Conjugated linoleic acids alleviate the immunosuppression of peripheral blood T lymphocytes in broiler chickens exposed to cyclosporin A

F. Y. Long,*† X. Yang,* Y. M. Guo,*[1] Z. Wang,* J. M. Yuan,* B. K. Zhang,* and D. Liu*

**The State Key Lab of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, 100193, PR China; and †National Engineering Research Center for Fruits and Vegetables Processing, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, 100083, PR China*

ABSTRACT The immunoregulatory actions of conjugated linoleic acids (CLA) of relevance immunosuppression were investigated. To test the hypothesis that CLA ameliorate immunosuppression, we developed the immunosuppressive model of peripheral blood T lymphocytes in broiler chickens induced by cyclosporin A. Peripheral blood T lymphocytes of broiler chickens were cultured with media containing various concentrations (25, 50, 100, and 200 μmol/L) of c9, t11-CLA and t10, c12-CLA to investigate the effects of CLA isomers on peripheral blood T lymphocyte proliferation, interleukin-2, the activity of phospholipase C, and protein kinase C production. Results suggested that CLA alleviated the immunosuppression of T lymphocytes in broiler chickens exposed to cyclosporin A through increasing of peripheral blood T lymphocyte proliferation and interleukin-2. The 2 CLA isomers enhanced T lymphocyte proliferation at low concentration and inhibited T lymphocyte proliferation at high concentration. In addition, the effect of c9, t11-CLA was better than that of t10, c12-CLA. At the cellular level, the effects of CLA on the alleviation of immunosuppression in T lymphocytes are mainly attributable to increasing the signaling molecules, such as phospholipase C and protein kinase C.

Key words: conjugated linoleic acid, immunosuppression, signaling molecule, chicken

2012 Poultry Science 91:2431–2437
http://dx.doi.org/10.3382/ps.2011-02022

INTRODUCTION

Immunosuppressive viral diseases threaten the poultry industry by increasing susceptibility to secondary infections and suboptimal response to vaccinations (Berg, 2000; Islam et al., 2001). Immunosuppressed flocks result in secondary infections, suboptimal response to vaccinations, and growth retardation, causing a great deal of economic loss to the poultry industry. Therefore, nutrition strategies may be desirable to alleviate immunosuppression in broiler chickens.

A possible application of immunomodulators, such as conjugated linoleic acids (**CLA**), to attenuate immunosuppression in animals has been reported (O'Shea et al., 2004). Conjugated linoleic acids refers to a class of positional and geometric conjugated dienoic isomers of linoleic acid, of which c9, t11-CLA and t10, c12-CLA are the main isomers (Evans et al., 2000; Shin et al., 2011). Our initial research led to the discovery that CLA, predominantly the c9, t11-CLA isomer, supplementation enhanced immune function under the cyclosporin A-induced (Long et al., 2010) and infectious bursal disease virus-induced immunosuppression in chickens (Long et al., 2011). Bassaganya-Riera et al. (2003) reported CLA ameliorated viral infectivity in a pig model of type-2 porcine circovirus-induced immunosuppression.

Lymphoid depletion is a hallmark immunological lesion associated with immunosuppressive viral infections, such as Marek's disease virus, infectious bursal disease virus, chicken infectious anemia virus, avian leucosis virus, and so on (Sharma et al., 2000; Islam et al., 2002). Studies on the effects of CLA on immune function have so far been conducted in the immunosuppressive status in animals. However, few studies were conducted to investigate the effects of CLA on the immune function in the T lymphocytes. Cyclosporin A (**CsA**), an immunosuppressive drug, prevents the synthesis of T-cell cytokines by blocking a late-stage signaling pathway initiated by the T-cell receptor, which affects the production of IL-2; hence, T-cell proliferation is affected (Hill et al., 1989; Schreiber and Crabtree, 1992; Sigal and Dumont, 1992).

Therefore, to investigate the alleviation immunosuppression role of CLA in T lymphocytes of chickens, we

Received November 15, 2011.
Accepted February 29, 2012.
[1]Corresponding author: guoym9899@yahoo.com.cn

created a model of CsA in T lymphocytes and determined the effects of dietary CLA on the lymphocyte proliferation, IL-2, the activity of phospholipase C (**PLC**), and protein kinase C (**PKC**) production.

MATERIALS AND METHODS

Dietary Treatments and Bird Management

The animal management protocol for this research was approved by the China Agricultural University Animal Care and Use Committee. Eighty 1-d-old male Arbor Acre broiler chickens were randomly allocated into 1 group with 8 replicates, 10 chicks per replicate (cage). A corn-soybean meal diet was used. Birds were fed for 3 wk. Chicks had free access to feed and water and were housed in wire cages and maintained on a 24-h constant-light program. Temperature in the chicken house was set at 35°C for the first 3 d and was reduced by 3°C each consecutive week until it reached 24°C. Compositions of the diets and nutrient levels for starters (d 1–21) are presented in Table 1. The diets were formulated to meet or exceed the NRC requirements.

Sample Collection

On d 21, one bird was randomly selected from each replicate, and heparinized blood samples were collected from the wing vein by venipuncture to measure the proliferation, IL-2 production, the activity of PLC, and PKC production of peripheral blood T lymphocytes immediately.

Preparation of Fatty Acids

Both isomers of CLA and linoleic acid (LA; ≥98% pure; Matreya Inc., Pleasant Gap, PA) were complexed to fatty acids-free at a 3:1 molar ratio using 1 mmol/L of BSA stocks as previously described (Ringseirs et al., 2008).

Table 1. Ingredients and compositions of dietary treatments on as-fed basis (%)

Ingredient	%	Nutrition level	%
Maize	55.32	ME (Mcal/kg)	2.95
Soybean meal	37.26	CP	21.00
Soybean oil	3.33	Calcium	1.00
Dicalcium phosphate	1.98	Available phosphorus	0.45
Limestone	1.18	Methionine	0.50
Sodium chloride	0.35	Lysine	1.15
Mineral premix[1]	0.20		
Choline chloride	0.16		
Methionine	0.18		
Lysine-HCl	0.051		
Vitamin premix[2]	0.03		
Antioxidants	0.02		

[1]Provided per kilogram of diet: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0.15 mg; I, 0.35 mg.

[2]Provided per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3, 2,500 IU; vitamin K_3, 2.65 mg; thiamine, 2 mg; riboflavin, 6 mg; vitamin B_{12}, 0.025 mg; vitamin E, 30 IU; biotin, 0.0325 mg; folic acid, 1.25 mg; pantothenic acid, 12 mg; niacin, 50 mg.

T Lymphocyte Isolation, Culture, and Treatment

Heparinized blood samples were collected from the wing vein. Lymphocytes were isolated from peripheral blood using lymphocyte density-gradient centrifugation medium (density = 1.077; HaoYang Biological Manufacture Co. Ltd., Tianjin, China). Lymphocytes were suspended in RPMI 1640 medium (Gibco, Grand Island, NY) containing 5% fetal calf serum and 25 m*M* HEPES (pH 7.0), and then applied to a nylon fiber column (Dainippon Pharmaceutical, Osaka, Japan) for purifying T lymphocytes according to the manufacturer's instructions. After that, T lymphocytes were washed 3 times in RPMI 1640 medium before being resuspended in RPMI medium supplemented with 5% fetal calf serum. Cell concentration was adjusted to 1 × 10^7 cells/mL of culture medium. One hundred microliters of cell suspension in a 96-well microtiter plate (Costar 3599, Corning Inc., Corning, NY) was incubated with lymphocyte mitogen concanavalin A (**Con A**; 10 μg/mL) in combination with CsA (20 ng/mL; Sigma Chemical Co., St. Louis, MO) for 2 h at 37°C with 5% CO_2 in an incubator. After incubation with CsA, cells were incubated with CLA isomers or LA for another 70 h. Experiments were carried out in triplicate and repeated at least twice.

The lymphocyte proliferation assay was performed as described previously (Lambrecht et al., 2004; Zhang et al., 2005; Rauw et al., 2007).

IL-2 Production of Peripheral Blood T Lymphocyte

A 950-μL cell suspension was placed into a 24-well plate and incubated with lymphocyte mitogen Con A (10 μg/mL) in combination with CsA (20 ng/mL) for 2 h at 37°C with 5% CO_2 in an incubator. After incubation with CsA, cells were incubated with CLA isomers or LA for another 46 h. Experiments were carried out in triplicate and repeated at least twice. Then, the supernatant was harvested and stored at −30°C until analyzed. Interleukin-2 production in lymphocyte culture supernatants was determined by IL-2 RIA kit (RapidBio Inc. 23830, Calabaras, CA), according to the manufacturer's instructions.

The Activity of PLC

The cells were collected and suspended in 1 mL of homogenate solution (pH 7.2) and homogenized in ice. After centrifugation at 750~800 × *g* at 4°C for 10 min, the supernatant was centrifuged again at 10,000 × *g* at 4°C for 1 h. The pellets were resuspended and treated by sonification several times for 2 min at 4°C and used as membranous PLC enzymes. Enzyme activity was ex-

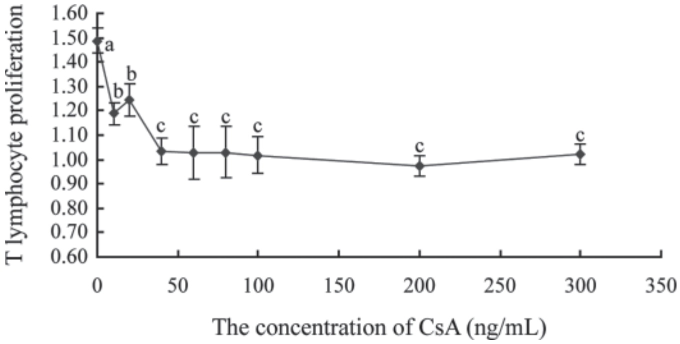

Figure 1. The cyclosporine A (CsA) at different concentration gradients induced the immunosuppression of T lymphocytes in broiler chickens. On d 21, one bird was randomly selected from each replicate, and heparinized blood samples were collected from the wing vein by venipuncture to measure the proliferation in peripheral blood T lymphocyte immediately. The results are presented as means of T lymphocyte proliferation in response to concanavalin A mitogen at different concentration gradients of CsA. [a–c]Means with different letters indicate a significant difference ($P < 0.05$).

pressed as nanomoles per minute per milligram of protein (nmol/min per mg). The PLC assay was performed as described previously (Wu et al., 1997).

PKC Assay

The cells were placed into a 24-well plate and incubated for 48 h with 10 μg/mL of Con A at 37°C with 5% CO_2 in an incubator. The supernatant was harvested and stored at −30°C until analyzed. The PKC production in T-lymphocyte culture supernatants was determined by a PKC kit (Promega Co., Madison, WI), according to the manufacturer's instructions.

Statistical Analysis

Data were reported as means and standard deviations and analyzed by one-way ANOVA of SPSS 10.0 (SPSS Inc., Chicago, IL). The significance of differences among different groups was evaluated by a least significant difference post hoc multiple comparisons test. A level of $P < 0.05$ was set as the criterion for statistical significance.

RESULTS

CsA-Induced Immunosuppressive Model in Peripheral Blood T Lymphocytes

The CsA-induced immunosuppressive effect of T lymphocyte proliferation in response to Con A mitogen is given in Figure 1. With the concentration gradient increase of CsA (0, 10, 20, 40, 60, 80, 100, 200, 300 ng/mL), T lymphocyte proliferation was significantly decreased ($P < 0.05$). Thus, in the following study, CsA at the concentration of 20 ng/mL was used to set up the model of immunosuppression.

Peripheral Blood T Lymphocyte Proliferation

The results showed that CsA significantly inhibited ($P < 0.05$) the peripheral blood T lymphocyte proliferation, and c9, t11-CLA and t10, c12-CLA alleviated the CsA-induced immunosuppression through increasing peripheral blood T lymphocyte proliferation in response to Con A mitogen (Figure 2). To some extent, under the CsA-induced immunosuppressive status, the 2 CLA isomers enhanced T lymphocyte proliferation at low concentration and inhibited lymphocyte proliferation at high concentration. In addition, the effect of c9, t11-CLA was better than that of t10, c12-CLA. The suitable concentrations of c9, t11-CLA were 25 and 50 μmol/L. Therefore, in the following studies, we only analyzed the effects of 25 μmol/L of c9, t11-CLA and t10, c12-CLA on the determinations of IL-2, PLC, and PKC in peripheral blood T lymphocyte.

IL-2 Production of Peripheral Blood T Lymphocyte

Compared with the control, CsA tended to inhibit IL-2 production of peripheral blood T lymphocyte ($P = 0.113$). Both CLA isomers at the concentration of 25 μmol/L tended to increase ($P = 0.081$) IL-2 production of T lymphocyte-induced immunosuppression by CsA (Figure 3). Thus, the results could indicate that c9, t11-CLA and t10, c12-CLA alleviated the CsA-induced immunosuppression.

The Activity of PLC in Peripheral Blood T Lymphocyte

Compared with the control, CsA had no effect on the activity of PLC of peripheral blood T lymphocyte (Fig-

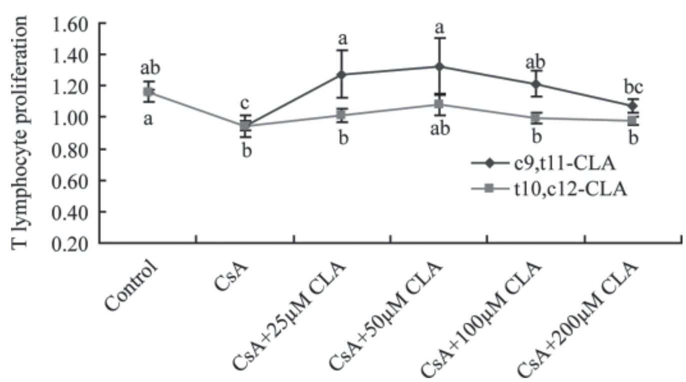

Figure 2. Effects of c9, t11-conjugated linoleic acid (CLA) and t10, c12-CLA on the peripheral blood T lymphocyte proliferation in response to concanavalin A mitogen under the cyclosporine A (CsA)-induced immunosuppression. On d 21, peripheral blood T lymphocyte of chickens were cultured with media containing various concentrations (25, 50, 100, and 200 μmol/L) of c9, t11-CLA and t10, c12-CLA, with linoleic acid as control, to investigate the effect of CLA isomers on the peripheral blood T lymphocyte proliferation. The results are presented as means of T lymphocyte proliferation in response to concanavalin A mitogen at different concentration gradients of CLA isomers. [a–c]Means with different letters indicate a significant difference ($P < 0.05$).

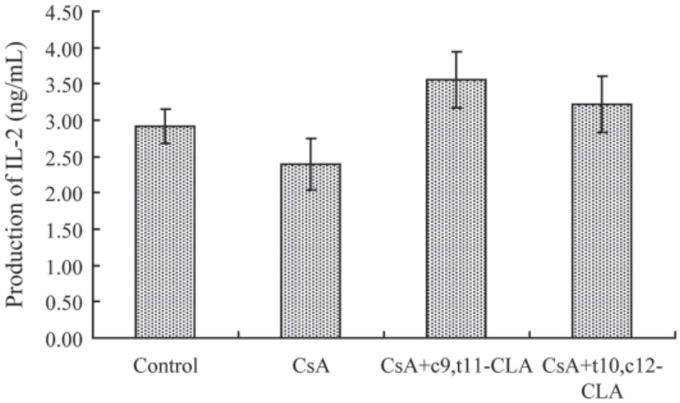

Figure 3. Effects of c9, t11-conjugated linoleic acid (CLA) and t10, c12-CLA on interleukin (IL)-2 production of peripheral blood T lymphocyte under the cyclosporin A (CsA)-induced immunosuppression. On d 21, peripheral blood T lymphocyte of chickens were cultured with media containing 25 μmol/L of c9, t11-CLA and t10, c12-CLA, with linoleic acid as control, to investigate the effect of CLA isomers on IL-2 production. The results are presented as means of IL-2 production of peripheral blood T lymphocyte at different CLA isomers.

ure 4). The c9, t11-CLA isomer significantly increased ($P < 0.05$) the Ca^{2+}-dependent and -independent PLC activities in the particulate and cytosolic fraction of peripheral blood T lymphocyte treated with CsA. The t10, c12-CLA tended to increase the activity of Ca^{2+}-independent PLC in the particulate and significantly increased ($P < 0.05$) Ca^{2+}-dependent and -independent PLC activities in the cytosolic fraction of peripheral blood T lymphocyte treated with CsA. And the c9, t11-CLA isomer had better effects on the activities of PLC of peripheral blood T lymphocyte treated with CsA than that of t10, c12-CLA.

The Production of PKC in Peripheral Blood T Lymphocyte

The CsA significantly decreased ($P < 0.05$) the production of PKC of peripheral blood T lymphocyte (Figure 5). Compared with the t10, c12-CLA isomer, c9, t11-CLA isomer significantly increased ($P < 0.05$) the production of PKC of peripheral blood T lymphocyte treated with CsA.

DISCUSSION

Many studies have shown a critical role of dietary CLA in the modulation of immune function in animals such as rat, pigs, and chickens (Sugano et al., 1998; Yamasaki et al., 2000; O'Shea et al., 2004; Zhang et al., 2005). The immune function of CLA was not only in the normal physiological status but also the immunosuppressive status (Bassaganya-Riera et al., 2003). In this study, to examine whether the supplementation of CLA attenuated the immunosuppression at the cellular level, we first used a model for inducing immunosuppression of peripheral blood T lymphocyte in chickens by culturing with media containing CsA. The CsA was reported to primarily suppress T lymphocyte proliferation. Here, we found that CsA significantly decreased the peripheral blood T lymphocyte proliferation, which was in line with the previous studies (Raj and Jones, 1997; Khehra and Jones, 1999; Isobe et al., 2000; Loa et al., 2002). Compared with t10, c12-CLA isomer, peripheral blood T lymphocytes culturing with media containing c9, t11-CLA isomer show significantly greater proliferation. These findings suggested the effect of c9, t11-CLA isomer on modulation of peripheral blood

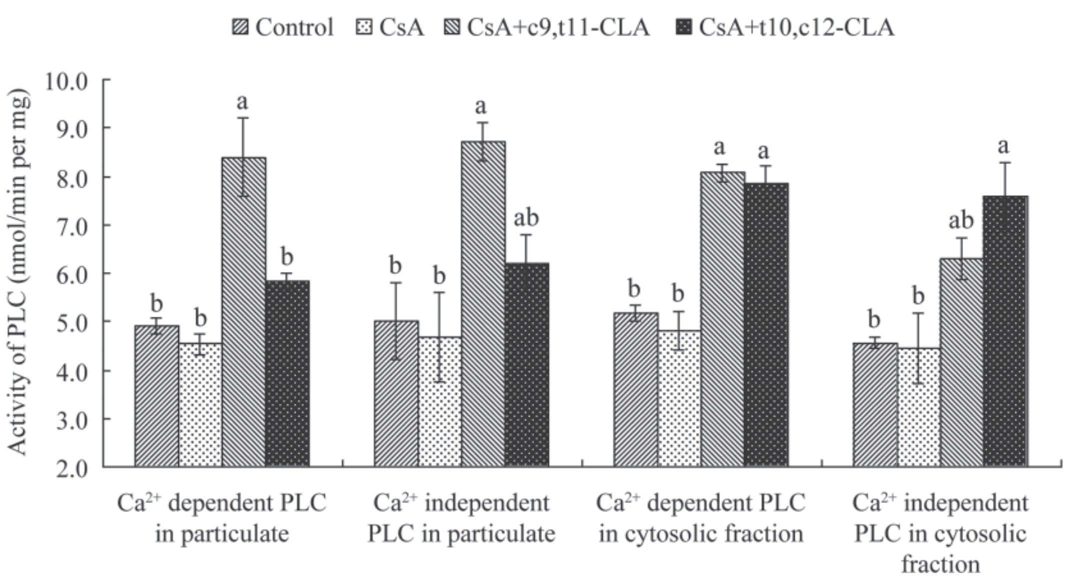

Figure 4. Effects of c9, t11-conjugated linoleic acid (CLA) and t10, c12-CLA on the activity of Ca^{2+}-dependent and -independent phospholipase C (PLC; nmol/min per mg) in particulate and cytosolic fractions of peripheral blood T lymphocyte under the cyclosporine A (CsA)-induced immunosuppression. On d 21, peripheral blood T lymphocyte of chickens were cultured with media containing 25 μmol/L of c9, t11-CLA and t10, c12-CLA, with linoleic acid as control, to investigate the effect of CLA isomers on the activity of PLC. The results are presented as means of the activities of PLC of peripheral blood T lymphocyte at different CLA isomers. [a,b]Means with different letters indicate a significant difference ($P < 0.05$).

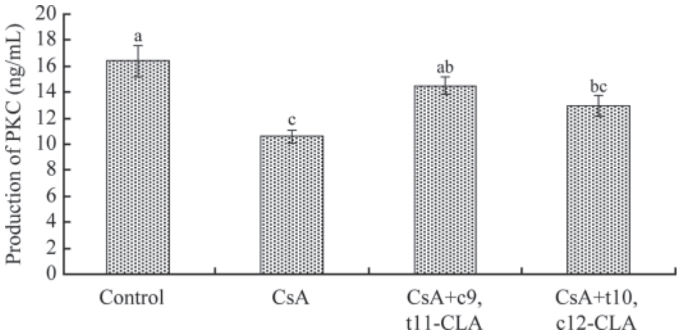

Figure 5. Effects of c9, t11-conjugated linoleic acid (CLA) and t10, c12-CLA on protein kinase C (PKC) production of peripheral blood T lymphocyte under the cyclosporin A (CsA)-induced immunosuppression. On d 21, peripheral blood T lymphocyte of chickens were cultured with media containing 25 μmol/L of c9, t11-CLA and t10, c12-CLA, with linoleic acid as control, to investigate the effect of CLA isomers on PKC production. The results are presented as means of PKC production of peripheral blood T lymphocyte at different CLA isomers. c = *cis*; t = *trans*. [a–c]Means in the same row without the same letter differ significantly ($P < 0.05$).

T lymphocytes proliferation was greater than that of t10, c12-CLA isomer under the CsA-induced immunosuppressive status. The results were basically in line with Soel et al. (2007) and Turpeinen et al. (2008), who showed that predominantly c9, t11-CLA isomer had the function of immune modulation in animals, whereas t10, c12-CLA isomer regulated the metabolism of lipid through changing the contents of fatty acid (Bissonauth et al., 2006; Zabala et al., 2006).

Cyclosporin A is an immunosuppressant agent blocking the transcription of the *IL-2* gene (Nowak et al., 1982), impairing IL-2-driven proliferation of activated T helper and cytotoxic T lymphocytes (Kim and Perfect, 1989). Interleukin-2 is a multifunctional protein, most notably known for the ability to induce the proliferation and maturation of activated T cells (Beadling and Smith, 2002; Beadling et al., 2002). In this study, we also found that CsA tended to decrease the IL-2 production in peripheral blood T lymphocytes, which was in line with the previous studies (Raj and Jones, 1997; Khehra and Jones, 1999; Isobe et al., 2000; Loa et al., 2002). In addition, peripheral blood T lymphocytes culturing with media containing 2 CLA isomers showed higher production of IL-2, which suggested the effect of CLA isomers had the function of alleviating the CsA-induced immunosuppression. In the previous study, we found that dietary CLA, predominantly c9, t11-CLA isomer, supplementation enhanced immune function in chickens under CsA-immunosuppressive status (Long et al., 2010) and alleviated immunosuppression in chickens infected with infectious bursal disease virus (Long et al., 2011) associated with lymphoid depletion. As the similar study, Bassaganya-Riera et al. (2003) reported CLA ameliorated viral infectivity in a pig model of type-2 porcine circovirus-induced immunosuppression, which also caused the B cell depletion in peripheral blood. This finding led to the conclusion that compared with t10,c12-CLA isomer, c9,t11-CLA isomer had the function of alleviating immunosuppression in animals. In the current study, both CLA isomers had the tendency of CsA-induced immunosuppression through increasing the production of IL-2 at the cellular level, and c9,t11-CLA isomer was better than t10,c12-CLA, which is basically consistent with the previous reports.

In T cell receptor signal transduction, a fraction of PLC, a cytosolic protein, is inducibly recompartmentalized to the lipid rafts, which was a part of cell membranes, including important signaling molecules influencing immune function. The PLC-mediated hydrolysis of phosphatidylinositol (4,5)-bisphosphate to inositol (1,4,5)-trisphosphate and diacylglycerol controls Ca^{2+} mobilization and PKC activation, respectively, critical steps that regulate *IL-2* transcription (Barker et al., 1998). In lymphocytes, PLC stimulates IL-2 receptor expression, activates PKC, and promotes cell growth in the presence of IL-2 (Schütze et al., 1994). In this study, both CLA isomers increased the PLC activities of peripheral blood T lymphocyte under CsA-induced immunosuppression, which led to increased the production of IL-2, but it was not significant. The reason may be that the signaling pathway of peripheral blood T lymphocyte was a complex course that was involved in the other pathway including MAPK, IP_3/Ca^{2+}, JAK/STAT, and so on (Cantrell, 1996; van der Bruggen et al., 1999; Bocca et al., 2007). In addition, other cytokines, such as TNF-α, IL-1, IL-6, IL-4, IL-10, also had effects on the production of IL-2 (Tomizawa et al., 1991; Narayan et al., 2011; Vinolo et al., 2011). To clarify this, further studies are necessary.

Protein kinase C plays a crucial role in the initial events of signal transduction (di Giacomo et al., 2010), which is important to the activation of T-lymphocyte (Arendt et al., 2002). In naive peripheral T cells, activation of PKC via the T cell receptor is required for IL-2 secretion, IL-2 receptor upregulation, and clonal expansion of CD4 and CD8 T cells (Sakowicz-Burkiewicz et al., 2008). Normal activation of PKC relies on the stimulation of phospholipid hydrolysis that follows a growth factor-receptor interaction. Activated PLC could hydrolyze membrane phosphatidylcholine into diacylglycerol and phosphocholine, and diacylglycerol is known to enhance the PKC activity (Way et al., 2000). Here, we found that CsA induced the decrease of PKC in peripheral blood T lymphocyte. Compared with t10,c12-CLA isomer, c9,t11-CLA isomer significantly increased the production of PKC. This showed that CLA, predominantly the c9, t11-CLA isomer, supplementation enhanced immune function under the CsA-immunosuppressive status in peripheral blood T lymphocyte, which was mediated by the enhancement of PKC.

In conclusion, peripheral blood T lymphocytes in chickens were cultured with media containing 2 CLA isomers, and predominantly the c9, t11-CLA isomer alleviated CsA-induced immunosuppression through increasing lymphocyte proliferation and the production of IL-2. The immune modulatory mechanism by which c9, t11-CLA had alleviating actions was in part via increasing the signaling molecules such as PLC and PKC.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Science Fund for Distinguished Young Scholars (No. 30425037) and the General Program from the National Natural Science Foundation of China (No. 30671520).

REFERENCES

Arendt, C. W., A. Björn, J. S. Timothy, and D. R. Littman. 2002. Protein kinase C-θ: Signaling from the center of the T-cell synapse. Curr. Opin. Immunol. 14:323–330.

Barker, S. A., K. K. Caldwell, J. R. Pfeiffer, and B. S. Wilson. 1998. Wortmannin-sensitive phosphorylation, translocation, and activation of PLC gamma1, but not PLC gamma2, in antigen-stimulated RBL-2H3 mast cells. Mol. Biol. Cell 9:483–496.

Bassaganya-Riera, J., R. M. Pogranichniy, S. C. Jobgen, P. G. Halbur, K. J. Yoon, M. O'Shea, I. Mohede, and R. Hontecillas. 2003. Conjugated linoleic acid ameliorates viral infectivity in a pig model of virally induced immunosuppression. J. Nutr. 133:3204–3214.

Beadling, C., and K. A. Smith. 2002. DNA array analysis of interleukin-2-regulated immediate/early genes. Med. Immunol. 1:2.

Beadling, C., K. W. Johnson, and K. A. Smith. 2002. Isolation of interleukin-2-induced immediate-early genes. Med. Immunol. 18:1–14.

Berg, T. P. 2000. Acute infectious bursal disease in poultry: A review. Avian Pathol. 29:175–194.

Bissonauth, V., Y. Chouinard, J. Marin, N. Leblanc, D. Richard, and H. Jacques. 2006. The effects of *t*10,*c*12 CLA isomer compared with *c*9,*t*11 CLA isomer on lipid metabolism and body composition in hamsters. J. Nutr. Biochem. 17:597–603.

Bocca, C., F. Bozzo, L. Gabriel, and A. Miglietta. 2007. Conjugated linoleic acid inhibits Caco-2 cell growth via ERK-MAKP signaling pathway. J. Nutr. Biochem. 18:332–340.

Cantrell, D. 1996. T-cell antigen receptor signal transduction pathways. Annu. Rev. Immunol. 14:259–274.

di Giacomo, V., M. Rapino, S. Sancilio, A. Patruno, S. Zara, R. D. Pietro, and A. Cataldi. 2010. PKC-δ signalling pathway is involved in H9c2 cells differentiation. Differentiation 80:204–212.

Evans, M., C. Geigerman, J. Cook, L. Curtis, B. Kuebler, and M. McIntosh. 2000. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3–L1 preadipocytes. Lipids 35:899–910.

Hill, J. E., G. N. Rowland, K. S. Latimer, and J. Brown. 1989. Effects of cyclosporine A on reovirus-infected broilers. Avian Dis. 33:86–92.

Islam, A. F., C. W. Wong, S. W. Walkden-Brown, I. G. Colditz, K. E. Arzey, and P. J. Groves. 2002. Immunosuppressive effects of Marek's disease virus (MDV) and herpes virus of turkeys (HVT) in broiler chickens and the protective effect of HVT vaccination against MDV challenge. Avian Pathol. 31:449–461.

Islam, A. F. M. F., S. W. Walkden-Brown, S. K. Burgess, and P. J. Groves. 2001. Marek's disease in broiler chickens: Effect of route of infection and herpes virus of turkey-vaccination status on detection of virus from blood or spleen by polymerase chain reaction, and on weights of birds, bursa and spleen. Avian Pathol. 30:621–628.

Isobe, T., S. Shimizu, S. Yoshihara, and Y. Yokomizo. 2000. Cyclosporin A, but not bursectomy, abolishes the protective immunity of chickens against *Leucocytozoon caulleryi*. Dev. Comp. Immunol. 24:433–441.

Khehra, R. S., and R. C. Jones. 1999. Investigation into avian pneumovirus persistence in poults and chicks using cyclosporin A immuno-suppression. Res. Vet. Sci. 66:161–163.

Kim, J. H., and J. R. Perfect. 1989. Infection and cyclosporine. Rev. Infect. Dis. 11:677–690.

Lambrecht, B., M. Gonze, G. Meulemans, and T. Van Den Berg. 2004. Assessment of the cell-mediated immune response in chickens by detection of chicken interferon-gamma in response to mitogen and recall Newcastle disease viral antigen stimulation. Avian Pathol. 33:343–350.

Loa, C. C., T. L. Lin, C. C. Wu, T. Bryan, T. Hooper, and D. Schrader. 2002. The effect of immunosuppression on protective immunity of turkey poults against infection with turkey coronavirus. Comp. Immunol. Microbiol. Infect. Dis. 25:127–138.

Long, F. Y., Y. M. Guo, Z. Wang, D. Liu, B. K. Zhang, and X. Yang. 2011. Conjugated linoleic acids alleviate infectious bursal disease virus-induced immunosuppression in broiler chickens. Poult. Sci. 90:1926–1933.

Long, F. Y., Z. Wang, Y. M. Guo, D. Liu, X. Yang, and P. Jiao. 2010. Conjugated linoleic acids alleviated immunosuppression in broiler chickens exposed to cyclosporin A. Food Agric. Immunol. 4:295–305.

Narayan, S., L. Kolly, A. So, and N. Busso. 2011. Increased interleukin-10 production by ASC-deficient $CD4^+$ T cells impairs bystander T-cell proliferation. Immunology 134:33–40.

Nowak, J. S., O. Kai, R. Peck, and R. M. Franklin. 1982. The effects of cyclosporin A on the chicken immune system. Eur. J. Immunol. 12:867–876.

O'Shea, M., J. Bassaganya-Riera, and I. C. Mohede. 2004. Immunomodulatory properties of conjugated linoleic acid. Am. J. Clin. Nutr. 79:1199S–1206S.

Raj, G. D., and R. C. Jones. 1997. Effect of T-cell suppression by cyclosporin on primary and persistent infection of infectious bronchitis virus in chickens. Avian Pathol. 26:257–276.

Rauw, F., B. Lambrecht, and T. Van Den Berg. 2007. Pivotal role of ChIFN in the pathogenesis and immunosuppression of infectious bursal disease. Avian Pathol. 36:367–374.

Ringseirs, R., N. Schulz, and K. Eder. 2008. Troglitazone but not conjugated linoleic acid reduces gene expression and activityy of matrix-metalloproteinases-2 and −9 in PMA-differentiated THP-1 macrophages. J. Nutr. Biochem. 19:596–603.

Sakowicz-Burkiewicz, M., G. Nishanth, U. Helmuth, K. Drögemuller, D. H. Busch, O. Utermöhlen, M. Naumann, M. Deckert, and D. Schlüter. 2008. Protein kinase C-theta critically regulates the proliferation and survival of pathogen-specific T cells in murine listeriosis. J. Immunol. 180:5601–5612.

Schreiber, S. L., and G. R. Crabtree. 1992. The mechanism of action of cyclosporin A and FK 506. Immunol. Today 13:136–142.

Schütze, S., T. Machleidt, and M. Kronke. 1994. The role of diacylglycerol and ceramide in tumour necrosis factor and interleukin-1 signal transduction. J. Leukoc. Biol. 56:533–541.

Sharma, J. M., I. J. Kim, S. Rautenschlein, and H. Y. Yeh. 2000. Infectious bursa disease virus of chickens: Pathogenesis and immunosuppression. Dev. Comp. Immunol. 24:223–235.

Shin, D., C. Narciso-Gaytán, J. H. Park, S. B. Smith, M. X. Sánchez-Plata, and C. A. Ruiz-Feria. 2011. Dietary combination effects of conjugated linoleic acid and flaxseed or fish oil on the concentration of linoleic and arachidonic acid in poultry meat. Poult. Sci. 90:1340–1347.

Sigal, N. H., and F. J. Dumont. 1992. Cyclosporine A, FK-506, and rapamycin: Pharmacologic probes of lymphocyte signal transduction. Annu. Rev. Immunol. 10:519–560.

Soel, S. M., O. S. Choi, M. H. Banga, J. H. Yoon Park, and W. K. Kim. 2007. Influence of conjugated linoleic acid isomers on the metastasis of colon cancer cells in vitro and in vivo. J. Nutr. Biochem. 18:650–657.

Sugano, M., A. Tsujita, M. Yamasaki, M. Noguchi, and K. Yamada. 1998. Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. Lipids 33:521–527.

Tomizawa, K., A. Ishizaka, K. Kojima, M. Nakanishi, Y. Sakiyama, and S. Matsumoto. 1991. Interleukin-4 regulates the interleukin-2 receptors on human peripheral blood B lymphocytes. Clin. Exp. Immunol. 83:492–496.

Turpeinen, A. M., N. Ylönen, E. V. Willebrand, S. Basuand, and A. Aro. 2008. Immunological and metabolic effects of *cis*-9, *trans*-11-conjugated linoleic acid in subjects with birch pollen allergy. Br. J. Nutr. 100:112–119.

van der Bruggen, T., S. Nijenhuis, E. V. Ruaij, J. Verhoef, and B. S. V. Asbeck. 1999. Lipopolysaccharide-induced tumor necrosis factor alpha production by human monocytes involves the Raf-1/MEK1–MEK1/ERK1–ERK2 pathway. Infect. Immun. 67:3824–3829.

Vinolo, M. A. R., H. G. Rodrigues, R. T. Nachbar, and R. Curi. 2011. Regulation of inflammation by short chain fatty acids. Nutrients 3:858–876.

Way, K. J., E. Chou, and G. L. King. 2000. Identification of PKC-isoform-specific biological actions using pharmacological approaches. Trends Pharmacol. Sci. 21:181–187.

Wu, X., H. Lu, L. Zhou, Y. Huang, and H. L. Chen. 1997. Changes of phosphatidylcholine-specific phospholipase C in hepatocarcinogenesis and in the proliferation and differentiation of rat liver cancer cells. Cell Biol. Int. 21:375–381.

Yamasaki, M., K. Kishihara, K. Mansho, Y. Ogino, M. Kasai, M. Sugano, H. Tachibana, and K. Yamada. 2000. Dietary conjugated linoleic acid increases immunoglobulin productivity of Sprague-Dawley rat spleen lymphocytes. Biosci. Biotechnol. Biochem. 64:2159–2164.

Zabala, A., M. P. Portillo, M. T. Macarulla, V. M. Rodríguez, and A. Fernández-Quintela. 2006. Effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers on liver and adipose tissue fatty acid profile in hamsters. Lipids 41:993–1001.

Zhang, H., Y. M. Guo, and J. M. Yuan. 2005. Conjugated linoleic acid enhanced the immune function in broiler chicks. Br. J. Nutr. 94:746–752.