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Effects of Combination Dietary Conjugated Linoleic Acid with Vitamin A (Retinol) and Selenium on the Response of the Immunoglobulin Production in Mice

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Abstract

The dietary effect of conjugated linoleic acid (CLA) on the response of the immunoglobulin (serum and tissue) production in Balb/C mice was examined at three doses: 0 %(control), 0.5% and 1.5%. The combination effects of CLA with vitamin ADE or selenium also were investigated.

CLA at 0.5% increased serum immunoglobulin A, G, mesenteric lymp node (MHN) and gut luminal IgA (secretory IgA) levels. However, 1.5% CLA decreased SIgG slightly. CLA both alone and combined with vitamin ADE and selenium did not affect serum IgE. The levels of immunoglobulin concentration in the 0.5% CLA group were higher than those in the1.5% CLA group. The level of serum IgG in 1.5% CLA combined with selenium was maintained at the same level as that of control. It is considered that overdoses of CLA (1.5%) even depressed the production of immunoglobulin but selenium and/or vitamin inhibited this activity to a certain extent.

In this study, dietary CLA increased immunoglobulin production in a dose-dependent manner. Vitamin ADE and Selenium combined with CLA also increased the immunoglobulin production response except serum IgE.

Key Words: immunoglobulin, conjugated linoleic acid, vitamin ADE, selenium

Introduction

Conjugated linoleic acid (CLA) is a derivative of a fatty acid linoleic acid, which is found in various ruminant-derived foodstuffs such as milk, cheese, and yogurt [7, 24]. It has been reported that CLA decreases carcinogenesis [14, 18, 19, 20, 21, 38], diabetes [16], and atherosclerosis [7, 41]. CLA also regulates immune parameters, for example, it modulates interleukin (IL)-2 productions by lymphocytes and phagocytotic activity of macrophages *in vitro* and *in vivo* [6, 28, 40]. CLA inhibits eicosanoid production and also modulates immunoglobulin in rats [25]. Since the immune system is central to defense against cancer, it is possible that the anticancer activity of CLA may be mediated through enhanced immune function [15]. According to the Cook and Pariza [8], CLA was found to be protective against the growth suppression associated with immune-stimulation.

Won *et al*. [40] studied the effect of CLA on the lymphocyte function and growth of a transplantable murine mammary tumor. In this study, they reported that dietary CLA modulated certain aspects of the immune defense but had no obvious effect on the growth of an established, and aggressive mammary tumor.

The oxidative status of biological tissue can be influenced by dietary components. The nutritive antioxidants of α-tocopherol (vitamin E), β-carotene (provitamin A), ascorbic acid (vitamin C) and selenium (cofactor for the antioxidant enzyme, glutathione peroxidase) can inhibit or delay the onset of atherosclerosis and cancer [2, 10, 22, 26, 31, 33]. Vitamin A (usually used in the form of ADE complex) also has immuno-stimulating activity [32] and inhibits secretion type 1 cytokines *in vitro* [12].

The earliest evidence that selenium is involved in immune function came in 1959 [34]. Many studies have suggested that adequate intake of selenium is required to prevent malignancy. Various components of the immune system fail to function correctly if dietary selenium is deficient [35]. However, very few studies were performed about the effects of CLA combined with other antioxidants or supplements in order to increase immunoglobulin productivity. Most of the CLA studies were about carcinogenesis [2, 18], reduction of body fat [39], and production of meat or egg that contain CLA [25, 36].

In this study, we examined the effects of two different doses of dietary CLA (0.5%, 1.5%) and its synergistic effects in combination with vitamin ADE and selenium on immunoglobulin production.

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Materials and Methods

Animals and treatment

Fifty-six male Balb/c mice, 6-week old, were obtained from Daehan Experimental Animal Center (Seoul, Korea). Mice were housed individually in polycarbonate cages in a room with controlled temperature and light level (23±2℃ and a 12-h light/12-h dark cycle). They were acclimatized immediately to a powdered commercial mouse diet (Lab-Rodent Diet, Purina, Korea) for 1 week before the initiation of the experimental studies and randomly placed into 7 different groups. Group 1, based diet with no CLA (control;CONT); Group 2, 0.5% CLA (based on body weight; CLA1); Group 3, 1.5% CLA (CLA2); Group 4, 0.5% CLA and vitamin ADE (vitamin A, 300 IU/kg/day; D, 100 IU/kg/day; E, 5mg /kg/day; CVA1); Group 5, 1.5% CLA and vitamin ADE (CVA2); Group 6, 0.5% CLA and selenium (1 ㎍/kg/day; CS1); Group 7, 1.5% CLA and selenium (CS2). Water and food were available ad libitum for the duration of the study.

Preparation of CLA

CLA was made by the method of Ip *et al*. [18] and extracted from corn oil. With a radiochemical purity of 76.1445%, the CLA (c9, t11 isomer) was composed of c9, t11, 27%; c12, t10, 26%; c18, t0, 5%; c18, t1, 27%; c18, t2, 0%; c16, t0, 13%. CLA at dose of 0.5% and 1.5% was given at 133.5 mg/kg/day and 400.5 mg/kg/day, respectively. For complete mixing of the ingredients, CLA was sprayed on the powder diet under nitrogen gas in a closed space before feeding.

Preparation of vitamin ADE and selenium

Vitamin A (as retinol) and selenium were obtained from Sigma (U.S.A.). These were sprayed on the powder diet just before feeding. Vitamin A at 300 IU/kg/day was given as ADE complex (D, 100 IU/kg/day; E, 5 mg/kg/day) and selenium at 1 ㎍/kg/day [23].

Vitamin E and D were used as antioxidants and supplements to vitamin A.

Preparation of serum

After 3 weeks of feeding, blood was withdrawn from the abdominal vena cava under light diethyl ether anesthesia. To estimate the levels of IgA, IgG, IgM and IgE, blood was incubated for 1 hour at 37℃ in the microfuge tube and then centrifuged at 3,000 rpm for 15 min at 4℃. The sample was allocated to the microfuge tube and analyzed with the sandwich ELISA method.

Preparation of mesenteric lymph node (MLN)

The removed MNL was torn in RPMI medium 1640(with L-glutamine without sodium bicarbonate, GIBCO-BRL, USA penicillin 100 U/ml, streptomycin 100 ㎍/ml), rinsed 3 times and then filtrated to eliminate tissue scum with 100 ㎛ mesh. The cell suspension was incubated at 37℃ for 30 minutes to eliminate fibroblast. Ten milliliter of cell suspension was suspended in 10 ml of histopaque-1077 (polysucrose, 5.7 g/dL, and sodium diatrizoate, 9.0 g/dL. aseptically filtered) and then centrifuged at 1,500g for 30 min.

The lymphocyte bands were carefully obtained from the tubes. The cells were washed again, the density was calculated as 1.5x106 cells/ml, and then cells were cultured in 96 well plates containing 10% fetal bovine serum.

Twelve hours later, 1.0 ㎍/ml of lipopolysaccharide was added. After reaction for 48 hours, the samples were allocated into 50㎕ at -80℃ until IgA analysis.

Preparation of the gut lumen lavage

After blood collection, the end of the duodenum and the cranial part of the cecum were tied and both ends were cut. One end of the small intestine was hung at the stand and a conical tube was placed at the other end. Then the lavage was collected by flushing with 2 ml cold PBS (4℃, containing soybean trypsin inhibitor 0.1mg/ml) from upper part to down part. And then it was centrifuged at 2,000g for 30 min and the suspension/supernatant was transferred to the microfuge tube. Measurement of secretory IgA concentration was done by the sandwich ELISA [8,32].

Statistical analysis

Data were analysed by one-way analysis of variance followed by Duncan's multiple-range test to identify significant differences (General Linear Model Procedure; SAS ver. 6.04, U.S.A.).

Results

The experiment was performed to examine whether or not CLA in combination with vitamin A (retinol) or selenium enforce the immunoglobulin productive activity.

Fifty-six male Balb/c mice were divided into 7 groups (CONT, CLA1, CLA2, CVA1, CVA2, CS1 and CS2) and were fed three different doses of dietary CLA (0, 0.5 and 1.5%), vitamin ADE and selenium for 3 weeks. After that, the mice were sacrificed and blood and tissues were obtained.

1. Serum immunoglobulin

Serum IgA

As shown in Fig. 1, the secretory IgA concentrations of CLA1, CLA2, CVA1, CVA2, CS1 and CS2 were 61.21±5.24, 60.25±3.55, 65.31±4.14, 63.72±2.34, 64.49±3.43, and 63.48±4.2 (㎍/ml), respectively. All experimental groups experienced a significant increase compared to the control group (48.88±5.67) ($p<0.05$). Although there was no significant difference, the 0.5% CLA treated groups showed a slightly higher increase than the 1.5% CLA groups.

Serum IgG

The serum IgG concentrations of CONT, CLA1, CLA2,

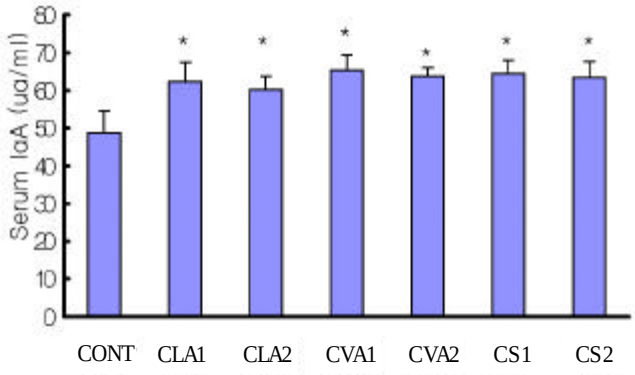

Fig. 1. Serum immunoglobulin A concentration.
CONT: groups fed control diet with no CLA. CLA1: groups fed diet supplemented with 0.5% CLA. CLA2: groups fed diet supplemented with 1.5% CLA. CVA1: groups fed diet supplemented with 0.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. CVA2: groups fed diet supplemented with 1.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. SC1: groups fed diet supplemented with 0.5% CLA and selenium: 1 μg/kg/day. SC2: groups fed diet supplemented with 1.5% CLA and selenium: 1 μg/kg/day. All values are expressed as mean± SD (n=8). * Significant difference from control group ($p<0.05$).

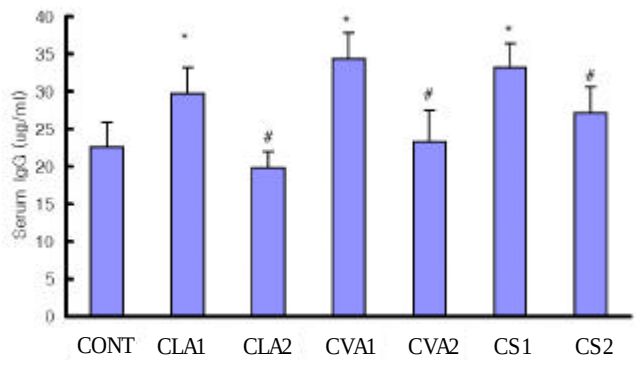

Fig. 2. Serum immunoglobulin G concentration.
CONT: groups fed control diet with no CLA. CLA1: groups fed diet supplemented with 0.5% CLA. CLA2: groups fed diet supplemented with 1.5% CLA. CVA1: groups fed diet supplemented with 0.5% CLA and vitamins A: 300 IU/kg/day. D: 100 IU/kg/day, E: 5 mg/kg/day. CVA2: groups fed diet supplemented with 1.5% CLA and vitamins A: 300 IU/kg/day. D: 100 IU/kg/day, E: 5 mg/kg/day. SC1: groups fed diet supplemented with 0.5% CLA and selenium: 1 μg/kg/day. SC2: groups fed diet supplemented with 1.5% CLA and selenium: 1 μg/kg/day. All values are expressed as mean± SD (n=8). * Significant difference from control group ($p<0.05$). # Significant difference between 0.5% and 1.5% CLA group, respectively ($p<0.05$).

CVA1, CVA2, CS1 and CS2 were 22.61 ±3.3, 29.76±3.5, 19.86±2.2, 34.36±3.45, 24.34±4.2, 33.23±3.15 and 27.23± 3.41 μg/ml, respectively (Fig. 2). CLA1, CVA1 and CS1 had a significant increase compare to control ($p<0.05$). However, CLA2 and CVA2 had a significant decrease compared to control. CS2 was slightly increased compared to other 1.5% CLA groups, but there was no significant difference among them.

Serum IgE

As shown in Fig. 3, the serum IgE concentrations of CONT, CLA1, CLA2, CVA1, CVA2, CS1 and CS2 were 1.83 ±0.16, 1.77±0.24, 1.85±0.31, 2.02±1.72, 1.90±1.22, 1.94 ±1.26, and 1.82±1.35 μg/ml, respectively. There was no significant difference among the groups.

2. MNL IgA

The MNL IgA concentrations of CONT, CLA1, CLA2, CVA1, CVA2, CS1 and CS2 were 0.25±0.03, 0.55±0.1, 0.48 ±0.3, 0.58±2.14, 0.53±1.4, 0.57±1.2, 0.56±0.34 μg/ml, respectively (Fig. 4). All of the treated groups had a significant increase compared to the control group ($p<0.05$). All the selenium and vitamin ADE treated groups had higher IgA concentrations than CLA-only treated groups.

3.Secretory IgA

The secretory IgA concentrations of CONT, CLA1, CLA2, CVA1, CVA2, CS1 and CS2 were 230.95±63.06, 300.2± 26.73, 292.3±19.56, 339.3±14.6, 311.6±62.17, 329.3±17.7, and 311±38.3 μg/ml, respectively (Fig. 5). In all of the treated groups, secretory IgA concentrations were increased compared to the control group ($p< 0.05$). The 0.5% CLA treated groups showed a tendency to have a slight increased over the 1.5% CLA groups.

Discussion

CLA has been found to be an effective antioxidant. An interesting property of CLA is its ability to suppress peroxide formation from unsaturated fatty acid in a test-tube model. CLA increased immunoglobulin production of spleen lymphocytes at doses under 0.5% [39].

It is considered that an increase of immunoglobulin production by dietary CLA may be achieved via regulation of IL-2 and PGE2 production [13, 25]. A large number of reports have appeared showing the inhibitory or stimulatory effects of retinoid (vitamin A) on various immune responses including the activity of lymphocytes [4, 5, 9]. Selenium deficiency caused by stress have reproduced neutrophil which has candidacidal and myeloperoxidase activities [1, 4, 5, 9].

It has been reported that a diet supplemented with 0.5% and 1.0% CLA enormously increases IgG and IgM production of MNL lymphocytes under lipopolysaccharide stimulation [41].

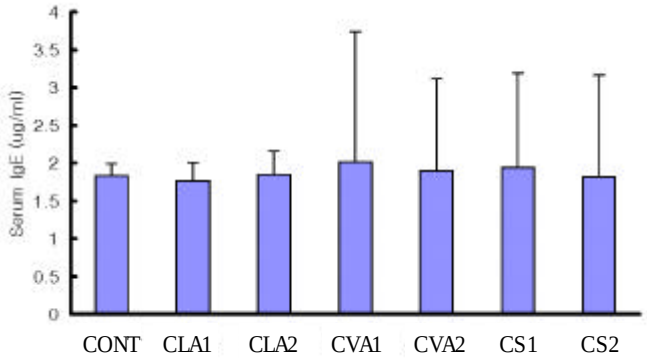

Fig. 3. Serum immunoglobulin E concentration.
CONT: groups fed control diet with no CLA. CLA1: groups fed diet supplemented with 0.5% CLA. CLA2: groups fed diet supplemented with 1.5% CLA. CVA1: groups fed diet supplemented with 0.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. CVA2: groups fed diet supplemented with 1.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. SC1: groups fed diet supplemented with 0.5% CLA and selenium: 1 μg/kg/day. SC2: groups fed diet supplemented with 1.5% CLA and selenium: 1 μg/kg/day. All values expressed as mean±SD (n=8).

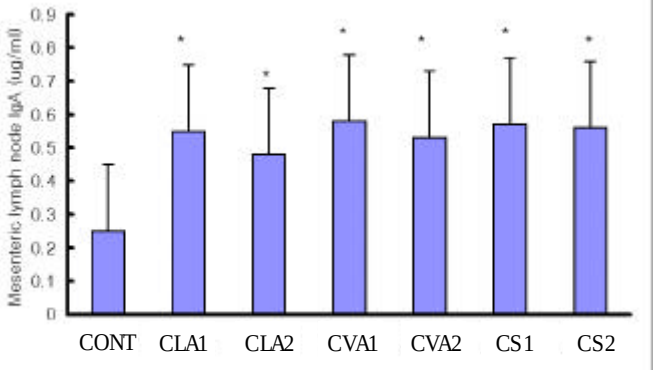

Fig. 4. Mesenteric lymph node IgA concentration.
CONT: groups fed control diet with no CLA. CLA1: groups fed diet supplemented with 0.5% CLA. CLA2: groups fed diet supplemented with 1.5% CLA. CVA1: groups fed diet supplemented with 0.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. CVA2: groups fed diet supplemented with 1.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. SC1: groups fed diet supplemented with 0.5% CLA and selenium: 1 μg/kg/day. SC2: groups fed diet supplemented with 1.5% CLA and selenium: 1 μg/kg/day. All values are expressed as mean± SD (n=8). * significant difference from control group ($p<0.05$).

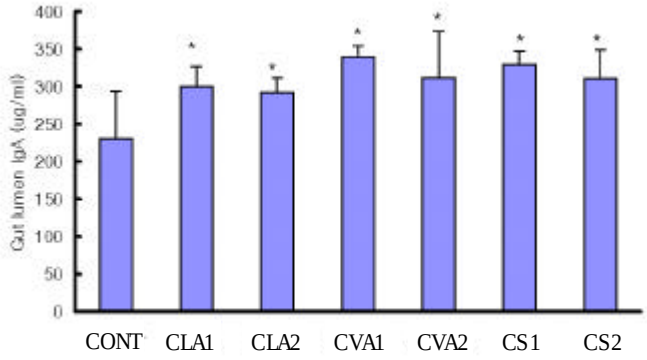

Fig. 5. Gut lumen IgA concentration.
CONT: groups fed control diet with no CLA. CLA1: groups fed diet supplemented with 0.5% CLA. CLA2: groups fed diet supplemented with 1.5% CLA. CVA1: groups fed diet supplemented with 0.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. CVA2: groups fed diet supplemented with 1.5% CLA and vitamins A: 300 IU/kg/day, D: 100 IU/kg/day, E: 5 mg/kg/day. SC1: groups fed diet supplemented with 0.5% CLA and selenium: 1 μg/kg/day. SC2: groups fed diet supplemented with 1.5% CLA and selenium: 1 μg/kg/day. All values are expressed as mean± SD (n=8). * Significant difference from control group ($p<0.05$).

Immunoglobulin A is produced locally by plasma cells in submucosal lymphoid tissues and regional lymph nodes. The functions of serum immunoglobulin A are well established: the ability to neutralize toxins, adhere to bacteria and viruses and interact with parasites and mucosal surface. The major effect of serum immunoglobulin A is to prevent the attachment of bacteria and viruses to the mucosal surface [11]. The major biologic function of serum immunoglobulin G *in vivo* is to promote the removal of microorganisms and neutralize toxins [11]. Serum immunoglobulin E is an immunoglobulin of major importance in mechanisms against parasites and in the immunopathogenesis of allergic disease.

In this experiment the levels of immunoglobulin A and secretory immunoglobulin (MNL IgA, gut lumen IgA) of the dietary CLA (0.5%, 1.5%) groups were higher than those of the control group that was fed a diet without CLA. Additionally, the two combination dietary groups (CLA and vitamin ADE, CLA and selenium) had higher levels of Ig and secretory IgA than those of the CLA-only group. However, the level of serum IgE was no significantly different between the control and experimental groups. CLA increased the production of IgA and IgG while reducing that of IgE in lymphocytes, in particular in MLN lymphocytes irrespective of the presence or absence of lipopolysaccharide, a cell activator [30].

An interesting observation is that CLA regulates the immunoglobulin production class specifically. Food allergy reaction is initiated by the production of allergen-specific IgE [27, 29, 37]. It is considered that treatment with a combination of CLA with vitamin ADE or selenium is more effective than CLA only. Ip *et al.* [17] reported that the protective effects of CLA were dose-dependent at a level of 1% CLA. Chronic feeding of up to 1.5% CLA produced no adverse consequences in the animals.

In this experiment, all the 1.5% CLA feeding groups had lower immunoglobulin concentrations than those of all the 0.5% CLA groups. Even serum IgG concentration in 1.5% CLA group was decreased compare to that of control.

Over-dosage of CLA may affect the depression of immune production. However, the addition of vitamin ADE and/or selenium to CLA increased the level of immunoglobulin production, even while reducing the inhibitory effect of excessively dosed CLA (1.5%) feeding groups.

The mechanism of vitamin A (retinol) in altering immune function has not been established. However it has been suggested that the immune-stimulating effects of vitamin A are mediated through its metabolites, which may play a role in lymphocytes proliferation, signaling and activation [29]. It is considered that treatment with a combination of CLA and vitamin A or selenium has a synergistic effect on the immunoglobulin production.

In conclusion, the optimal dose of CLA to simulate immunoglobulin productivity was 0.5% CLA in this experiment. In addition, CLA in combination of vitamin complex of ADE or selenium could be effective supplements for the elevation of immunoglobulin production in serum and tissues.

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