A mechanistic approach to understanding conjugated linoleic acid’s role in inflammation using murine models of rheumatoid arthritis

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Butz DE, Li G, Huebner SM, Cook ME. A mechanistic approach to understanding conjugated linoleic acid’s role in inflammation using murine models of rheumatoid arthritis. Am J Physiol Regul Integr Comp Physiol 293: R669–R676, 2007. First published June 6, 2007; doi:10.1152/ajpregu.00005.2007.—A naturally occurring fatty acid, conjugated linoleic acid (CLA), reduces immune-induced TNF and inducible cyclooxygenase (COX-2) expression; key mediators of inflammation in rheumatoid arthritis (RA). On the basis of previous work, it was hypothesized that dietary CLA would act as an anti-inflammatory agent in select animal models of RA. In the collagen antibody-induced arthritis (CAIA) model, mice fed CLA (mixed isomers of c9, t11, and t10, c12-CLA) for 3 wk before anticollagen antibody injection had reduced lipopolysaccharide-induced plasma TNF levels and had arthritic scores that were 60% of mice fed corn oil (CO). In the collagen-induced arthritis (CIA) model, mice fed mixed isomers of CLA for 21 days before immunization had lower IgG1 titers, earlier signs of joint inflammation, but similar arthritis scores compared with CO-fed mice during the remaining 70-day post-injection period. Beginning on day 80 to 133, CLA-fed mice had arthritis scores 70% that of the CO-fed mice. In a second CIA experiment, CLA was fed only after the booster injection. Plasma IgG1 levels were not reduced and arthritis onset was delayed 4 days in CLA-fed mice compared with the CO-fed mice. Peak arthritis score was similar between CLA and CO-fed mice from day 35 to 56. Because CLA reduced inflammation in the CIA model, delayed onset of arthritis in the CIA model (CIA experiment 2) and reduced arthritis score after day 80 in the CIA model (CIA experiment 1), we concluded that dietary CLA exhibited anti-inflammatory activity that was dependent on antibody.

CONJUGATED LINOLEIC ACID (CLA) isomers are naturally occurring fatty acids (C18:2) derived primarily from microbial biohydrogenation of linoleic acid (C18:2) and linoleic acid (C18:3) (6, 26). However, CLA synthesized by alkali isomerization has been the primary source for assessment of its immune modifying properties. Dietary CLA (predominantly equal parts c9, t11 and t10, c12 CLA) was shown to alleviate clinical signs of inflammatory diseases such as atherosclerosis (23, 38), lupus (55, 57), type I airway hypersensitivity (51, 52), endotoxin-induced cachexia (8, 31), and models of inflammatory bowel disease (2, 18). Potential anti-inflammatory properties of CLA may be attributable to its regulation of TNF and inducible cyclooxygenase (COX-2). Dietary CLA reduced LPS or dextran sulfate-induced release of TNF (2, 56) and inducible nitric oxide synthase (56), and antigen-induced release of eicosanoid products of COX-2 (52). Signaling pathways for TNF and COX-2 such as peroxisome proliferator-activated receptor γ (PPARγ) (58), NF-κB (2, 24), and MAPK (25, 41) have been implicated to be affected by CLA.

Rheumatoid arthritis (RA) is a type III hypersensitivity immune disorder (like lupus) characterized by articular joint inflammation and remodeling leading to severe pain and disability (42). Collagen antibody-induced arthritis (CAIA) and collagen-induced arthritis (CIA) are animal models of RA that have been extensively studied (34, 48–50) and have been shown to be suitable for providing insights into the basic pathological mechanisms of chronic inflammation (27). CAIA is the simplest model of RA and involves passive transfer of monoclonal antibodies directed at artiogenic epitopes of the type II collagen molecule (CII). When these anti-CII antibodies are injected into mice, a severe erosive arthritis similar to RA occurs (49). Unlike the CIA model, the CAIA model eliminates the need for acquired immunity, and results in joint inflammation within 24 h after initiation (47). In the CIA model of RA, articular joint inflammation appears after repeated immunization with CII (12, 50), a putative autoantigen in RA (21, 43), and through the priming of the adaptive immune response (12, 50). Following immunization against CII, susceptible mice develop a severe erosive articular joint destruction similar in pathology to RA (34). Autoantibodies against CII play a key role in joint destruction in both the CAIA and CIA models of RA (44, 49).

Inflammatory mediators such as COX-2 and TNF play important roles in the pathology of RA, CAIA, and CIA. Myers et al. (33) reported that DBA/1 COX-2 knockout mice are resistant to CAIA and CIA, whereas COX-1 (associated with housekeeping functions) knockout, and wild-type mice were shown to develop clinical signs of arthritis as expected. Blockade of the COX pathway using NSAIDs was an effective treatment of acute and chronic inflammation such as in RA (42). The overexpression of the human TNF transgene in arthritis-susceptible mice led to the spontaneous development of severe erosive arthritis similar to CIA and RA (3). Inflammation in both RA and its animal models can be attenuated by COX inhibitors (30) or by anti-TNF therapies (28, 53). Anti-TNF antibody administration in CIA model ameliorated disease (53), and several humanized anti-TNF therapeutics are now approved for RA therapy (14, 15).

On the basis of evidence that dietary CLA reduces inflammatory mediators, such as TNF and COX-2 and inhibiting these mediators decreases autoimmune-induced inflammation of articular joints in models of RA, we hypothesized that...
dietary CLA would reduce inflammation induced by anti-CII antibodies in articular joints. Previous experiments involving the autoimmune disease systemic lupus erythematosus (55, 57) suggested that dietary CLA’s effects on adaptive immune responses may confound an interpretation of CLA’s role in inflammation. Hence, murine models of inflammation that were dependent or independent of an acquired immune response to CII were used.

MATERIALS AND METHODS

All studies were conducted in accordance with protocols approved by the College of Agricultural and Life Sciences Animal Care and Use Committee. Figure 1 illustrates the timeline for each of the three experiments performed in the current work.

Anticollagen antibody-induced arthritis (CAIA) model. Four-week-old male BALB/c mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in a small laminar flow animal isolator in littered shoebox units (3 mice per unit) with a 12:12-h light-dark cycle. After 3 days of acclimation to the environment, mice were switched to a semipurified 99% complete diet (TD94060; Harlan-Teklad, Madison, WI) supplemented with 0.5% CLA (mixed isomers) and 0.5% corn oil (CO) or 1% CO (Table 1; n = 12 per diet). CLA and CO fatty acid content (Table 2) was assessed by gas chromatography using an HP-88 100-m fused silica capillary column (0.25 mm inside diameter, 0.2-μm film thickness) as previously described (22).

After 3 wk of dietary supplementation, dietary groups were further divided into monoclonal antibody and sham-injected groups to complete a two-by-two factorial design (n = 6 per group, 24 total). On day designated zero (Fig. 1A), mice were injected intravenously (via the tail vein) with 100 μl of either 10 mg/ml antibody cocktail (4 monoclonal antibody clones against distinct CII epitopes [Chemicon International, Temecula, CA] or vehicle (sterile PBS). Twenty-four-hours later, all mice were injected intraperitoneally with 50 μg LPS in PBS (1 mg/ml). Blood was collected from the retroorbital venous plexus, while animals were under light isoflurane anesthesia 1 h after LPS injection. Plasma was separated and samples were stored at −80°C. Mice were examined daily by a trained observer (unaware of treatment) for evidence of inflammation and joint swelling. The inflammation (arthritis) score was based on a scale of 0 to 4 using the

![Fig. 1. Illustration of experimental designs and timeline used in each experiment. A: collagen antibody-induced arthritis (CAIA) study was conducted as a two-by-two factorial arrangement of treatments with diet (conjugated linoleic acid, CLA or corn oil, CO) and injection (antibody cocktail, or vehicle) as main effects. Diet feeding began 21 days before the injection protocol, and arthritis was scored daily following the completed injection protocol. B: first collagen-induced arthritis (CIA) experiment (design 1) was conducted as a two-by-two factorial arrangement of treatments with diet (CLA or CO) and immunization type II collagen molecule [(CII)-immunized or sham-immunized] as the main effects. Diet feeding began 21 days before the immunization protocol, and arthritis was scored every 2 or 3 days after the initial injection. C: second CIA experiment (design 2) was a completely randomized design. Treatments were dietary CLA+CII-immunization; dietary CO+CII-immunization; and dietary CO+sham immunization. In design 2 of the CIA model all animals were fed the CO diet (21 days) before the initial injection. After the booster injection, mice were either fed the CO or the CLA diet for the remaining 56 days of the study. Arthritis scores were determined every 2 or 3 days during the feeding period.](http://ajpregu.physiology.org/Downloadedfrom.html)
On ally. The booster injection consisted of either 100 dilution of anti-mouse IgG antibody coupled to horseradish peroxi-
plate in serial dilutions and incubated for 2 h. The plate was washed
monoclonal antibody clone MAB8887 (Chemicon) were added to the
PBS. Either test plasma samples or a known amount of anti-CII
(100 Scientific, Pittsburgh, PA) were incubated overnight with chick CII
clinical inflammation (arthritis) score was monitored three times per
day 21, mice received 100 emulsified in a 1:1 ratio with complete Freund's adjuvant (2 mg/ml
injection intradermally at the base of the tail. The primary injection
separated and samples were stored at
plexus, while mice were under light isoflurane anesthesia. Plasma was
separated and samples were stored in 10% buffered formalin. Samples were
embedded in paraffin and sectioned. Slides were stained
with hematoxylin and eosin stains, and slides were photographed under ×4 magnification.

Statistics. A moving average of clinical arthritis scores was deter-
rmined by calculating the mean of two consecutive observations for a
given paw at each time point (CIA experiment 1 only). Arthritis score
data were analyzed using the SAS program (SAS Institute, Cary, NC) with
the mixed procedure accounting for autocorrelation of repeated
measures. Antibody titers and TNF concentrations were analyzed
using ANOVA. Results were considered significant with a $P$ value
<0.05 unless otherwise stated.

RESULTS

CIA model. Inflammation in the CIAA model began develop-
ing within 24 h after the LPS injection. At the peak of
inflammation, occurring on days 4–6, arthritis scores in the
CO-fed antibody-injected group scored near 6 (Fig. 2). A
statistically significant reduction in inflammation in the CLA-
fed antibody-injected group occurred on days 5 and 6 with arthritis scores 60% of CO fed antibody-injected group scores (Fig. 2). Figure 3 illustrates joint histology of right-rear hind
paws of randomly selected mice in each group. The ankle joint
of the mAb-CO animal (Fig. 3B) shows significant cell infiltration
and joint remodeling compared with the same joint in the
sham-CO animal (Fig. 3A). The ankle joint from the
mAb-CLA animal (Fig. 3C) shows a well-structured ankle

<table>
<thead>
<tr>
<th>Ingredient, g/100 g</th>
<th>CLA</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>47.6</td>
<td>47.6</td>
</tr>
<tr>
<td>Casein</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Corn starch</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5.5</td>
<td>6</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AIN-76 Mineral mix</td>
<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>AIN-76 Vitamin mix</td>
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<td>1</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Choline bitartrate</td>
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<td>0.2</td>
</tr>
<tr>
<td>Ethoxyquin</td>
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<td>0.001</td>
</tr>
<tr>
<td>CLA90</td>
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Table 1. Experimental diet contents for CLA and CO diets

Table 2. Fatty acid contents of dietary oils

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>CLA</th>
<th>CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>c16:0</td>
<td>0.6</td>
<td>12.8</td>
</tr>
<tr>
<td>c18:0</td>
<td>n/d</td>
<td>2.4</td>
</tr>
<tr>
<td>c18:1 c9</td>
<td>6.4</td>
<td>29.1</td>
</tr>
<tr>
<td>c18:1 c11</td>
<td>0.3</td>
<td>0.8</td>
</tr>
<tr>
<td>c18:2 c9, c12</td>
<td>0.3</td>
<td>53.1</td>
</tr>
<tr>
<td>c18:2 c9, t11</td>
<td>42.7</td>
<td>n/d</td>
</tr>
<tr>
<td>c18:2 t10, c12</td>
<td>43.5</td>
<td>n/d</td>
</tr>
<tr>
<td>c18:2 cc*</td>
<td>1.9</td>
<td>n/d</td>
</tr>
<tr>
<td>c18:2 tt†</td>
<td>4.1</td>
<td>n/d</td>
</tr>
<tr>
<td>c18:3 c9, c12, c15</td>
<td>n/d</td>
<td>1.2</td>
</tr>
<tr>
<td>c20:0</td>
<td>n/d</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Analysis of test oils by gas chromatography test oil. *Conjugated cis, cis
isomers; †conjugated trans, trans isomers; n/d, not detectible.

CLA’S ROLE IN MODELS OF RHEUMATOID ARTHRITIS

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juvant with or without the vehicle, sham-immunized mice did not exhibit signs of arthritis at any time regardless of diet. Arthritis score became apparent in the CO-fed mice only after the booster injection with CII, and scores increased in both CLA- and CO-fed mice until peaking at a score of 2 to 3 from day 21 to day 49 (Fig. 4). Apart from the early appearance of inflammation signs in the CLA-fed mice, there was no overall significant difference in arthritis score between CII-immunized mice fed CLA, and mice continued to be fed their dietary treatments and scored for inflammation until day 133 post-CII injection; however, no additional sampling was done during this period. Beginning on day 80 and to the end of the observation period, a relapse of joint inflammation occurred. Mice in the CLA-fed group had a 30% reduction in arthritis scores compared with the CO-fed group (differences were nonsignificant).

CLA’s Role in Models of Rheumatoid Arthritis

In the first CIA experiment, mice were fed CLA or CO diets beginning 21 days before the immunization protocol (Fig. 1B). On day 0, one-half of the mice from each diet group were immunized against chick CII and the other half of the mice were sham-immunized (2 × 2 factorial design; n = 4 per group). Clinical signs of arthritis were not apparent before the booster injection in the CO-fed mice; however, the CLA-fed mice exhibited signs of inflammation before day 21, booster injection of CII. Apart from mild inflammation of the injection site typically associated with Freund’s complete adjuvant with or without the vehicle, sham-immunized mice did not exhibit signs of arthritis at any time regardless of diet. Arthritis score became apparent in the CO-fed mice only after the booster injection with CII, and scores increased in both CLA- and CO-fed mice until peaking at a score of 2 to 3 from day 21 to day 49 (Fig. 4). Apart from the early appearance of inflammation signs in the CLA-fed mice, there was no overall significant difference in arthritis score between CII-immunized mice fed CLA, and mice continued to be fed their dietary treatments and scored for inflammation until day 133 post-CII injection; however, no additional sampling was done during this period. Beginning on day 80 and to the end of the observation period, a relapse of joint inflammation occurred. Mice in the CLA-fed group had a 30% reduction in arthritis scores compared with the CO-fed group (differences were nonsignificant).

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In the second CIA experiment, mice were maintained on a CO diet prior to and up to the 21-day CII booster injection. At the time of booster injection, one-third of the mice \((n = 9)\) were switched to a CLA diet, while the other mice \((n = 18)\) were maintained on a CO diet (Fig. 1C). The CLA mice and one-half of the CO mice were immunized against chick CII, while one-half of the CO fed mice were sham-immunized to complete a three treatment completely randomized design \((n = 9\) per group). As observed in the first CIA experiment, CO-fed mice began showing signs of inflammation within about 5 days after the booster injection of CII. CLA-fed mice showed an \(\sim 4\)-day delay in reaching arthritic scores of 1, 2, 3, and 4. Apart from the delayed signs of inflammation in the CLA-fed mice, the arthritis score increased after the booster injection in both CII-immunized CLA- and CO-fed mice in a similar manner and reached a plateau (score of \(\sim 4\)) until day 56 (Fig. 5). Sham-immunized mice did not exhibit signs of arthritis at any time. CO-fed CII-immunized mice were statistically different from the sham group by day 28, while CLA-fed mice were not statistically different from the sham-immunized mice until day 33.

Plasma total anti-CII IgG, anti-CII IgG\(_1\), and anti-CII IgG\(_{2a}\) titers were measured in both CIA experiments. CII-specific antibody was not detected in plasma prior to immunization (day \(-7\), data not shown) or in sham-injected groups from samples taken on day 35 in both experiments. In the first CIA experiment, there was a reduction in antigen-specific total anti-CII IgG and anti-CII IgG\(_1\) titer in the CLA-fed and CII-immunized mice compared with the CO-fed and CII-immunized mice \((P = 0.06\) and \(P = 0.08\), respectively; Table 3). There was no difference in anti-CII IgG\(_{2a}\) titer between CLA- and CO-fed and CII immunized mice. (However, it may be important to note that there was a 1.4-fold increase in IgG\(_{2a}\) as a result of CLA). In the second CIA experiment, total anti-CII IgG (CLA = 1,403 vs. CO = 3,506) and anti-CII IgG\(_1\) (CLA = 784 vs. CO = 979) were not statistically different, even though the number of mice was increased, and while anti-CII IgG\(_{2a}\) (CLA = 3,676 vs. CO = 2,413) titters were not affected by diet, a 1.5-fold increase was evident as in the first CIA experiment (see DISCUSSION).

**DISCUSSION**

To study the effects of CLA on inflammation in RA, two models were used: CAIA and CIA. The CAIA model of RA is solely dependent on antibody-initiated inflammation of joints, whereas the CIA model is dependent on both the acquired immune response to the injected CII antigen and the resulting antibody. Hence, use of these two models of arthritis allowed for differentiation between CLA’s effects on antibody and acquired immunity-mediated events in joint inflammation (35). Mice fed CLA for 3 wk before application of CAIA methods showed a significant decrease in joint inflammation. COX-2 null mice showed reduced inflammation when CAIA was applied (33). Because dietary CLA has been shown to decrease COX-2 expression, and resulting eicosanoids in response to both reoccurring antigen exposure (51, 52) and endotoxin (24),

**Table 3. Anti-type II collagen IgG reactive titer on day 35 of the first CIA experiment**

<table>
<thead>
<tr>
<th>Collagen Immunized</th>
<th>Sham Injected</th>
<th>Main Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA</td>
<td>CO</td>
<td>Diet</td>
</tr>
<tr>
<td>Total IgG</td>
<td>1277 (82)</td>
<td>458 (32) n/d</td>
</tr>
<tr>
<td>IgG1</td>
<td>434 (188) 1290 (400) n/d n/d</td>
<td>0.0771 0.0021 0.0771</td>
</tr>
<tr>
<td>IgG2a</td>
<td>669 (276) 485 (105) n/d n/d</td>
<td>0.5474 0.0022 0.5474</td>
</tr>
</tbody>
</table>

Values are expressed as means \(\pm\) SE.
a possible mechanism of CLA-induced suppression of inflammation associated with CAIA may have involved COX down-regulation. In addition, dietary CLA has also been shown to reduce TNF in response to LPS [shown here and elsewhere (4, 5, 17, 56)]. TNF is an integral proinflammatory cytokine in CAIA (20). Inhibition of TNF through the use of anti-TNF antibodies, soluble TNF receptors, or antagonist of TNF-converting enzyme (TACE) have been used as therapeutic strategies for RA (36, 37, 54). The CLA-induced reduction in TNF, found 1 h after LPS injection in the CAIA model, may be relevant to the anti-inflammatory effects observed days later. Newton et al. (37) showed that inhibition of TACE-dependent release of soluble TNF in the CAIA model (TNF released independent of downstream gene transcription and translation) reduced the cascade of cytokines released subsequent to TNF release and reduced clinical scores of inflammation in the 11 days following the LPS activation of the disease. Coppieters et al. (10) showed the use of camelid antibodies to TNF resulted in decreased inflammation of joints in the murine model of CIA similar to what was shown in this study (Fig. 3). The use of anti-TNF or anti-IL-1 antibodies before the onset of CIA was also effective at delaying the onset of arthritis but was ineffective at preventing the clinical signs of arthritis (54).

Hence, dietary CLA’s effects on TNF should also be considered as a possible mechanism by which CLA reduced CAIA. CLA-induced suppression of antibody-dependent inflammatory responses have also been demonstrated in the airway (51, 52), where antigen-induced contraction of trachea (% of maximum contraction) was decreased when harvested from guinea pigs fed CLA. Anaphylaxis in response to egg white lysozyme was decreased by dietary CLA in mice (19). Longevity of NZB/W F1 mice that spontaneously develop lupus erythematosus was extended 1.5-fold if the mice were fed CLA compared with corn oil (55). Hence, anti-inflammatory effects of dietary CLA in the antibody-dependent model of CAIA were anticipated and consistent with previous reports in other antibody-dependent disease models.

A second study was conducted using the same feeding strategy described for CAIA to investigate CLA’s effects during an operative acquired immune response. Mice were subjected to the CIA protocol 3 wk after being fed a CO- or CLA-supplemented diet. We hypothesized that the inflammatory response in the CIA model would be different from the CAIA model because the adaptive immune response was engaged in CIA (1). This anticipated difference was based on previous work that had shown that dietary CLA has a broad array of effects on acquired immune responses (7, 8, 16, 40). Recent work has also suggested that dietary CLA may shift an acquired immune response toward a Th-1 type reaction (7, 56).

Evidence supporting a shift toward a Th-1 reaction was decreased IgE antibody production in lymphocytes from rats fed CLA (45) and decreased IL-4 and increased IL-2 in concanavalin-A stimulated splenocyte harvested from mice fed CLA (56). RA and CIA are well recognized as Th-1 diseases (9, 13, 28, 29). Our findings in the CIA experiment of an effect of dietary CLA on IgG1 (66% decrease) and IgG2a (1.4-fold increase) were supportive of a shift toward a Th-1 response. Cooper et al. (9) showed that a change in IgG2a anti-CII levels (1.6-fold difference) was associated with the incidence of CIA disease (increased anti-CII IgG2a was associated with more severe disease). In another study, decreasing anti-CII IgG2a by binding TNF, with soluble TNF receptor, reduced lesion scores in the CIA model (32). Hence, previous reports suggesting that CLA may select for a Th-1 response and the antibody profiles reported here would be supportive of increased clinical signs of CIA in CLA-fed mice. Indeed, mice fed CLA showed their first clinical signs of disease 10 days before control fed mice. In another Th-1 disease, lupus erythematosus, NZB/W F1 mice fed CLA developed antinuclear antibodies and proteinuria earlier than control fed mice (57).

Hence, the earlier clinical signs in both the CIA (here) and lupus (57) models of inflammation supported a CLA-induced shift toward a Th-1 type response. Peak inflammation in the CIA model was not affected by diet (power analysis was sufficient to detect the 40% change observed in CAIA). Although no differences in long-term clinical signs of arthritis were detected, it is possible that long-term changes in joint structure could have occurred had histological analysis of joint tissue been examined (not done). An earlier appearance of anti-nuclear antibodies and proteinuria in CLA-fed NZB/W F1 mice was not associated with a decreased life span, as would be predicted based on earlier appearance of disease signs (57). It was possible that the antibody-dependent anti-inflammatory effects observed in the CAIA model may have counteracted the enhanced disease-causing effects of a shift toward Th-1 responses in mice fed CLA in a manner similar to what was found in the lupus model (56).

The CIA study described above was continued (133 days) longer than typically reported using this model (a length of less than 70 days is typical in CIA studies). The extended study of the CIA mice afforded an opportunity to observe the effects of dietary CLA during a “relapse” of joint inflammation. By day 70, the mean arthritis scores for both control- and CLA-fed mice had declined below a score of 1 [a pattern consistent with Myers et al. (33)]. Beginning ~80 days after the first CII-injection, an unexpected relapse of joint inflammation was observed, a response that has not been well studied (1, 46); hence, antibody titers were not measured. During the relapse, joint swelling in the CLA-fed mice was reduced ~30% (not statistically significant possibly because the study was powered to detect a 40% reduction in arthritis scores). Because no serological analysis was done at this time, any comment on these data would be speculative. It has been reported that DBA/1 mice may develop arthritis spontaneously (11, 39). The possible effect of dietary CLA during the relapse period could be similar to what has been reported in the lupus model of autoimmunity. In a study involving spontaneous lupus, NZB/W F1 mice had increased longevity (1.5-fold) and reduced end-stage body weight wasting following proteinuria if fed CLA (55, 57). Hence, it is possible that an anti-inflammatory response associated with feeding CLA during the relapse period was antibody dependent and similar to what was observed in the CAIA model described above and the lupus model described previously (57).

In the third experiment, an attempt was made to test the hypothesis that CLA may be protective against CIA if the CLA were fed after the first injection of CII (feeding commences with the second or booster injection). The results of this experiment showed a delay (~4 days) in the appearance of clinical signs of joint swelling in the CLA-fed mice compared with the control-fed mice, but peak scores were similar for mice fed the two diets. The IgG2a was increased 1.5-fold.
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(non-significant) in the CLA-fed mice compared with the control-fed mice, suggesting that feeding CLA at the booster injection may have remained too early to test the intended hypothesis. However, these data were supportive of the first CIA experimental findings that CLA did not exacerbate the clinical signs of arthritis.

Commercially prepared mixed isomers of CLA (predominantly c9, t11, and t10, c12-CLA) were used in the experiments presented. Currently, dietary supplements of mixed isomers with the two major isomers shown are the only CLA supplements available to the general public. Research has shown that the biological activity of the individual isomers may yield results very different than the pure isomers. For example, the c9, t11 CLA isomer was the isomer reported to inhibit the release of TNF in LPS-injected mice (t10, c12 had minimal effects) (56), whereas, the t10, c12 isomer appeared to be the isomer most effective in the regulation of COX-2 expression (24). Butz et al. (4) showed that the mixed isomers of CLA were necessary for the prevention of LPS-induced weight loss (neither isomer alone was effective); however, when mice were fed the t10, c12 isomer following LPS injection, gain following weight loss was compensatory. It is anticipated that the response of mice to individual isomers may be different than what is presented in this study. However, since each isomer alone (c9, t11-CLA affects TNF and t10, c12-CLA affects COX-2) has an effect on inflammatory mediators involved in arthritis (24, 56), it was decided that a study of commercially available sources of CLA should be studied and reported.

In conclusion, the results of these experiments suggested that dietary CLA might have had a moderate ability to prevent CIA antibody-dependent joint inflammation. CLA’s anti-inflammatory affect may have been less evident if fed during the development of an acquired immune response to CIA than after the acquired immune event was complete. The diminished anti-inflammatory affect of dietary CLA during the acquired immune response may have been due to CLA’s tendency to drive a Th-1 type of immune response. The antibody-dependent anti-inflammatory effects of CLA may have counteracted CLA’s possible drive toward a Th-1 type of immune response, which, in turn, may have explained why arthritis scores were not increased when CLA was fed prior to CIA.

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