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CLA reduces antigen-induced histamine and PGE_2 release from sensitized guinea pig tracheae

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Departments of [1]Nutritional Sciences and [6]Animal Sciences, College of Agriculture and Life Sciences; [2]Department of Medicine, Medical School; [4]Department of Surgical Sciences, School of Veterinary Medicine, and the [5]Food Research Institute, University of Wisconsin, Madison, Wisconsin 53706; and [3]Department of Internal Medicine, University of Texas, Galveston, Texas 77555

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Whigham, Leah D., Ellen B. Cook, James L. Stahl, Ricardo Saban, Dale E. Bjorling, Michael W. Pariza, and Mark E. Cook. CLA reduces antigen-induced histamine and PGE_2 release from sensitized guinea pig tracheae. *Am J Physiol Regulatory Integrative Comp Physiol* 280: R908–R912, 2001.—Conjugated linoleic acid (CLA) has been shown to enhance immune reactions such as lymphocyte blastogenesis and delayed-type hypersensitivity. We investigated the role of CLA in type I (immediate) hypersensitivity, using a guinea pig tracheal superfusion model for measuring antigen-induced airway smooth muscle contraction and inflammatory mediator release. Female Hartley guinea pigs were fed a diet supplemented with 0.25 g corn oil or linoleic acid/100 g of diet (control) or 0.25 g CLA/100 g of diet for at least 1 wk before and during active sensitization to ovalbumin antigen. Tracheae from sensitized guinea pigs were suspended in air-filled water-jacketed (37°C) tissue chambers in a superfusion apparatus. Tracheae were superfused with buffer containing antigen, and tissue contraction was recorded. Superfusate was collected at 90-s intervals for evaluation of histamine and PGE_2 release. CLA did not affect antigen-induced tracheal contractions when expressed as gram contraction per gram tissue. CLA significantly reduced antigen-induced histamine and PGE_2 release. CLA appears to decrease release of some inflammatory mediators during type I hypersensitivity reactions.

type I hypersensitivity; conjugated linoleic acid; immunity; allergies; asthma

CONJUGATED LINOLEIC ACIDS (CLA) are naturally occurring isomers of linoleic acid in which the double bonds are in a conjugated formation (i.e., a 1,3-diene, not methylene interrupted). Notable biological effects include anticarcinogenesis (13), antiatherogenesis (15), body fat reduction (22), lean body mass enhancement (22), anticachectic effects (10), and immune enhancement, including increased lymphocyte blastogenesis (6, 10, 19, 32), increased lymphocyte cytotoxic activity (6), and increased delayed-type hypersensitivity (10).

The mechanism by which CLA affects these aspects of the immune system is not completely understood. The conversion of arachidonic acid into eicosanoids by cyclooxygenase or lipoxygenase is hypothesized to be affected by CLA (10). CLA is incorporated into the *sn*-2 position of phospholipids (21) and can be metabolized into 20 carbon molecules with either three or four double bonds (2, 26). However, to the best of our knowledge, no conjugated forms of cyclooxygenase or lipoxygenase products have been detected. Nugteren (20) showed that *cis*-8, *trans*-12, *cis*-14 eicosatrienoate and *cis*-5, *cis*-8, *trans*-12, *cis*-14 eicosatetraenoate (the elongated and desaturated fatty acid that would form from *trans*-10, *cis*-12 CLA) were able to competitively inhibit cyclooxygenase.

The use of CLA as a dietary supplement has increased in recent years, leading to a need for understanding the biological effects of CLA under different physiological conditions. Dietary fatty acids have been hypothesized to play a role in type I hypersensitivity reactions by affecting the eicosanoid pathway (3, 4). Because of the potential for CLA to modulate the eicosanoid pathway, its previously reported effects on immune function and the high incidence of type I hypersensitivity in humans [over 20% of the US population (1)], the present study was conducted to describe the effects of supplemental dietary CLA in a guinea pig model of type I hypersensitivity.

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MATERIALS AND METHODS

Diets and sensitization. Female Hartley guinea pigs (Harlan Sprague Dawley, Madison, WI) weighing 200–350 g were randomly divided into two dietary treatment groups, control and CLA. Because of experimental limitations in managing the number of tissues, the experiment was done in three blocks. Diets consisted of standard commercial alfalfa-based guinea pig chow[1] (7006, Harlan-Teklad, Madison, WI) supplemented with 0.25 g/100 g of either control oil or CLA. The control oil was either corn oil (~55% linoleic acid) or linoleic acid (95%, Nu-Check Prep, Elysian, MN). Both corn oil and linoleic acid are typical control oils used in dietary CLA experiments (5, 8, 23, 25). There were no statistical differences in effects of the two control diets, so results were pooled (Fig. 1). CLA[2] was synthesized from linoleic acid (Nu-Check Prep) by published methods (7). Guinea pigs were housed in a temperature- and humidity-controlled room with a 12:12-h light-dark cycle in compliance with the University of Wisconsin-Madison Research Animal Resources Center. Guinea pigs were given free access to the experimental diets at least 1 wk before and during active sensitization to chicken egg ovalbumin (OVA, Sigma, St. Louis, MO) antigen. Guinea pigs were sensitized with an initial intraperitoneal injection of 50 μg OVA in PBS with 1 mg aluminum hydroxide followed 2 wk later by a subcutaneous injection (flank) of 200 μg OVA in PBS emulsified with equal volume of incomplete Freund's adjuvant. This sensitization protocol enhances production of IgG_1, the reaginic antibody in guinea pigs (31). Guinea pigs were killed 4 days after the second injection with an intraperitoneal injection of pentobarbital sodium (100 mg/kg).

Tracheal superfusion. Tracheae (n = 15 per treatment) were removed, placed in physiological saline solution (PSS), and trimmed of excess tissue with care being taken not to stretch or abrade the tracheae. The PSS was a bicarbonate buffer solution containing (in mM) 118 NaCl, 1.0 NaH_2PO_4, 4.7 KCl, 2.5 $CaCl_2$, 0.5 $MgCl_2$, 11 glucose, and 25 $NaHCO_3$. Each trachea was cut longitudinally into a spiral at a 45° angle (9) and suspended isotonically in an air-filled, water-jacketed (37°C) tissue chamber (30). The tracheae were superfused at a rate of 2.2 ml/min with PSS (37°C; gassed with 95% O_2 and 5% CO_2) while being maintained at a constant tension of 5 g for an equilibration period of 90 min (11). Changes in tensions (expressed as g of contraction) were measured with Grass FT 03 electrical force-displacement transducers and plotted with a Grass polygraph (model 7D, Grass Instruments, Quincy, MA). After equilibration, tracheae were challenged by continuously superfusing PSS containing 0.01 g/l OVA (2.2 ml/min). Superfusates were collected in separate aliquots at 90-s intervals for 15 min beginning 90 s before antigen challenge (designated collection *period 0*) and stored at 4°C. Peak changes in tracheal tensions were determined for each 90-s collection period. After collection, superfusates were stored at −20°C until they were analyzed for histamine and PGE_2 content (analyses were performed within 3 days of superfusion). After antigen challenge, tracheae were continuously superfused with PSS containing 10^{-5} M carbachol (carbamylcholine chloride, Sigma) to produce maximal contraction. After carbachol-induced contraction, tracheae were weighed, minced with scissors, homogenized in 0.4 N perchloric acid, and placed in a boiling water bath for 10 min. After centrifugation, the supernatants were collected for determination of residual histamine.

Mediator analysis. Histamine contents from superfusates and tracheae were determined by enzyme immunoassay (EIA, Immunotech, Westbrook, ME) for nine guinea pigs from each treatment. The sensitivity of the assay is 0.05 μg/l. PGE_2 contents of superfusates from 15 tracheae from each treatment were analyzed using an EIA (Amersham Life Science, Arlington Heights, IL) with a sensitivity of 40 pg/ml. In this assay, cross-reactivity with PGE_1, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, and arachidonic acid is 25%, 0.04%, <0.1%, and <0.001%, respectively.

Fatty acid analysis. Fatty acid composition of the diet and CLA isomer composition of the diet and tracheae were determined by gas chromatography using previously published methods (7). Tracheae used for CLA isomer analysis were obtained in a separate experiment using the same feeding and sensitization protocol described above.

Statistical analysis. Data were analyzed using SAS (Cary, NC). ANOVAs with repeated measures were performed to test for diet effect on tracheal contractions, histamine release, and PGE_2 release.

RESULTS

Tracheal contractions. Carbachol, an acetylcholine analog, is typically used in superfusion experiments to determine the maximal contraction of tissues to express contraction as a percent of the maximal carbachol-induced contraction. This method is frequently preferred, but assumes that the treatment being studied does not affect the maximum contraction. Another way to express contractions is as grams of contraction per gram of trachea. This method works best for tracheae of approximately the same size, and the assumption made is that muscle distribution is constant. In these experiments, there was a moderate treatment effect on the maximal carbachol-induced contractions (in g contraction/g trachea: control, 14.82; CLA, 17.37; SE = 0.94, P = 0.06). Therefore, contractions were expressed both as grams contraction per gram wet trachea and as a percent of maximal (carbachol induced) contraction. There were no significant differences in tracheal contraction due to diet (Fig. 1*A*) when expressed as gram per gram trachea, but the CLA-supplemented guinea pig tracheae had significantly lower contractions during time *periods 1* and *2* relative to control guinea pigs when expressed as percent of maximal carbachol-induced contraction.

Histamine release. There was a significantly lower amount of histamine released by tracheae from CLA-supplemented guinea pigs relative to controls during the first 90 s of antigen challenge (Fig. 1*B*, collection *period 1*, $P < 0.0001$). The peak histamine release at this time point corresponded with peak tracheal contraction for both treatment groups. There were no significant differences in histamine release at all other time points measured. Total histamine release and total tissue histamine (total released plus residual in trachea) were not significantly different between groups (control, 7,355 ng/g; CLA, 7,075 ng/g; $P > 0.5$).

[1] Proximate composition (provided by company; in g/kg): 17.0 crude protein, 2.5 crude fat, 16.0 crude fiber. Fatty acid composition by gas chromatography analysis of basal diet (as %total fatty acids): 34% 18:2, 28% 18:1, 20% 16:0, 7% 18:3, 6% 18:0, 2% 16:1.

[2] Isomer composition of CLA: 43% *trans*-10, *cis*-12; 42% *cis*-9, *trans*-11/*trans*-9, *cis*-11; 10% *trans*,*trans* isomers, 3% *cis*,*cis* isomers. Pure isomers were not available in sufficient quantity.

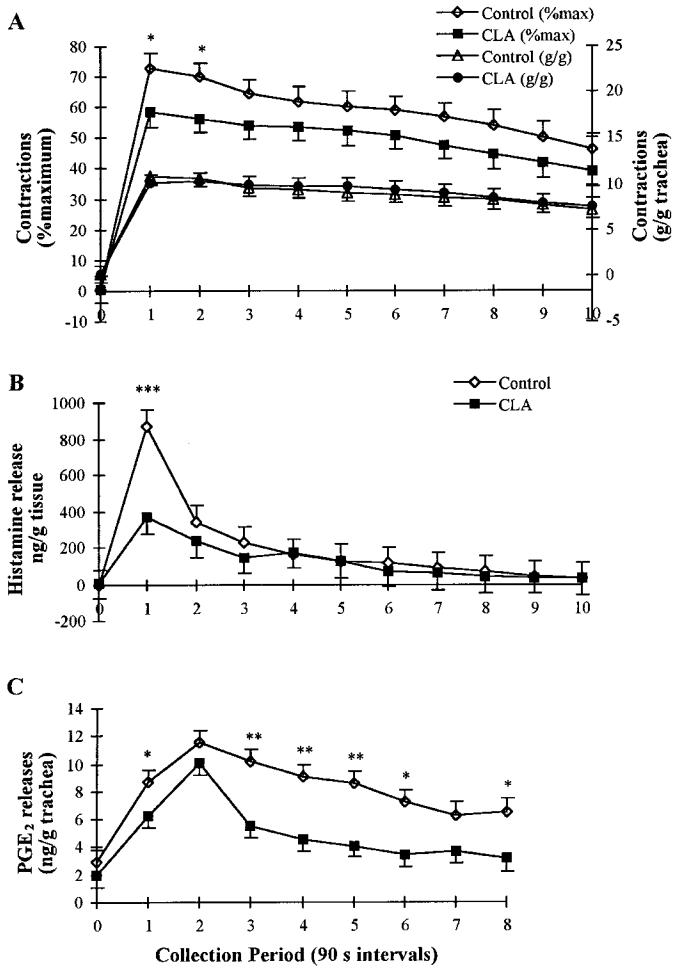

Fig. 1. Measurement of airway hyperresponsiveness over time from tracheae of guinea pigs fed a control diet or a conjugated linoleic acid (CLA)-supplemented diet. *A*: tracheal contractions (left axis: % maximal contraction, right axis: g contraction/g trachea, $n = 15$). *B*: histamine release ($n = 9$). *C*: PGE_2 release ($n = 15$). Values are least-squared means from 3 experiments. Error bars represent SE of the least-squared means. $^*P < 0.05$, $^{**}P < 0.001$, $^{***}P < 0.0001$.

PGE_2 release. Overall release of PGE_2 was significantly lower in CLA-supplemented guinea pigs relative to controls (Fig. 1*C*). Peak PGE_2 release (collection *period 2*) followed peak contraction and histamine release (collection *period 1*).

Weight gain, feed consumption, and tissue analysis. There were no significant differences between dietary treatments in weight gain or feed consumption during these experiments (data not shown). CLA content of control tracheae was in the ranges of 0–0.23% and 0.11–0.16% of total fatty acids for phospholipids and neutral lipids, respectively; and for CLA tracheae, 1.83–2.20% and 1.99–2.64% of total fatty acids for phospholipids and neutral lipids, respectively.

DISCUSSION

Histamine release from tracheae of CLA-supplemented guinea pigs was significantly reduced during the time interval of maximal release. Histamine is an inflammatory mediator released from antigen-activated mast cells in airway tissue, and histamine causes smooth muscle contraction, increased epithelial permeability, and increased mucous secretion (33). Despite higher levels of histamine release in the control group during the first 90-s interval after OVA challenge, tracheal contractions (g contraction per g trachea) were similar to the CLA group. It is possible that histamine was released at a greater concentration than required for maximal tracheal contractions or that other inflammatory mediators induced contraction or relaxation. CLA feeding did not affect total tissue histamine in our study. Sugano and coworkers (27) did not observe an influence of CLA on histamine release from rat peritoneal exudate cells, but did notice a decreased trend in stored histamine in cells with increasing dietary CLA levels. Noteworthy differences between the study by Sugano and coworkers and this study are that the animals used in the former study were not sensitized to an antigen, and isolated cells, not tissues, were used.

PGE_2 release by tracheae from CLA-fed guinea pigs was consistently decreased during superfusion. This is consistent with other reports of CLA decreasing PGE_2 in serum (27, 28), bone (17), spleen (28), and cultured keratinocytes (19). PGE_2 plays an important role in type I hypersensitivity by inhibiting the formation of interferon-γ, a cytokine responsible for downregulating production of IgE (12). PGE_2 can also act directly on B cells to increase the formation of IgG_1 (the reaginic Ig in guinea pigs) and IgE (24). Therefore, decreased PGE_2 may result in decreased sensitization to the allergen. In fact, previous reports (27) indicate that CLA-supplemented rats had decreased serum IgE (the reaginic Ig in rats) compared with control-fed rats.

PGE_2 is also an airway relaxant (14). We did not evaluate this effect in the current study. However, CLA decreased the release of both contractile (histamine) and relaxant (PGE_2) substances, leading to a net mechanical response to antigen stimulation that was not different from controls.

The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of CLA are the two most abundant CLA isomers in most commercial sources. Based on prior evidence that *cis*-8, *trans*-12, *cis*-14 eicosatrienoate and *cis*-5, *cis*-8, *trans*-12, *cis*-14 eicosatetraenoate inhibit cyclooxygenase (20), we hypothesize that the *trans*-10, *cis*-12 isomer of CLA is the isomer responsible for decreasing the amount of PGE_2 measured. Recent availability of pure isomers will allow for the testing of this hypothesis.

Maximal contractions in response to carbachol were moderately increased in CLA-supplemented guinea pigs. CLA was previously shown to be incorporated into the phospholipids of cell membranes (21) and to decrease delta-9 desaturase (16). Membrane lipid composition has been shown to have an important effect on acetylcholine receptor function (29). Therefore, the increased carbachol contraction could reflect an altered responsiveness of the receptor due to changes in membrane fatty acid composition by CLA and/or by altered delta-9 desaturase activity. Alternatively, CLA has been shown to increase lean muscle mass (22), and, therefore, guinea pigs fed CLA may have increased

smooth muscle mass, which in turn could have resulted in increased carbachol-induced contraction. The observed reduction in maximal contraction of tracheae when expressed as a percent of maximal carbachol-induced contraction appears to be the result of increased carbachol responsiveness of tracheae from CLA-supplemented guinea pigs.

In conclusion, in this guinea pig model of type I hypersensitivity, supplemental CLA reduced release of the inflammatory mediators measured (histamine and PGE_2). Histamine release may be decreased as a result of decreased sensitization to the allergen. The reduction in PGE_2 release is probably due to an altered eicosanoid synthesis pathway. CLA did not affect antigen-induced tracheal contractions (g/g trachea). These data suggest that CLA may play a role in downregulating type I hypersensitivity reactions.

Perspectives

The results from this study have an important broad implication. CLA, a naturally occurring component in our diet, may have downregulatory effects on type I hypersensitivity reactions such as allergies and asthma, conditions that affect a large number of people. The CLA isomer occurring at highest levels in our diet is the *cis*-9, *trans*-11/*trans*-9, *cis*-11. However, this study was done with a mixture of isomers containing approximately equal amounts of the *trans*-10, *cis*-12 and *cis*-9, *trans* 11/*trans*-9, *cis*-11 isomers, and at this point we do not know if one isomer or both are responsible for the effects seen. Further work is needed to better understand the interactions of the various CLA isomers with immune function and to determine if CLA downregulates type I hypersensitivity responses in other whole animal models and humans.

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REFERENCES

1. **American Academy of Allergy, Asthma, and Immunology.** *Executive Summary*. Milwaukee, WI: Task Force on Allergic Disorders, 1998.
2. **Banni S, Carta G, Contini MS, Angioni E, Deiana M, Dessi MA, Melis MP, and Corongiu FP.** Characterization of conjugated diene fatty acids in milk, dairy products, and lamb tissues. *J Nutr Biochem* 7: 150–155, 1996.
3. **Black PN.** The prevalence of allergic disease and linoleic acid in the diet. *J Allergy Clin Immunol* 103: 351–352, 1999.
4. **Black PN and Sharpe S.** Dietary fat and asthma: is there a connection? *Eur Respir J* 10: 6–12, 1997.
5. **Brodie AE, Manning VA, Ferguson KR, Jewell DE, and Hu CY.** Conjugated linoleic acid inhibits differentiation of pre- and post-confluent 3T3–L1 preadipocytes but inhibits cell proliferation only in preconfluent cells. *J Nutr* 129: 602–606, 1999.
6. **Chew BP, Wong TS, Shultz TD, and Magnuson NS.** Effects of conjugated dienoic derivatives of linoleic acid and beta-carotene in modulating lymphocyte and macrophage function. *Anticancer Res* 17: 1099–1106, 1997.
7. **Chin SF, Liu W, Storkson JM, Ha YL, and Pariza MW.** Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. *J Food Comp Anal* 5: 185–197, 1992.
8. **Chin SF, Storkson JM, Albright KJ, Cook ME, and Pariza MW.** Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J Nutr* 124: 2344–2349, 1994.
9. **Constantine JW.** The spirally cut tracheal strip preparation. *J Pharm Pharmacol* 17: 384–385, 1965.
10. **Cook ME, Miller CC, Park Y, and Pariza MW.** Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. *Poult Sci* 72: 1301–1305, 1993.
11. **Fishleder RI and Buckner CK.** Studies on the relationship between contraction and mediator release produced by ovalbumin in superfused trachea isolated from the actively sensitized guinea pig. *J Pharmacol Exp Ther* 230: 534–540, 1984.
12. **Gold KN, Weyand CM, and Goronzy JJ.** Modulation of helper T cell function by prostaglandins. *Arthritis Rheum* 37: 925–933, 1994.
13. **Ha YL, Grimm NK, and Pariza MW.** Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. *Carcinogenesis* 8: 1881–1887, 1987.
14. **Kleeberger SR and Freed AN.** Prostanoids. In: *Asthma and Rhinitis*, edited by Busse WW and Holgate ST. Cambridge: Blackwell Scientific Publications, 1995, p. 825–837.
15. **Lee KN, Kritchevsky D, and Pariza MW.** Conjugated linoleic acid and atherosclerosis in rabbits. *Atherosclerosis* 108: 19–25, 1994.
16. **Lee KN, Pariza MW, and Ntambi JM.** Conjugated linoleic acid decreases hepatic stearoyl-CoA desaturase mRNA expression. *Biochem Biophys Res Commun* 248: 817–821, 1998.
17. **Li Y and Watkins BA.** Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E_2 biosynthesis in rats fed n-6 or n-3 fatty acids. *Lipids* 33: 417–425, 1998.
18. **Liu KL and Belury MA.** Conjugated linoleic acid modulation of phorbol ester-induced events in murine keratinocytes. *Lipids* 32: 725–730, 1997.
19. **Miller CC, Park Y, Pariza MW, and Cook ME.** Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. *Biochem Biophys Res Commun* 198: 1107–1112, 1994.
20. **Nugteren DH.** Inhibition of prostaglandin biosynthesis by 8 *cis*, 12 *trans*, 14 *cis*-eicosatrienoic acid and 5 *cis*, 8 *cis*, 12 *trans*, 14 *cis*-eicosatetraenoic acid. *Biochem Biophys Acta* 210: 171–176, 1970.
21. **Park Y.** *Regulation of Energy Metabolism and the Catabolic Effects of Immune Stimulation by Conjugated Linoleic Acid*. Madison, WI: University of Wisconsin, 1996 (PhD thesis).
22. **Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, and Pariza MW.** Effect of conjugated linoleic acid on body composition in mice. *Lipids* 32: 853–858, 1997.
23. **Park Y, Storkson JM, Albright KJ, Liu W, and Pariza MW.** Evidence that the *trans*-10, *cis*-12 isomer of conjugated linoleic acid induces body composition changes in mice. *Lipids* 34: 235–241, 1999.
24. **Roper RL and Phipps RP.** Prostaglandin E_2 and cAMP inhibit B lymphocyte activation and simultaneously promote IgE and IgG1 synthesis. *J Immunol* 149: 2984–2991, 1992.
25. **Satory DL and Smith SB.** Conjugated linoleic acid inhibits proliferation but stimulates lipid filling of murine 3T3–L1 preadipocytes. *J Nutr* 129: 92–97, 1999.
26. **Sebedio JL, Juaneda P, Dobson G, Ramilison I, Martin JD, and Chardigny JM.** Metabolites of conjugated isomers of linoleic acid (CLA) in the rat. *Biochim Biophys Acta* 1345: 5–10, 1997.
27. **Sugano M, Tsujita A, Yamasaki M, Noguchi M, and Yamada K.** Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats. *Lipids* 33: 521–527, 1998.
28. **Sugano M, Tsujita A, Yamasaki M, Yamada K, Ikeda I, and Kritchevsky D.** Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. *J Nutr Biochem* 8: 38–43, 1997.

29. **Sunshine C and McNamee MG.** Lipid modulation of nicotinic acetylcholine receptor function: the role of membrane lipid composition and fluidity. *Biochim Biophys Acta* 1194: 59–64, 1994.
30. **Undem BJ, Buckner CK, Harley P, and Graziano FM.** Smooth muscle contraction and release of histamine and slow-reacting substance of anaphylaxis in pulmonary tissues isolated from guinea pigs passively sensitized with IgG_1 or IgE antibodies. *Am Rev Respir Dis* 131: 260–266, 1985.
31. **Wong DTO and Barbaro JF.** Production of guinea pig IgG1 homocytotropic antibodies to hapten-conjugated homologous serum albumin with different adjuvant combinations. *Int Arch Allergy Appl Immunol* 50: 155–163, 1976.
32. **Wong MW, Chew BP, Wong TS, Hosick HL, Boylston TD, and Shultz TD.** Effects of dietary conjugated linoleic acid on lymphocyte function and growth of mammary tumors in mice. *Anticancer Res* 17: 987–993, 1997.
33. **Wood-Baker R.** Histamine and its receptors. In: *Asthma and Rhinitis*, edited by Busse WW and Holgate ST. Cambridge: Blackwell Scientific Publications, 1995, p. 791–800.