Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n−3 fatty acids1–4

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ABSTRACT
Conjugated linoleic acid (CLA) and the long-chain polyunsaturated n−3 fatty acids have been shown in vivo and in vitro to reduce tumor growth. Tumor growth could occur by slowing or stopping cell replication (by interfering with transition through the cell cycle), increasing cell death (via necrosis and/or apoptosis), or both. The anticancer effects of fatty acids, shown in vivo, could also be mediated by effects on the host’s immune system. Although it is widely recognized that n−3 fatty acids can alter immune and inflammatory responses, considerably less is known about CLA. For n−3 fatty acids, several candidate mechanisms have been proposed for their immune effects, including changes in 1) membrane structure and composition, 2) membrane-mediated functions and signals (eg, proteins, eicosanoids), 3) gene expression, and 4) immune development. Considerable work has been done that shows the potential importance of CLA as an anticancer treatment; however, many questions remain as to how this effect occurs. This review summarizes the CLA and cancer literature and then uses the evidence for the anticancer immune and tumor properties of the long-chain n−3 fatty acids docosahexaenoic and eicosapentaenoic acids to suggest future research directions for mechanistic studies on CLA and cancer. Am J Clin Nutr 2004;79:1190S–8S.

KEY WORDS Docosahexaenoic acid, eicosapentaenoic acid, cancer, mammary tumors, apoptosis, necrosis, cell cycle, rodents

INTRODUCTION
Fats are adversely implicated in the etiology of many cancers, yet evidence is abundant that certain fatty acids, such as the highly polyunsaturated n−3 fish oil fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have potential anticancer activity [reviewed by Bartsch et al (1), Rose and Connolly (2), and Hardman (3)]. More recently, anticancer activity was demonstrated for conjugated linoleic acid (CLA) in both human tumor cell lines and in well-accepted rodent models of carcinogenesis (4–10). Human epidemiologic data support the anticancer potential of n−3 fatty acids as an inverse association between dietary n−3 intake (and the intake of fish) and the incidence of several forms of cancer, including breast and colorectal cancers [reviewed by de Deckere (11)]. Although an inverse relation was observed between CLA accumulation in breast tissue and the incidence of breast cancer in postmenopausal women (12), estimated CLA intake was reported to demonstrate a positive (albeit weak) relation with breast cancer incidence in the Netherlands Cohort Study (13). Thus, there is insufficient evidence from epidemiologic studies in humans, at this time, to support the anticarcinogenic properties of CLA demonstrated in animal and tissue culture studies.

The biochemical mechanisms whereby dietary CLA and n−3 fatty acids inhibit carcinogenesis are not established. Fatty acids could influence tumor growth by way of a direct effect on the tumor or by way of their effects on immunosurveillance in the host. This report uses the literature about n−3 fatty acids to explore mechanisms by which CLA might influence tumor growth and immune function.

EFFECTS OF FATTY ACIDS ON TUMORIGENESIS
It is well established that feeding DHA and EPA (from 5% to 20% wt:wt, as fish oil) reduces the growth of tumors in rodents, including tumors of the mammary gland (2, 14), colon (2), prostate (15), liver (16) and pancreas (17). Work in human cancer cell lines has convincingly demonstrated that both the long-chain polyunsaturated n−3 fatty acids DHA and/or EPA can reduce the growth of many different human tumor types, including breast (18), colon (19), pancreatic (20), chronic myelogenous leukemic (21), and melanoma (22) cell lines.

Considerable evidence demonstrates that dietary CLA inhibits the initiation, after initiation, or promotion stages of carcinogenesis, as well as some evidence that it can influence cancer progression [reviewed by Belury (23)]. Feeding a synthetic mixture of CLA isomers (45% c9,t11-CLA, 42% c10,t12-CLA, with several other remaining isomers comprising minor amounts) at 0.5–1.5% (wt:wt) in nutritionally complete semipurified diets...
either during or after chemical carcinogen treatment inhibited tumorigenesis in the mammary gland, colon, skin, and prostate (5–7, 24, 25). Although there is considerable evidence of the antitumor growth effects of CLA on tumor cell lines and rodent models, CLA was not demonstrated to inhibit tumor growth in all animal models. Feeding CLA did not impede the development of aberrant crypt foci after azoxymethane treatment (26), the occurrence of liver metastasis in ductal pancreatic cancer in rodents (27), tumorigenesis in the ApcMin mouse (28), or the growth of an aggressive mammary (29) or prostatic (30) tumor. Recently, it was reported that the t10,c12-CLA might even act as a cancer promoter in colon carcinogenesis in the Min mouse, possibly through pathways affecting nuclear factor-κB and cyclin D1 (31). Both the c9,t11- and the t10,c12-CLA isomers and mixtures of these isomers were demonstrated to reduce the proliferation of many tumor cell lines in culture (32, 33), and adding either of the major isomers of CLA to the diet results in a similar inhibition of mammary tumorigenesis (34, 35). In vivo CA was also demonstrated to inhibit the growth of transplanted prostatic (10) and mammary (9) tumor cell lines. Feeding CLA (1.0 g/100 g diet) reduced the proliferation of terminal-end bud and lobuloalveolar bud structures in the mammary gland of rats (36). These are the sites at which tumors form in both rat and human mammary cancers. Evidence also suggests that CLA could influence the progression (metastasis) of mammary rodent tumors (5, 37).

Fatty acids and their effect on tumor cell growth

As illustrated in Figure 1, fatty acids could alter the growth of tumor cells by 1) influencing cell replication by interfering with components of the cell cycle or 2) increasing cell death, either by way of necrosis or apoptosis.

Effects on cell replication

Cellular replication is composed of several distinct phases (Figure 1): G1 is an initial growth phase that leads to DNA synthesis (S phase), followed by a gap phase (G2), and finally by mitosis (M phase), the actual segregation of chromosomes and cytoplasm (38). Two important families of regulatory molecules promote progression through the cell cycle, the cyclins and the cyclin-dependent kinases (cdk’s) (38). Normal cells progress through the cell cycle after stimulation of these regulatory molecules by exogenous agents such as growth factors, hormones, or cytokines (38). Cancerous cells, however, appear to lose their dependency on these external signals and often progress, unregulated, through many cell cycles (39). Multiple specific mutations in the genes encoding proteins that normally play a role in regulating the progression of cells through the cell cycle are identified in tumor cells (40).

Some evidence exists that n–3 fatty acids have an effect on tumor cell progression through the cell cycle, but the evidence in vivo is still preliminary. In vitro DHA treatment arrested progression through the cell cycle in human-derived MCF-7 breast cancer (41) and malignant melanoma (22) cell lines. Similarly, EPA treatment in vitro is reported to arrest the growth of K-562 human leukemic (21), pancreatic (42), and colon (19) cancer cell lines in different phases of the cell cycle, correlating with a downregulation of cyclin protein expression in some instances (21). In vivo, fish oil fed to rats implanted with a mammary tumor cell line prolonged the DNA replication time of the tumor cells, supporting the hypothesis that n–3 fatty acids could slow down progression through S phase (43).

Considerable in vitro work suggests that incubating tumor cells with CLA alters the expression of key proteins that regulate the cell cycle [reviewed by Belury (23)]. An in vitro study suggests c9,t11-CLA affects cyclins, cdk inhibitors, and check point proteins (44). Work by Ip et al (45) demonstrated that feeding CLA or c9,t11-CLA–rich butter fat for 4 wk reduces the expression of cyclins D and A in the terminal-end buds and alveolar clusters of the mammary epithelium. Cyclins D and A are key proteins involved in facilitating entry of the cells into the cell cycle and progression through S phase, respectively (46). CLA feeding was also shown to up-regulate the expression of p53 [reviewed by Belury (23)], the protein product of a tumor suppressor gene that is frequently mutated in many tumor cells (47).

p53 Is involved in monitoring the quality of DNA after G1 phase and, if DNA is damaged, will block entry of the cell into S phase (Figure 1) by altering the expression of genes involved in growth arrest and promotion (38). p53 Can also induce genes belonging to a family of regulatory molecules known as cdk inhibitors (46). In the study by Ip et al (45), there was a trend toward an increase, although not statistically significant, in the proportion of cells from the CLA-fed rats that expressed the p16 and p27 cdk inhibitors. Together, these data suggest that CLA could reduce tumor cell proliferation by modifying cell cycle proteins that regulate this process.

Effects on cell death

Cell death can occur by way of necrosis or apoptosis. Necrosis generally results from an insult or toxicity reaction and triggers inflammation, whereas apoptosis describes the distinct energy-requiring process of programmed cell death, characterized by DNA fragmentation, chromosome condensation, nuclear membrane...
fragmentation, formation of apoptotic bodies, and inversion of phosphatidylserine in the plasma membrane.

**Necrosis.** It was reported that many tumor cells do not possess sufficient antioxidant defense systems when compared with healthy cells, and so they are more susceptible to oxidative and peroxidative damage (48). Polyunsaturated fatty acids (PUFAs) are the main intracellular substrates for lipid peroxidation; thus, PUFAs-derived reactive lipid compounds could damage cell membranes, change the cellular composition or cytoskeletal assembly, modify membrane transport systems or enzymes, or inhibit polymerase reactions and/or polyamine synthesis (49). Therefore, it is reasonable to expect that PUFAs-enriched tumor cells might have an increased susceptibility to oxidant stress. There is evidence for an oxidative effect of n–3 fatty acids from both in vitro (20, 50, 51) and in vivo studies (49, 52). However, it is not entirely clear that lipid peroxidation is cytotoxic to cells (51), and, recently, the specificity of this effect in the in vitro work was questioned (53). Studies in our own laboratory demonstrated that the addition of an antioxidant (vitamin E) to the culture media does not abrogate the growth-inhibitory effects of n–3 fatty acids on breast cancer cell growth (unpublished data, 2004), suggesting that the growth inhibition observed with n–3 fatty acids cannot be attributed to lack of oxidative defense.

Early studies suggested that an oxidative mechanism was involved in the growth-suppressive effects of CLA. Supplementation of cell culture medium with mixed isomer CLA (17–71.5 μmol/L) was reported to increase the susceptibility of tumor cells to lipid peroxidation (54–56). Despite this early interest in oxidative stress on tumor growth, subsequent studies suggested that CLA does not act directly as a pro-oxidant (57). However, CLA enrichment in membranes can result in the production of conjugated diene hydroperoxides (58). These compounds could result in cytotoxic effects or could simply contribute to generating an internal cellular pro-oxidant milieu that influences growth-regulatory signals (59). This indirect oxidative function is supported in vivo in which feeding CLA to healthy subjects was reported to induce both nonenzymatic and enzymatic lipid peroxidation (60). Contrary to the tumor peroxidation hypothesis, CLA enrichment in nontumor tissues was reported to increase these tissues’ oxidative stability (54). This stability is suggested to be due to the decrease in linoleic acid metabolites (particularly arachidonic acid) when CLA concentrations are increased in tissues (61). It was suggested that the different isomers could have different oxidative properties (in healthy tissues), and the proportion of n9,11-CLA to other CLA isomers, in particular 3,12-CLA, could alter the balance between anti- and pro-oxidant susceptibility (54).

**Apoptosis.** In vivo, feeding DHA, EPA, or a mixture was demonstrated to increase the rate of apoptosis of tumor cells in rodent models, including tumors of the mammary gland (62, 63), liver (16), and colon (64, 65). Similarly, adding EPA or DHA to culture media was demonstrated to induce apoptosis in breast (66), colon (19, 64, 67, 68), lymphoma (69), leukemic (70, 71), pancreatic (20, 42), and melanoma (22) human cancer cell lines. The mechanism of induction of apoptosis by n–3 fatty acids is unknown but was suggested to involve n–3–mediated changes in membrane fluidity or structure; products of PUFA metabolism such as lipid peroxides, aldehydes, prostataglandins, or leukotrienes; or synthesis of reactive oxygen species (20).

Similarly, feeding CLA was reported to induce apoptosis in mammary (72), colon (73), and adipose (74) tissues. Providing CLA in vitro induced apoptosis in breast (75), SGC-7901 (46), and HT-29 (76) tumor cells. Although most studies used a mixture of isomers, the effects of CLA on breast or stomach tumors were shown for the c9,t11-CLA (44, 75, 77) and c10,c12-CLA (75, 77) isomers. Recently, it was suggested that a 50:50 mixture of the 2 main CLA isomers was more effective than individual isomers at inducing apoptosis in breast cancer cell lines (75). As with n–3 fatty acids, the mechanism for the effects of CLA on apoptosis is not established. Data suggest that CLA could down-regulate ErbB3 signaling and the phosphoinositide 3-kinase and Akt pathway (76) and that it can decrease expression of bcl-2, a gene involved in suppression of apoptosis (72).

Recently, feeding CLA (as the 2 major isomers or as a mixture) was demonstrated to inhibit the expression of extracellular-regulated kinase 1 protein and to promote the expression of mitogen-activated protein kinase phosphatase-1 protein in a rodent model of forestomach neoplasia (77). Only a small effect of CLA was reported on induction of the apoptosis-promoting Bax protein (23). Recently, it was reported that the apoptosis induced by c9,t11-CLA in SGC-7901 cells could be due to the ability of this isomer to block progression through the cell cycle (44). Considerable data support that CLA can increase peroxisome-proliferator activated receptor-γ (PPARγ) expression in tissues [reviewed by Belury (78)], and PPARγ is reported to promote apoptosis in many tumor cell lines (79).

**Fatty acids and their effect on the immune system**

Immune surveillance, the ability to detect and destroy tumor cells, is an important role of the cellular arm of the immune system (80). T helper (CD4+1) and cytotoxic T lymphocytes (CD8+1) play a central role in tumor surveillance (80). There is a progressive decrease in many immune surveillance defenses in animal models of cancer (81) and humans with cancer (82). The influence of various fatty acids, most extensively n–3 PUFAs, on the functional responses of cells of the immune system was examined in vitro, in animal feeding studies, and in human intervention studies.

n–3 Fatty acids and immune function

Although it is well established that long-chain n–3 PUFAs can up-regulate anticancer defenses such as natural killer cell cytotoxicity and humoral and T cell responses [reviewed by Yaqoob (83)], the application of studies in healthy humans and animals to cancer may not be as straightforward. For example, our research has demonstrated that the influence of dietary n–3 PUFAs on the immune response differs between healthy animals and animals with suppressed immune systems (14, 84). Additionally, the amount and the mixture of fatty acids in the diet, particularly the content of n–6 fatty acids, influences the immune effect that results after feeding n–3 fatty acids. Tumor-bearing rats fed long-chain n–3 PUFAs as part of a low-PUFA diet had significantly increased natural killer cell cytotoxicity, a higher proportion of CD8+1 and CD28+1 cells that were activated (ie, expressing CD25) and increased nitric oxide and interleukin 2 (IL-2) production after mitogen stimulation, whereas these immune enhancements were not found when n–3 PUFA was supplemented in a high-PUFA diet (84).

**Conjugated linoleic acid isomers and immune function**

Several studies reported immunologic effects of mixtures of CLA isomers in poultry, rodents, guinea pigs, and pigs [reviewed
by Sebedio (85)]. Although not directly related to anticancer defense, there are reports of beneficial effects of feeding CLA to animals and rodents on inflammatory-induced growth suppression (86), endotoxin-induced anorexia (87), mucosal damage and growth failure in experimental colitis (88), and antigen-induced type 1 hypersensitivity response (89, 90). However, feeding mixtures of CLA did not affect the resistance of mice to infection with *Listeria monocytogenes* (91). The effects on various immune characteristics from feeding and in vitro experiments are presented in Table 1.

Although it is not always possible to translate in vitro measures specifically to in vivo function against a tumor, all of the findings reported in Table 1 would generally be regarded as beneficial to cancer prevention. Interestingly, in the one study, the effects of CLA on cellular immunity were found to remain for some time beyond the period of dietary supplementation (96). To our knowledge no studies examined the effect of feeding CLA on immune function in the presence of a tumor. As evident in Table 1, few studies were conducted with use of single isomers. However, the results of a recently published randomized double-blinded clinical trial suggest that, unlike most of the tumor studies, the individual isomers could act differently on components of the immune system. In that study, providing 1.6 g/d CLA for 12 wk, a 50:50 mixture of CLA, but not an 80:20 (51:50) mixture, improved the proportion of individuals producing a protective antibody titer to hepatitis B vaccination. Interestingly, in those healthy subjects, other aspects of immune function (delayed-type hypersensitivity responses, natural killer cell activity, lymphocyte proliferation, and production of tumor necrosis factor α, IL-1α, IL-6, interferon-α, IL-2, IL-4, and prostaglandin E2) were not affected (100). These results are consistent with an earlier report (101).

### MECHANISMS TO EXPLAIN THE EFFECTS OF FATTY ACIDS ON IMMUNE AND TUMOR CELLS

Although it is widely recognized that dietary fatty acids can potentially alter immune and inflammatory responses and tumorigenesis, current understanding of the cellular mechanisms is incomplete. Several candidate mechanisms are proposed, including alterations in membrane structure and composition, changes in membrane-mediated functions and signals (eg, proteins, eicosanoids), changes in gene expression, and effects on the development of the immune system. As the evidence for the potential mechanisms for n−3 fatty acids on tumor growth and immune function is the subject of several excellent recent reviews (83, 102–104), this section uses that information to explore the evidence for CLA.

#### Changes in membrane composition

Immune cell activation [cell proliferation, phagocytosis (105)] and tumor growth [malignancy (106)] result in an increased rate of novo synthesis and turnover of membrane phospholipids. These processes require a constant supply of fatty acids, the main supply being those consumed in the diet. It is well established that both the amount and type of fat consumed in the diet influence the lipid composition of immune (107, 108) and tumor (16, 109–111) cell membranes. Changes in membrane composition would affect growth, interaction with other cells (immune system), and the function of proteins and other components that are in the membrane. The function of the immune system depends on interactions between different cell types and through effects on membrane composition; dietary fatty acids have the potential to influence these interactions (83). Considerable evidence supports this mechanism for n−3 fatty acids [reviewed by Yaqoob (83)]. Although little work demonstrates the incorporation of CLA isomers into immune cell membranes,

### TABLE 1

<table>
<thead>
<tr>
<th>Immune defense</th>
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<tr>
<td><strong>T-cell function</strong></td>
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<tr>
<td>Improved blastogenesis response to mitogens</td>
<td>Mixtures of isomers: Wong et al (29); t10,c12-CLA reviewed in Pariza et al (92)</td>
</tr>
<tr>
<td>Increased IL-2 production in response to mitogens</td>
<td>Mixtures of isomers: Wong et al (29), Hayek et al (93)</td>
</tr>
<tr>
<td>Increased production of other cytokines in response to mitogens</td>
<td>c9,t11-CLA: Kelley et al (94), Yamasaki et al (95); t10,c12-CLA: Kelley et al (94)</td>
</tr>
<tr>
<td>Increased delayed-type hypersensitivity response</td>
<td>Mixtures of isomers: Whigham et al (89), Miller et al (87), Wong et al (29)</td>
</tr>
<tr>
<td>Improved cytotoxic T-cell function: increased ability of CD8αβ lymphocytes to proliferate and release granzyme with stimulation</td>
<td>Mixtures of isomers: Bassaganya-Riera et al (96)</td>
</tr>
<tr>
<td>No effect on lymphocytes cytotoxicity</td>
<td>Mixtures of isomers: Wong et al (29)</td>
</tr>
<tr>
<td>Increased proportion of CD8+</td>
<td>Mixtures of isomers: Bassaganya-Riera et al (96, 97); c9,t11-CLA: Yamasaki et al (95)</td>
</tr>
</tbody>
</table>

**Humoral function**

| Temporary increase in IgA production                                           | Mixed isomers: Yamasaki et al (95, 98); t10,c12-CLA: Yamasaki et al (95) |
| Mixed isomers: Bassaganya-Riera et al (96, 97); c9,t11-CLA: Yamasaki et al (95) |

**Macrophage function**

| Reduced the production of inflammatory mediators (including eicosanoid synthesis and production) | Mixtures of isomers: Sebedio et al (85), Cook et al (86), Miller et al (87) |
| Reduced inflammatory cytokine production after stimulation (TNF-α, IL-1, IL-6, nitric oxide) | Mixed isomers: Yu et al (99), Yang et al (25); c9, t11-CLA: Yang et al (25) |
| Reduced nitric oxide production after stimulation                                 | Mixed isomers: Yang et al (25) |

**Tumor cell function**

| Reduced nitric oxide production after stimulation (including eicosanoid synthesis and production) | Mixtures of isomers: Sebedio et al (85), Cook et al (86), Miller et al (87) |
| Reduced the production of inflammatory mediators (including eicosanoid synthesis and production) | Mixed isomers: Yu et al (99), Yang et al (25); c9, t11-CLA: Yang et al (25) |

| Reduced the production of inflammatory mediators (including eicosanoid synthesis and production) | Mixed isomers: Yu et al (99), Yang et al (25); c9, t11-CLA: Yang et al (25) |
| Reduced the production of inflammatory mediators (including eicosanoid synthesis and production) | Mixed isomers: Yu et al (99), Yang et al (25); c9, t11-CLA: Yang et al (25) |

**IL, interleukin; TNF-α, tumor necrosis factor-α.
it can likely be assumed, as it is well established, that feeding CLA isomers is associated with accumulation of CLA and its metabolites in many other tissues and cell types (61, 112). Our work (33) and that of others (59) demonstrated that CLA is rapidly incorporated into the functionally important tumor cell membrane lipids (phospholipids). Our results suggest that CLA isomers (both major isomers) are specifically replacing the essential fatty acids arachidonic and linoleic acids in phospholipids (33). Data exist to suggest that the different isomers might be incorporated at different rates (4, 5, 33).

Lipid rafts are dynamic microenvironments in the exoplasmic leaflets of the phospholipid bilayer of plasma membranes, which are thought to preferentially group transmembrane proteins according to their function (113). Several proteins involved in signaling are commonly found in lipid rafts, and many of these proteins are palmitoylated (114). Activation of the proteins within rafts by an extracellular ligand can result in rapid clustering of the membrane protein stearoyl-CoA desaturase (131) and replacement of arachidonate-derived eicosanoids (prostaglandin F2, leukotriene B4, and thromboxane A2) by CLA (115, 116), offering a logical yet unexplored link between changes in the CLA content of cell membranes and changes in cellular function.

Changes in membrane-mediated signals (signal transduction) and proteins

Changes in plasma membrane structural characteristics in mammalian cells can change the activity of proteins that serve as ion channels (117), transporters (117), receptors (118), signal transducers (119), or enzymes (120). Dietary lipids were demonstrated to influence the pattern of fatty acids released from lymphocytes (ie, arachidonic acid) (121), which would ultimately influence the synthesis of eicosanoids (prostaglandins, leukotrienes, thromboxanes). In addition to their role in regulation of immune and inflammatory responses (83), eicosanoids may also be needed to sustain growth of tumor cells (122).

Long-chain n−3 fatty acids were shown in immune cells to alter cell surface costimulatory and activation markers or molecules (123, 124), calcium signaling (125), and protein kinase C translocation in the membrane (126). Similarly, in other cell types, membrane incorporation of n−3 fatty acids can alter membrane permeability (127), membrane fluidity (128, 129), and hormone and growth factor binding (130).

It is well accepted that genotype influences the metabolism of most nutrients and the susceptibility to cancer. Although it is well established that the immune system is responsive to changes in nutrient intake and that it plays an important role in tumor surveillance, few studies examine the effect of CLA on immune surveillance during cancer or in people at high risk.

The results of many of the studies suggest that the underlying mechanism for CLA isomers may not be that different. Further work on membrane-mediated effects (ie, lipid rafts) and PPAR-dependent and-independent mechanisms are needed.

1 PPAR, peroxisome-proliferator activated receptor.

**TABLE 2**

<table>
<thead>
<tr>
<th>Question</th>
<th>Explanation/rational</th>
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<tr>
<td>Do the isomers of CLA have different physiologic effects?</td>
<td>Studies tended to use CLA preparations that contained mixtures of many isomers, making it impossible to ascertain which isomer was responsible for the observed physiologic effects. Although both isomers appear to have anticancer properties, there is evidence that the mechanisms could differ. There is a need to conduct animal and human studies that use highly pure preparations of single isomers of CLA.</td>
</tr>
<tr>
<td>Does the biological form of CLA consumed influence its activity?</td>
<td>CLA is available in synthetic form and in ruminant products. Although the chemical isomers could be the same, the form in which they are consumed is different (free fatty acids esterified or nonesterified, phospholipid, positional triacylglycerol). The effect of this form on the digestion, absorption, and incorporation into specific lipids in tumors and immune cell lipids has not been established.</td>
</tr>
<tr>
<td>What is the effect of an intake of CLA over a lifetime?</td>
<td>Studies are needed to characterize the effect of consumption of CLA during development and differentiation of the immune system and the tissues of common cancer sites.</td>
</tr>
<tr>
<td>What is the influence of genotype on the metabolism and physiologic effect of consuming CLA?</td>
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</tr>
<tr>
<td>What is the impact of dietary CLA on cancer surveillance?</td>
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<tr>
<td>What are the mechanisms by which CLA inhibits tumor growth?</td>
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**Effect of fatty acids on gene expression**

n−3 Polyunsaturated fatty acids

Considerable evidence indicates that n−3 PUFAs are capable of inducing changes in gene expression in several different cell
types, including tumor and immune cells. The list of genes whose expression appears to be affected by fish oil or purified n-3 fatty acids continues to grow, and excellent reviews have been published (136–138). Although the exact way in which n-3 fatty acids alter gene transcription is not known, there is considerable speculation and new evidence that this alteration might involve a class of nuclear receptors called PPARs. PPARs are ligand-activated transcription factors present in a variety of cell types, with diverse actions, mainly in lipid metabolism (83, 139). Activators of both PPARα and PPARγ were shown to inhibit the activation of several inflammatory genes [reviewed by Berger and Moller (140)]. n-3 Fatty acids can activate PPARs by directly binding to them (141) or by binding their cyclooxygenase and lipoygenase metabolites (142).

**Conjugated linoleic acid**

Evidence supports an effect of CLA on gene expression in tumor cells, because CLA was demonstrated to influence the expression of genes of the cycle (described in Effects on cell replication), thereby regulating cell growth and differentiation (78). Recently, a mixture of CLA isomers was reported to regulate the expression of major oncoproteins involved in cell survival and programmed cell death signaling in human mammary cell lines [MCF-7, MDA-MB-231, and MCF-10a cells (75)]. Isomers of CLA have moderate affinity, compared with n-3 fatty acids, for PPARs [reviewed by Belury (78)]. Evidence is accumulating that activators of PPARs are protective against cancers arising in the mammary gland, colon, and prostate (143). It was suggested that CLA could both change the level and alter the activation of several PPARs (78). Although there is not a great deal of experimental support at the present time for CLA modulation of PPARs on immune metabolism, data do suggest that the anti-inflammatory effects of CLA in a macrophage cell line are mediated, at least in part, through changes in PPAR expression (99).

**Effect of fatty acids on development of the immune system**

Adult immune defenses develop during the first few years of life (144) and are influenced to some extent by the intake of polyunsaturated fats (108, 144). Epidemiologic data suggest that diet, particularly lipids, early in life influences cancer incidence (145, 146). To our knowledge no work is aimed at determining the effects of feeding CLA on immune development. Exciting data suggest that feeding CLA early in life alters the development of the mammary gland in rodents (147). Feeding CLA during mammary gland development in rats resulted in diminished mammary epithelial branching, possibly contributing to the reduction in mammary cancer risk in these rats (45). Thus, in rats, optimal CLA nutrition during pubescence could conceivably control the population of cancer-sensitive target sites in the mammary gland.

**Conclusions and remaining questions**

Considerable work has been done to demonstrate the potential importance of CLA as an anticancer treatment. There are many clues as to how this molecule might mediate its effects on the tumor and immune system. Many of these effects parallel our current understanding of the anticancer effects of long-chain polyunsaturated n-3 fatty acids. Despite the growth in research in this area, many questions remain (Table 2). Answers to these questions are required to provide the rationale to move CLA and cancer research from the culture plate and animal model to human trials.

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