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Long-Term Feeding of the *cis*-9,*trans*-11 Isomer of Conjugated Linoleic Acid Reinforces the Specific Immune Response in Rats[1,2]

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Abstract

Several effects on the immune system have been ascribed to the *cis*9,*trans*11 conjugated linoleic acid (CLA) isomer. We studied whether feeding a diet enriched with an 80:20 CLA isomer mix of *cis*9,*trans*11 and *trans*10,*cis*12 CLA from gestation to adulthood affects the capacity of adult rats to achieve a specific immune response. Pregnant Wistar rats were fed a 1% CLA diet or a control diet beginning on d 7 of gestation. Weaned pups received the same diet as dams until they were 15 wk old. Rats from both groups were immunized with ovalbumin (OVA) when they were 9 wk old. Dietary CLA enhanced splenocyte OVA-specific proliferation by ~50% ($P < 0.05$) and decreased the mitogen-induced proliferative responses of these cells by ~10–20% ($P < 0.05$). The diminished splenocyte proliferative response was accompanied by a lower interleukin-2 secretion ($P < 0.05$). Long-term CLA supplementation did not increase serum, spleen, or mesenteric lymph node production of OVA-specific antibodies (Ab) or the number of spleen anti-OVA Ab-secreting cells. Interestingly, dietary CLA increased intestinal anti-OVA IgA production by ~75% ($P < 0.05$). In conclusion, a 1% CLA diet administered from gestation to adulthood enhanced specific systemic cell-mediated immunity as well as the mucosal IgA immune response, whereas it downregulated the polyclonal activation of the immune system. These data support the long-term effects of dietary *cis*9,*trans*11 CLA isomer on the immune system. J. Nutr. 139: 76–81, 2009.

Introduction

The influence of dietary fatty acids on the immune function was first studied by Meade and Mertin (1), who focused on the effects of fatty acids on in vitro lymphocyte proliferation. Many studies have subsequently been published and currently it is widely known that dietary fatty acids are able to modify immune responses. The mechanisms involved in these effects influence cell signaling, gene expression, cell membrane structure and function, and the profile of lipid mediator production (2–5).

Conjugated linoleic acid (CLA)[6] is a lipid of great importance. The *cis*9,*trans*11 CLA isomer, also called rumenic acid, is the predominant isoform and is naturally found in beef and dairy products. Several other CLA isomers are industrially produced during vegetable oil processing, with the most abundant among these isomers being the *trans*10,*cis*12 CLA isomer. Many health benefits are ascribed to CLA, including anticancer (6–8), anti-atherogenic (9), antidiabetogenic (10,11), and modifying body composition (12) and bone mass (13). However, results from studies in rodents and humans indicate an increase in the concentration of blood sugar and insulin, insulin resistance, VLDL, and reduced blood leptin and HDL (14). In overweight human subjects, the *trans*10,*cis*12 CLA isomer caused a several-fold increase in lipid peroxidation and serum C-reactive protein (15). Additionally, CLA isomer mixtures have been shown to have immunomodulatory properties, ranging from activation to inhibition (16,17). These discrepancies are mainly due to the isomer mixtures used for supplementation. The *trans*10,*cis*12 CLA isomer is responsible for body fat reductions (18), whereas both the latter and the *cis*9,*trans*11 CLA isomer have probable immunomodulatory properties (16). CLA immunomodulation has often been examined by identifying changes in the mitogen-induced immune response (19), but little attention has been drawn to the interaction between CLA and the adaptive immunity after specific antigen (Ag) challenge and even less work has been carried out studying the mucosal compartment.

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[6] Abbreviations used: Ab, antibody; Ag, antigen; CAS, casein; CCM, complete culture media; CLA, conjugated linoleic acid; IL-2, interleukin-2; MLN, mesenteric lymph node; OVA, ovalbumin; PMA/Io, phorbol myristate acetate/Ionomycin; SC, secreting cell; TPS, total period of supplementation; US, unstimulated cell.
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The intestinal immune system is the largest and most complex part of the immune system and its responses are elaborated by interaction of regulatory mechanisms that ensure the maintenance of gut homeostasis. Secretory IgA is the main immunoglobulin on the mucosal surfaces (80–90%) and has the shared role of protecting against both ubiquitous foreign substances and microbes while not subjecting the mucosa to undue inflammation (20).

Because previous studies have suggested that CLA intake during developmental phases may have effects later in life (21,22), and that the *cis*9,*trans*11 CLA isomer prevails in breast milk and constitutes ~80% of the total CLA isomers present (23), this study was performed from gestation to adulthood. The aim of this study was to ascertain whether the capacity to produce a specific immune response in ovalbumin (OVA)-sensitized adult rats is influenced by long-term feeding of an enriched diet containing an 80:20 CLA isomer mix of *cis*9,*trans*11 and *trans*10,*cis*12 CLA, respectively.

Materials and Methods

Rats. Pregnant Wistar rats at 7 d of gestation were obtained from Harlan. The rats were housed in individual cages under controlled temperature and humidity conditions in a 12-h-light:12-h-dark cycle and consumed food and water ad libitum. Rats were monitored daily and allowed to deliver at term. Litters were unified to 10 pups per lactating dam; pups had free access to the nipples and rat diet. On the day of weaning, rats were separated by gender and distributed to 3 per cage. Body weight and food intake were monitored weekly. Handling was done in the same time range to avoid the influence of biological rhythms. At the end of the study, we measured body and tail length for each rat, as well as thymus, spleen, and liver weight. Body weight and body length (nose-anus length) were used to determine BMI (kg/m^2). Studies were performed in accordance with the institutional guidelines for the care and use of laboratory animals established by the Ethical Committee for Animal Experimentation at the University of Barcelona and approved by the Catalonian Government (UB 302–05/DMA 3241).

Experimental groups and diets. Pregnant Wistar rats at 7 d of gestation were assigned to 1 of the 2 dietary groups and after delivery, litters were kept with their dams until weaning (d 21). Thereafter, pups consumed the same diet as their mothers. The 2 dietary groups were the CLA group ($n = 20$) and the control group ($n = 20$). The CLA group were rats whose dams were fed a 1% CLA-enriched diet during gestation (2 wk) and suckling (3 wk); pups received CLA through the placenta and milk, respectively. From weaning until the end of the study (15-wk-old rats), rats were also fed 1% CLA diet [total period of supplementation (TPS), 17 wk]. The control group rats were fed a control diet throughout the 17 wk of study (TPS, 0 wk).

The control diet corresponded to the AIN-93G formulation (24). The 1% CLA diet was obtained from modified standard flour (AIN-513, Harlan) containing 10 g CLA/kg flour (**Table 1**), using a CLA isomer mixture of ~80% *cis*9,*trans*11 and 20% *trans*10,*cis*12 among the total of CLA isomers in the oil (79.5%). CLA oil was kindly supplied by Loders Croklaan, Lipid Nutrition, Wormerveer, The Netherlands. The CLA mixture had 0.69% FFA as oleic acid, a peroxide value of 0.2 mEq/kg, 5.6% SFA, and <5% of CLA minor isomers. The standard flour AIN-513 was modified with 1% less soybean oil and replaced with the same amount of CLA (Table 1). The mixture was pelletized (1-cm-diameter pellets) and dried in a 40°C oven for 24 h. The pelleted diet was vacuum-packed to prevent oxidation and contamination by fungi and kept at 4°C until use. For humidity control, periodic tests were performed in an electronic humidity analyzer (Sartorius MA-45) for 15 min at 105°C, which showed a 5% weight loss because of drying. This diet was examined and 1% CLA content and absence of PUFA oxidation was confirmed. The 1% CLA (wt:wt) pelleted diet was produced in the Medicine Development Service of the Faculty of Pharmacy at the University of Barcelona.

OVA immunization. Nine-week-old rats received 10 mg/kg of OVA (grade V, Sigma Immunochemicals) emulsified with alum adjuvant (Inject Alum, Pierce) by intraperitoneal injection.

Sample obtaining. Six weeks after immunization, rats were killed and a macroscopic organ observation was performed. Blood was obtained by cardiac puncture and serum was separated and stored at −20°C until use. Spleen and mesenteric lymph nodes (MLN) were excised for lymphocyte isolation. The small intestine was also removed and a distal portion was weighed, longitudinally opened, cut in 5-mm pieces, and incubated with PBS for 20 min at 37°C in a shaker. After centrifugation, the intestinal wash was stored at −20°C until IgA quantification by ELISA.

Lymphocyte isolation and culture

Lymphocytes from spleen and MLN were immediately isolated after organ excision. Spleen cell suspensions were obtained by passing the tissue through a steel mesh in cold sterile conditions as previously described (25). Then, cells were centrifuged at 600 × *g*; 5 min at 4°C and resuspended in PBS (pH 7.2). Erythrocytes were lysed by adding distilled water to the cell suspension and, after restoring tonicity, cells were washed and resuspended in complete culture media (CCM): RPMI-1640 medium, supplemented with 10% fetal bovine serum (Sigma), 0.1 IU/L streptomycin-penicillin (Sigma), 2 mmol/L L-glutamine (Sigma), and 0.05 mmol/L 2-β-mercaptoethanol (Merck).

MLN cell suspensions were obtained in sterile conditions by passing the tissue through a steel mesh. Cell suspensions were kept on ice for 10 min to remove tissue debris by sedimentation and later cells were centrifuged at 600 × *g*; 5 min at 4°C. Cells were then resuspended in CCM. We determined the number and viability of spleen and MLN lymphocytes by double staining with acridine orange and ethidium bromide (Sigma).

Determination of anti-OVA antibody concentration. Indirect ELISA assays were performed to determine total OVA-specific antibodies (Ab)

TABLE 1 Composition of the experimental diets

Ingredient	AIN-93G[1]	1% CLA
Total energy, *kJ*	15,767	15,763
Fat, *% energy*	16.700	16.700
CLA oil, *% energy*		2.3900
Soybean oil, *% energy*	16.700	14.310
	g/kg	
CAS	200.00	199.98
L-Cysteine	3.0000	2.9900
Corn starch	397.50	397.50
Maltodextrin	132.00	131.90
Sucrose	100.00	99.990
Cellulose	50.000	49.990
Mineral mix	35.000	34.990
Vitamin mix	10.000	9.9900
Choline bitartrate	2.5000	2.5000
Ter-butylhydroquinone	0.014	0.014
Soybean oil	70.000	59.990
16:0	7.7000	6.7000
18:0	2.8000	2.4000
18:1	16.400	14.200
18:2	37.200	32.200
18:3	5.6000	4.8000
80:20 *cis*9,*trans*11:*trans*10,*cis*12 CLA oil[2]		10.000[3]

[1] The diet was prepared according to AIN guidelines (24).
[2] From total oil, 78% corresponded to CLA in triacylglycerol form containing an isomer ratio of 80:20 *cis*9,*trans*11:*trans*10,*cis*12. The CLA mixture had 0.69% FFA as oleic acid, a peroxide value of 0.2 mEq/kg, 5.6% SFA, and <5% of minor CLA isomers.
[3] CLA isomer content after the manufacturing process was ~0.8% and the *cis*9,*trans*11:*trans*10,*cis*12 proportion was 80:20.

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in serum, intestinal washes, and spleen and MLN cell supernatants as previously reported (26). Because a standard amount of anti-OVA Ab was not available, we used a pool of OVA-immunized rat serum in each plate to normalize OD results. Results were expressed relative to the control group, which was set at 100%.

Quantification of anti-OVA Ab-secreting cells. We used a previously described enzyme-linked immunosorbent spot technique to quantify anti-OVA IgA-, IgG-, and IgM-secreting cells (SC) from spleen (26).

Specific and polyclonal lymphocyte proliferative response. Specific proliferative response from spleen and MLN cells was determined by culturing 1×10^5 cells in 100 μL of CCM after OVA (10 mg/L) stimulation for 96 h (37°C, 5% CO_2). Addition of control protein [casein (CAS), 10 mg/L] and only medium [unstimulated cells (US)] were used as negative reference controls for each sample.

Polyclonal proliferative response was also quantified by incubating spleen and MLN cells (1×10^5 cells/200 μL CCM) after stimulating with phorbol myristate acetate [(PMA), 250 μg/L] plus ionomycin [(Io), 250 μg/L] or without stimulus in 96-well plates for 72 h.

In both cases, cell proliferation was determined by ELISA using Cell Proliferation Biotra from Amersham Biosciences according to the manufacturer's instructions.

Detection of interleukin-2 in culture supernatants. Interleukin-2 (IL-2) was quantified in 24-h supernatant cultures of spleen and MLN cells (10^6 cells per well) after stimulation with PMA/Io (250 μg/L). Cytokine concentrations were determined using rat ELISA kits from Biosource. ELISA were performed according to the manufacturers' instructions.

Statistical analysis. Data were analyzed by 2-way ANOVA (diet × stimulation) and when the interaction was significant, by the post hoc Scheffé test. Repeated-measures 2-way ANOVA was used to analyze body weight data in male and female rats. Differences were considered significant at $P < 0.05$.

Results

Morphometrical variables. Body weight was monitored throughout the study according to animal gender due to sexual features differentiation after wk 3 of life. Dietary CLA did not modify body weight of males but increased that of females ($P < 0.05$) from 6 wk of age until the end of the study at age 15 wk (**Fig. 1**). Food intake did not differ between the groups (data not shown). At the end of the study, despite differences in females' body weight, BMI of females (5.5 ± 0.1) and males (7.3 ± 0.1) were similar in the dietary groups. Body and tail lengths of males (44 ± 1 cm and 21 ± 1 cm, respectively) and females (40 ± 2 cm and 21 ± 1 cm, respectively) were not affected by dietary CLA. Thymus, spleen, and liver weights in females did not differ between groups (data not shown). However, the spleen weight of males fed CLA was ~17% lower than that of males from the control group (data not shown; $P < 0.05$).

Mitogen-induced cell immune response. Although the main goal was to determine the effect of CLA supplementation on the capacity to generate an Ag-specific immune response, the mitogen-induced immune response was also evaluated.

Spleen lymphoproliferative capacity was evaluated under in vitro conditions (**Fig. 2***A*). Splenocytes from control and CLA rats had a higher proliferation after mitogen stimulation than US ($P < 0.05$). Splenocytes from rats fed CLA throughout the study (TPS, 17 wk) had a ~10% lower proliferative response than control rats after PMA/Io stimulation ($P < 0.05$). This down-regulatory effect by dietary CLA was not due to cell viability loss, because viability from the CLA after PMA/Io addition was comparable to that of control cells.

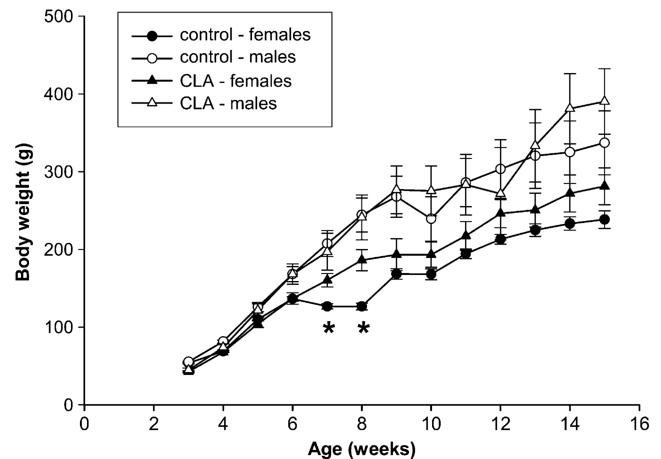

FIGURE 1 Body weight of male and female Wistar rats fed a CLA or control diet from weaning until 15 wk of age. Data are means ± SEM, $n = 20$. *Different from females fed CLA at that time, $P < 0.05$.

IL-2 cytokine secretion was evaluated in 24-h spleen cultures after polyclonal stimulation. The production of this cytokine, responsible for lymphocyte proliferation, was lower in cell cultures of CLA-fed rats than in those of rats fed the control diet ($P < 0.05$; Fig. 2*C*).

The MLN lymphoproliferative response was also evaluated after mitogen stimulation (Fig. 2*D*). MLN cells from rats fed CLA all their lives had a similar proliferative response to rats fed the control diet after mitogen stimulation (Fig. 2*D*). However, IL-2 production and cell viability, measured in 24-h cultures, were not affected by either PMA/Io addition or dietary CLA (Fig. 2*E*,*F*).

Specific anti-OVA cell immune response. To determine the long-term effects of dietary CLA supplementation on specific Ag responses, the lymphoproliferative capacity after OVA addition was evaluated. Control and CLA groups had a higher (2- to 3-fold) splenocyte proliferation after OVA stimulation than unstimulated and control protein addition ($P < 0.05$) (**Fig. 3***A*). In terms of specific proliferative response (mean percentage of increase compared with unstimulated conditions), splenocytes recovered from OVA-immunized rats fed CLA had higher (~275%) lymphoproliferative response to OVA than splenocytes recovered from OVA-immunized rats fed the control diet (~165%) ($P < 0.05$).

The effect of dietary CLA was also established on MLN lymphoproliferative capacity after OVA stimulation (Fig. 3*B*). MLN cells from all OVA-immunized rats proliferated after OVA stimulation compared with unstimulated conditions, but the diet groups did not differ.

Specific anti-OVA humoral immune response. To ascertain long-term CLA diet effects on humoral immune response, we have quantified serum OVA-specific Ab concentration, in vitro anti-OVA Ab (spleen and MLN) production, and spleen anti-OVA-Ab-SC number. Both experimental groups had high anti-OVA Ab concentrations in serum and in splenocyte supernatants (data not shown). Long-term dietary CLA did not modify the humoral response against the OVA-specific challenge in these compartments. Although long-term CLA supplementation tended to increase the in vitro capacity of spleen to produce anti-OVA Ab by 35%, ($P = 0.1$), this was not reflected in the serum concentration of anti-OVA Ab. Moreover, serum total Ig levels were not affected by dietary CLA (data not shown).

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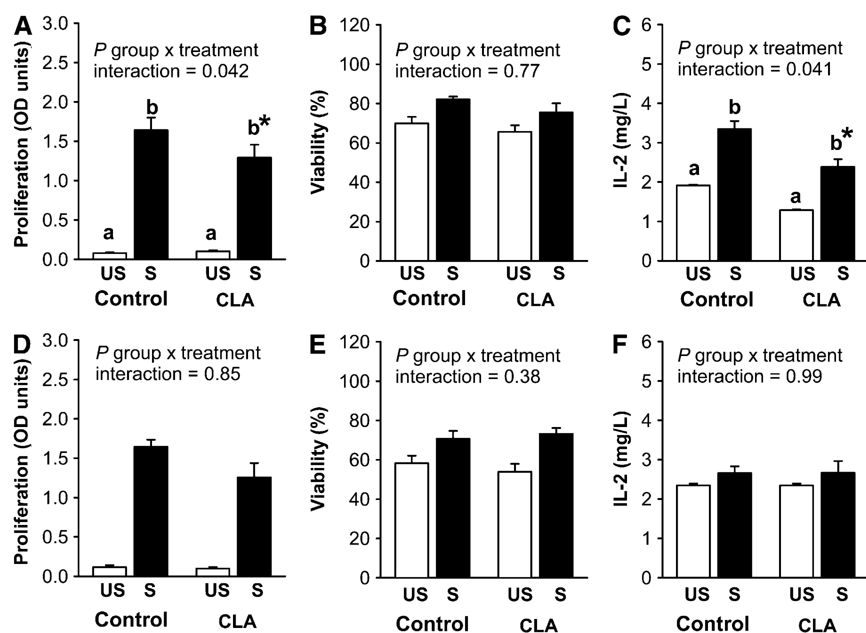

FIGURE 2 Proliferative response (*A,D*), viability (*B,E*), and IL-2 production (*C,F*) of US and PMA/Io-stimulated (S) spleen (*A–C*) and MLN (*D–F*) cells from rats fed a CLA or control diet. Data are means ± SEM, $n = 20$. Within a diet group, labeled means without a common letter differ, $P < 0.05$. *Different from the corresponding control, $P < 0.05$.

In addition, we counted spontaneous anti-OVA IgG-, IgM-, and IgA-SC in spleens. OVA-immunized rats had more spleen anti-OVA IgG- and IgM-SC than IgA-SC. This pattern was not affected by long-term dietary CLA, although the number of anti-IgA-SC tended to be greater (15.6 ± 3.5) in CLA-fed rats than in control rats (11.9 ± 1.9) ($P = 0.09$).

Anti-OVA Ab levels were also quantified in MLN cell supernatants. These results were similar between both groups (data not shown). However, analysis of intestinal washes showed that dietary CLA modulated mucosal IgA production. Long-term dietary CLA increased the anti-OVA IgA levels in the intestinal mucosa ~75% ($P < 0.05$), although CLA did not modify total gut IgA (data not shown). These data suggest that the 1% CLA diet had a restricted enhancement effect on OVA-specific IgA intestinal production and not a general effect on humoral immunity.

Discussion

In this study, we reported on the effects of long-term feeding a CLA mixture of *cis*9,*trans*11 and *trans*10,*cis*12 isomers (80:20) on the specific and polyclonal immune responses of Wistar rats. We demonstrated that a long-term CLA diet because gestation enhances some aspects of Ag-specific responses, whereas it downregulates polyclonal activation of the immune system.

Because CLA supplementation lasted 17 wk, it was important to assess whether CLA affected rat growth or had toxic effects. The CLA diet did not modify BMI, showing typical values of Wistar rats fed a control diet (27). However, females fed CLA seemed to have improved feed efficiency, because they achieved the plateau adult body weight (~250 g) at least 2 wk earlier than females fed a control diet. In addition, long-term dietary CLA did not cause macroscopic adverse effects compared with control rats. These results agree with those obtained in male rats fed a *cis*9,*trans*11 CLA-enriched diet for 8 wk (28). Conversely, CLA toxic effects were found in subjects fed 3.4 g/d of purified *trans*10,*cis*12 CLA isomer (14). However, as shown here, 20% of this isomer was well tolerated by Wistar rats for 17 wk beginning with the gestation period.

Although the main goal of this study was to examine whether a long-term 1% CLA diet modulates the capacity to generate an Ag-specific immune response, the in vitro capacity to generate a mitogen-induced response was also evaluated. It is the first time, to our knowledge, that this kind of global analysis allows showing that data obtained from the evaluation of an Ag-specific challenge are due to direct effects of CLA on the specific mechanism of immune response and excludes the possibility that the observed findings are due to a global effect on the immune system.

Previous studies concerning other PUFA have demonstrated lymphocyte proliferation reduction (5,29). Accordingly, CLA-fed rats in the present study had lower splenocyte proliferative response and IL-2 production than rats fed the control diet. These results agree with those of Tricon et al. (30), who showed that peripheral blood mononuclear cells from subjects fed either

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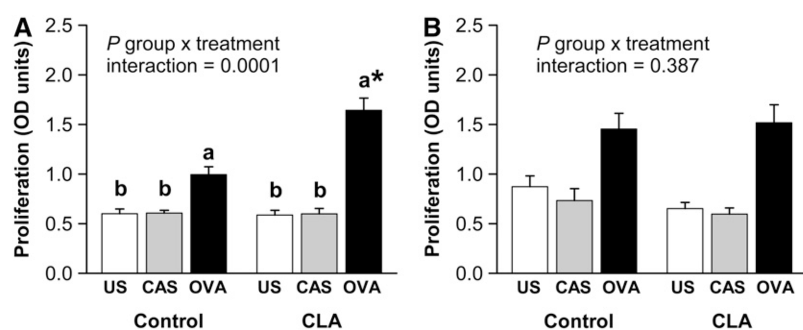

FIGURE 3 OVA-specific immune responses of spleen (*A*) and MLN (*B*) cells from rats fed a CLA or control diet. The proliferative responses were evaluated in US and CAS- and OVA-stimulated cells. Data are means ± SEM, $n = 20$. Labeled means within a diet group without a common letter differ, $P < 0.05$. *Different from corresponding control, $P < 0.05$.

*cis*9,*trans*11 or *trans*10,*cis*12 CLA isomers, after ConA stimulation, decreased CD69 expression, which strongly correlates with lymphocyte proliferation. However, there are other studies using diverse CLA isomer mixtures that described either increased splenocyte proliferation or no effect after stimulus addition (29,31–33).

Besides the polyclonal immunomodulator effects of CLA, the specific anti-OVA immune response also deserves major attention. Some features of the specific systemic and mucosal immune response in OVA-immunized rats have been evaluated. Systemic response was considered by means of specific spleen proliferative response as well as its ability to produce anti-OVA Ab and the serum concentration of anti-OVA Ab. A long-term CLA diet enhanced OVA-specific splenocyte proliferation. This result agrees with that reporting a higher specific proliferative response of T CD8+ lymphocytes from pigs fed a CLA diet (~50:50 isomers mix) (34,35). In addition, following hepatitis B vaccination, specific lymphocyte proliferation was higher in humans fed CLA 50:50 than in the control group (31). Conversely, Kelley et al. (36) showed no effect on influenza-specific proliferation in humans after feeding CLA, but in this case, the 2 main isomers used contributed only 40% of total CLA isomers, whereas in most of the studies affecting proliferative response, the main isomers made up ~80% of all CLA isomers.

OVA-primed spleen B cells produced specific anti-OVA Ab after later OVA contact. Nevertheless, rats fed a CLA diet did not generate a higher systemic (serum and spleen) humoral response against OVA. This might suggest that the presence of 1% CLA in the diet increased neither the number of primed memory B cells nor their ability to produce specific Ab. Our results agree with others carried out in humans and animals fed CLA (35–37), although Albers et al. (31) showed a higher concentration of anti-B hepatitis Ab in subjects consuming CLA 50:50 capsules. On the other hand, CLA feeding did not modify total serum Ig concentrations. This result agrees with many others (33,36,38) but disagrees with a human study that reported increased IgM and IgA plasma concentrations after consuming CLA (39). Nevertheless, better humoral enhancing effects were observed after feeding CLA to young rodents, which reportedly increased concentrations of spleen IgG, IgM, and IgA (40,41), although specific adaptive responses were not addressed in such studies.

Regarding mucosal sites, we found interesting CLA results in this particular immune compartment. CLA supplementation modified neither MLN cell OVA-specific proliferation nor its anti-OVA-Ab production in culture. Nevertheless, a long-term CLA diet increased anti-OVA IgA production at the intestinal level, whereas it did not modify the total IgA concentration in the same samples. The boost of specific intestinal IgA is of great importance, because this Ig is the main isotype present in all mucosa and confers high protection against foreign substances and microbe entry through the intestine, as well as by other mucosal compartments, due to specific secretory IgA homing among mucosal sites (42). Thus, to our knowledge, this is the first time that a CLA supplementation enhancement of Ag-specific mucosal responses has been reported.

Because the CLA diet increased only intestinal-specific IgA, but not spleen, serum, or MLN Ab, it is plausible to suggest that CLA may be enhancing B cells present in the lamina propia or even promoting the IgA-SC migration to the intestine from other immune compartments. This particular type of immunoenhancement induced by CLA, acting on a specific cell subset, is likely, because Bassaganya-Riera et al. (43) reported a higher percentage of a particular immune cell subset, but not of others, in swine fed CLA. Additional experimentation is required to elucidate the mechanism(s) through which CLA enhances specific IgA production in the intestine and whether the *cis*9,*trans*11 CLA isomer is the main isomer responsible for this action.

In conclusion, the present study demonstrates that long-term feeding an 80:20 *cis*9,*trans*11 and *trans*10,*cis*12 CLA mixture influences host immune responses to both polyclonal and specific stimuli. Overall, a long-term 1% CLA diet (from gestation to adulthood) downregulates polyclonal reaction of the immune system, as described for other PUFA, but enhances some aspects of specific cell-mediated immunity as well as the specific IgA intestinal production. These data highlight the evidence of the immunomodulatory effects of CLA, particularly of mixtures rich in the *cis*9,*trans*11 CLA isomer, and suggest that this fatty acid naturally present in dairy products may contribute specifically to mucosal immune defense and/or to counteract situations of immune imbalance.

Literature Cited

1. Meade CJ, Mertin J. Fatty acids and immunity. Adv Lipid Res. 1978;16:127–65.
2. Miles EA, Calder PC. Modulation of immune function by dietary fatty acids. Proc Nutr Soc. 1998;57:277–92.
3. Yaqoob P. Fatty acids and the immune system: from basic science to clinical applications. Proc Nutr Soc. 2004;63:89–104.
4. Yaqoob P, Calder PC. Fatty acids and immune function: new insights into mechanisms. Br J Nutr. 2007;98:S41–5.
5. Calder PC. Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. Mol Nutr Food Res. 2008;5:885–97.
6. Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis. 1987; 8:1881–7.
7. Ip C, Jiang C, Thompson HJ, Scimeca JA. Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis. Carcinogenesis. 1997; 18:755–9.
8. Kelley NS, Hubbard NE, Erickson KL. Conjugated linoleic acid isomers and cancer. J Nutr. 2007;137:2599–607.
9. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery. 1997;22: 266–77.
10. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, Belury MA. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun. 1998;244:678–82.
11. Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. Am J Clin Nutr. 2004;79: S1164–8.
12. Park Y, Pariza MW. Mechanisms of body fat modulation by conjugated linoleic acid (CLA). Food Res Int. 2007;40:311–23.
13. Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids. 1998;33:417–25.
14. Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care. 2002;25:1516–21.
15. Kelley DS, Erickson KL. Modulation of body composition and immune cell functions by conjugated linoleic acid in humans and animal models: benefits vs. risks. Lipids. 2003;38:377–86.
16. Pariza MW, Park Y, Cook ME. The biologically active isomers of conjugated linoleic acid. Prog Lipid Res. 2001;40:283–98.
17. O'Shea M, Bassaganya-Riera J, Mohede IC. Immunomodulatory properties of conjugated linoleic acid. Am J Clin Nutr. 2004;79: S1199–206.

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18. Terpstra AH. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. Am J Clin Nutr. 2004;79:352–61.
19. Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids. 1998;33:521–7.
20. Brandtzaeg P, Johansen FE. Mucosal B cells: phenotypic characteristics, transcriptional regulation, and homing properties. Immunol Rev. 2005;206:32–63.
21. Bassaganya-Riera J, Reynolds K, Martino-Catt S, Cui Y, Hennighausen L, Gonzalez F, Rohrer J, Benninghoff AU, Hontecillas R. Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology. 2004;127:777–91.
22. Frazier AL, Ryan CT, Rockett H, Willett WC, Colditz GA. Adolescent diet and risk of breast cancer. Breast Cancer Res. 2003;5:R59–64.
23. Luna P, Juarez M, Angel de la Fuente M. Fatty acid and conjugated linoleic acid isomer profiles in human milk fat. Eur J Lipid Sci Technol. 2007;109:1160–6.
24. Reeves PG, Nielsen FH, Fahey GC Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr. 1993;123:1939–51.
25. Pérez-Cano FJ, Castellote C, Marín-Gallén S, González-Castro A, Franch À, Castell M. Phenotypic and functional characteristics of rat spleen lymphocytes during suckling. Dev Comp Immunol. 2007;31: 1264–77.
26. Pérez-Berezo T, Ramiro-Puig E, Pérez-Cano FJ, Castellote C, Permanyer J, Franch À, Castell M. Influence of a cocoa-enriched diet on specific immune response in ovalbumin-sensitized rats. Mol Nutr Food Res. 2009;53: In press DOI 10.1002/mnfr.200700396.
27. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC, Novelli Filho JL. Anthropometrical parameters and markers of obesity in rats. Lab Anim. 2007;41:111–9.
28. Turpeinen AM, von Willebrand E, Salminen I, Linden J, Basu S, Rai D. Effects of cis-9,trans-11 CLA in rats at intake levels reported for breast-fed infants. Lipids. 2006;41:669–77.
29. Calder PC, Yaqoob P, Thies F, Wallace FA, Miles EA. Fatty acids and lymphocyte functions. Br J Nutr. 2002;87:S31–48.
30. Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Grimble RF, Williams CM, Calder PC, Yaqoob P. Effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on immune cell function in healthy humans. Am J Clin Nutr. 2004;80:1626–33.
31. Albers R, van der Wielen RP, Brink EJ, Hendriks HF, Dorovska-Taran VN, Mohede IC. Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid (CLA) isomers on immune function in healthy men. Eur J Clin Nutr. 2003;57:595–603.
32. Hayek MG, Han SN, Wu D, Watkins BA, Meydani M, Dorsey JL, Smith DE, Meydani SN. Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrlBR mice. J Nutr. 1999;129:32–8.
33. Kelley DS, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL. Dietary conjugated linoleic acid did not alter immune status in young healthy women. Lipids. 2000;35:1065–71.
34. Bassaganya-Riera J, Hontecillas R, Zimmerman DR, Wannemuehler MJ. Long-term influence of lipid nutrition on the induction of CD8(+) responses to viral or bacterial antigens. Vaccine. 2002;20:1435–44.
35. Bassaganya-Riera J, Pogranichniy RM, Jobgen SC, Halbur PG, Yoon KJ, O'Shea M, Mohede I, Hontecillas R. Conjugated linoleic acid ameliorates viral infectivity in a pig model of virally induced immunosuppression. J Nutr. 2003;133:3204–14.
36. Kelley DS, Simon VA, Taylor PC, Rudolph IL, Benito P, Nelson GJ, Mackey BE, Erickson KL. Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells, but did not alter their function. Lipids. 2001;36:669–74.
37. Jaudszus A, Krokowski M, Mockel P, Darcan Y, Avagyan A, Matricardi P, Jahreis G, Hamelmann E. Cis-9,trans-11-conjugated linoleic acid inhibits allergic sensitization and airway inflammation via a PPARγ-related mechanism in mice. J Nutr. 2008;138:1336–42.
38. Kelley DS, Warren JM, Simon VA, Bartolini G, Mackey BE, Erickson KL. Similar effects of c9,t11-CLA and t10,c12-CLA on immune cell functions in mice. Lipids. 2002;37:725–8.
39. Song HJ, Grant I, Rotondo D, Mohede I, Sattar N, Heys SD, Wahle KW. Effect of CLA supplementation on immune function in young healthy volunteers. Eur J Clin Nutr. 2005;59:508–17.
40. Yamasaki M, Kishihara K, Mansho K, Ogino Y, Kasai M, Sugano M, Tachibana H, Yamada K. Dietary conjugated linoleic acid increases immunoglobulin productivity of Sprague-Dawley rat spleen lymphocytes. Biosci Biotechnol Biochem. 2000;64:2159–64.
41. Yamasaki M, Chujo H, Hirao A, Koyanagi N, Okamoto T, Tojo N, Oishi A, Iwata T, Yamauchi-Sato Y, et al. Immunoglobulin and cytokine production from spleen lymphocytes is modulated in C57BL/6J mice by dietary cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid. J Nutr. 2003;133:784–8.
42. Fagarasan S. Evolution, development, mechanism and function of IgA in the gut. Curr Opin Immunol. 2008;20:170–7.
43. Bassaganya-Riera J, Hontecillas-Magarzo R, Bregendahl K, Wannemuehler MJ, Zimmerman DR. Effects of dietary conjugated linoleic acid in nursery pigs of dirty and clean environments on growth, empty body composition, and immune competence. J Anim Sci. 2001;79:714–21.

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