid aggregates occasionally invading the submucosa (*B*), conspicuous crypt abscesses (*C*), and epithelial ulcers (*D*) could be seen (hematoxylin and eosin).

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TABLE 3 Cell subpopulations (flow cytometry) of IEL and LPL in IL-10 (−/−) mice fed control or MCT diets for 8 wk[1]

	MCT	Control
IEL		
CD3+,[2] *% of IEL*	0.9 (0.6–1.1)	12.3 (9.7–14.5)*
CD3+CD4+, *% of CD3+*	8.3 (6.1–19.7)	14.1 (10.6–15.2)
CD3+CD8+, *% of CD3*	61.7 (54.0–63.8)	60.5 (59.8–64.4)
CD3+CD4+:CD3+CD8+	0.12 (0.07–0.17)	0.31 (0.25–0.70)*
LPL		
CD3+,[2] *% of LPL*	1.4 (1.1–3.9)	7.2 (4.5–8.0)*
CD3+CD4+, *% of CD3+*	19.1 (15.4–23.0)	80.7 (62.8–81.8)*
CD3+CD8+, *% of CD3+*	17.9 (13.9–37.6)	53.1 (27.0–68.8)
CD3+CD4+:CD3+CD8+	0.8 (0.22–5.5)	2.3 (0.7–4.6)

[1] Values are medians (IQR), $n = 12$. *Different from MCT, $P < 0.05$.
[2] Those cells fulfilling criteria of lymphocytes on the basis of size and granular complexity in the cytometric plot.

TABLE 5 Cytokine production by explants of colonic biopsies in IL-10 (−/−) mice fed control or MCT diets for 8 wk[1,2]

	MCT	Control diet
	mg/L of supernatant	
IL-1	0 (0–0)	0 (0–3.9)
IL-2	1.5 (0–12)	17.5 (5.6–28.0)
IL-4	0 (0–2.5)	0 (0–4.6)
IL-5	6 (0–45)	50 (12–119)
IL-6	1 (0–3)	16.5 (5–33)*
IL-12	0 (0–2.9)	0 (0–0)
IFNγ	0.8 (0–58)	110 (80–782)*
TNFα	0 (0–7.7)	4.9 (0–45)

[1] Values are medians (IQR), $n = 12$. *Different from MCT, $P < 0.05$.
[2] Detection limits: 3.1×10^{-3} for IL-2, IL-4, IL-10, and IL-12; 6.3×10^{-3} for IL-5; 12.5×10^{-3} for IL-1 and TNFα; 21.9×10^{-3} for IL-6; and 31.3×10^{-3} for IFNγ.

This effect appears to be at least in part due to increased apoptosis of these cells.

As in human inflammatory bowel disease (33,34), proinflammatory cytokines are upregulated in IL-10(−/−) colitic mice (35). Of these, IFNγ is particularly relevant in the early (36) but not in the late colitis stages (37) in these mice. In the present study, IFNγ production was extremely high in IL-10(−/−) mice fed the control diet and its production, as well as that of IL-6, was markedly downregulated by feeding the MCT diet.

TLR are key mediators of innate host defense in the intestinal mucosa. In health, TLR signaling protects the intestinal epithelial barrier and confers tolerance to commensal bacteria. In disease, aberrant TLR signaling may stimulate inflammatory responses, leading to acute and chronic intestinal inflammation (38). In the present study, we assessed the mRNA of TLR-2 and TLR-9 as sensors of products of commensal intestinal bacteria (mainly gram-positive) instead of gram-negative pathogens (i.e. TLR-4), because the mice were on SPF conditions (39). Moreover, TLR-2 and TLR-9 signaling occurs solely through a MyD88 activation pathway (40). We observed increased TLR-9 and TLR-2 mRNA in IL-10(−/−) colitic mice receiving the control diet, which is in agreement with recent work showing that TLR signaling is a key step in the development of colitis in this but not in other genetically engineered models of intestinal inflammation (22,41). Feeding the MCT diet was associated with a marked decrease in TLR-9 mRNA. Whether this is a major mechanism for preventing colitis or a mere epiphenomenon cannot be ascertained. However, recent data indicate that TLR-9 signaling in intestinal epithelial cells varies in a domain-specific manner; whereas basolateral TLR-9 activates nuclear factor-κB, apical TLR-9 suppresses this pathway (42). We can speculate that mice with less severe colitis (and therefore increased epithelial integrity) basolateral TLR-9 expression could be reduced. Unfortunately, no further colonic tissue was available to help us explore this issue.

Although MCT are traditionally considered as merely an easy-to-oxidize energy source, recent data support the idea that they can also exhibit immunomodulatory properties. For instance, MCT-containing lipid emulsions for i.v. use have been reported to modulate the function of neutrophils in peripheral blood both in vitro and ex vivo (43,44)

The effects of enteral MCT administration in rats undergoing different proinflammatory challenges are even more interesting. Several reports indicate that orally administered MCT attenuate ethanol-induced liver damage in rats (45–48). This effect was associated with a decrease in oxidative stress (45,46), nuclear factor-κB activation (48), TNFα production (45,47,48), and cyclooxygenase 2 expression in the liver (48,49), as well as with

TABLE 4 Percentage of apoptotic cells (flow cytometry) of IEL and LPL in IL-10 (−/−) mice fed control or MCT diets for 8 wk[1]

	MCT	Control
IEL	*%*	
CD3+	54.0 (48.5–62.5)	8.7 (7.7–10.5)*
CD3+CD4+	66.7 (50–75.1)	8.8 (7.9–10.5)*
CD3+CD8+	46.9 (46.4–48.0)	8.4 (7.4–9.1)*
LPL		
CD3+	36.8 (28–39.9)	17.8 (16.8–23.0)*
CD3+CD4+	37.7 (33.4–42.2)	20 (13.3–30.8)*
CD3+CD8+	21.0 (13.5–25.1)	16.0 (11.1–21.7)

[1] Values are medians (IQR), $n = 12$. *Different from MCT, $P < 0.04$.

TABLE 6 FA profile in the colon of IL-10(−/−) mice fed control or MCT diets for 8 wk[1]

	MCT	Control diet
	% of total FA	
MCFA[2] (C8-C12)	5.9 (5.7–9.0)	3.7 (0.2–5.8)*
LCFA[3] (C14-C24)	39.3 (34–46)	52.2 (38.6–59)
MUFA[4]	13 (11.6–15)	10.2 (6.5–13.5)
(n-6) FA	25.6 (8.3–29.4)	18.4 (0.4–25)
18:2(n-6)	6.3 (5.5–7.3)	2.2 (0.08–3.4)*
20:3(n-6)	0.05 (0.05–4.7)	1.9 (0.05–5.2)
20:4(n-6)	13.5 (0.1–19.6)	8.5 (0.08–13.2)
(n-3) FA	0.2 (0.14–13.2)	4.2 (0.16–14.1)
18:3(n-3)	0.05 (0.03–0.05)	0.05 (0.03–0.09)
20:5(n-3)	0.05 (0.03–0.05)	0.08 (0.05–0.81)
22:6(n-3)	0.07 (0.05–13.2)	3.7 (0.06–11.5)
MCFA:LCFA	0.17 (0.15–0.19)	0.07 (0.004–0.1)*
(n-3) FA:(n-6) FA	0.027 (0.006–0.5)	0.6 (0.5–0.6)*

[1] Values are medians (IQR), $n = 12$. *Different from MCT, $P < 0.05$.
[2] MCFA, Medium-chain FA.
[3] LCFA, Long-chain FA.
[4] MUFA, Monounsaturated FA.

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lower endotoxin levels (49). Indeed, MCT were also effective in preventing mortality, intestinal damage, liver injury, and CD14 expression in the Kupffer cells of rats challenged with endotoxin (50). Furthermore, an MCT-enriched diet resulted in both the lowest plasma proinflammatory (TNFα, IL-1β) and the highest antiinflammatory (IL-10) cytokine response in mice challenged with endotoxin compared with diets enriched with (n-6), (n-9), and (n-3) FA (51).

The effect of MCT in experimental models of intestinal inflammation has been not very much evaluated. In a study comparing the effect of MCT with isocaloric amounts of long-chain triglycerides [mainly containing (n-6) FA] in rats with TNBS-induced ileitis, the damage score at d 8 was significantly lower, and the intestinal levels of TNFα and leukotriene (LT) B_4 tended to be lower as well, in rats receiving the MCT diet (52). Further work by the same authors, comparing MCT and long-chain triglyceride administration for 3 d after the induction of TNBS ileitis, failed to disclose differences in the damage score, but both myeloperoxidase activity and the intestinal content of IL-8 were significantly diminished in MCT-treated rats (29). Previous work from our laboratory showed that feeding MCT before and during the induction of TNBS colitis resulted in an intracolonic LTB_4:LTB_5 ratio at d 3 significantly lower than that in rats fed (n-6) FA, and as low as that obtained with fish oil [(n-3) FA], despite the lack of differences in the damage score (19).

The cellular and molecular mechanisms for the immunomodulatory actions of MCT are far from being elucidated. It has been hypothesized that MCT could influence membrane fluidity, cell mobility, receptor function, activation of intracellular signaling either directly or through the formation of eicosanoids, gene expression, and cell differentiation (50). In vitro studies in rat colonocytes indicate that MCT are as well oxidized as butyrate (53), suggesting that they have epithelial trophic effects leading to improved mucosal barrier function. In endotoxin-challenged rats, feeding MCT resulted in increased epithelial cell size, a higher number of goblet cells, and decreased permeability of the small bowel compared with corn oil (50). This was associated with a significant decrease of the fecal bacterial content (50). Finally, it has been reported that MCT reduce the oxidation rate of long-chain FA both in rats (54) and humans (55), thus facilitating their incorporation in cell membranes. This could explain the paradoxical increase in the colonic content of linoleic acid that we observed in mice fed MCT.

One can argue that the effects of replacing (n-6) FA with MCT on the development of experimental colitis might be due to the decrease in the (n-6) FA supply rather than to immunomodulatory actions of MCT. Indeed, linoleic acid is the precursor of arachidonic acid, which in turn is the substrate of proinflammatory eicosanoids (56). Also, arachidonic acid and other linoleic acid derivatives seem to exert other eicosanoid-independent proinflammatory actions (57–59).

In fact, linoleic acid has been reported to upregulate IL-8 in intestinal cells from Crohn's disease patients in vitro via arachidonate metabolites (60,61). Also, some clinical trials suggest that low- or very low-fat diets might be particularly useful in inducing remission in active Crohn's disease (15,62–64), although significant differences were not found in a recent meta-analysis (4).

Tsujikawa et al. (65) compared low-fat with MCT-enriched enteral nutrition in rats with TNBS-induced colitis or ileitis. Both diets were equally effective in preventing ileitis, but the MCT diet was better than the low-fat formula for preventing colitis, suggesting that MCT per se should have an additional effect to the removal of (n-6) FA from the diet.

In conclusion, the present study shows that partially replacing dietary (n-6) FA with MCT decreased the incidence of colitis in a model of spontaneous intestinal inflammation and provides experimental arguments for a possible primary therapeutic effect of MCT in human inflammatory bowel disease. These results should be confirmed in other experimental models of intestinal inflammation with different pathogenic backgrounds. Particularly, it will be of interest to assess the role of MCT in models involving intestinal barrier function such as dextran sulfate sodium colitis.

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