Conjugated Linoleic Acid Induces Mast Cell Recruitment during Mouse Mammary Gland Stromal Remodeling¹,²

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Abstract

Conjugated linoleic acid (CLA) is a dietary chemopreventive agent that induces apoptosis in the mammary adipose vascular endothelium and decreases mammary brown adipose tissue (BAT) and white adipose tissue (WAT). To determine onset and extent of stromal remodeling, we fed CD2F1/Cr mice diets supplemented with 1 or 2 g/100 g mixed CLA isomers for 1–7 wk. BAT loss, collagen deposition, and leukocyte recruitment occurred in the mouse mammary fat pad, coincident with an increase in parenchymal-associated mast cells in mice fed both levels of CLA. Feeding experiments with purified isomers (0.5 g/100 g diet) demonstrated that these changes were induced by trans-10, cis-12 CLA (10,12-CLA), but not by cis-9, trans-11 CLA (9,11-CLA). This stromal remodeling did not require tumor necrosis factor (TNF)-α, a major cytokine in mast cells, as TNF-α null mice demonstrated collagen deposition, increased leukocytes, and BAT loss in the mammary fat pad in response to 10,12-CLA. To test the hypothesis that mast cells recruited in response to 10,12-CLA were required for stromal remodeling, Steel mice [WBB6F1/J-kitW/kitW-V], which lack functional mast cells, were examined for their stromal response to 10,12-CLA. Both wild-type and Steel mice showed a significantly increased leukocytic adipose infiltrate, collagen deposition, and decreased adipocyte size, although BAT was maintained in Steel mice. These results demonstrate that 10,12-CLA induces an inflammatory and fibrotic phenotype in the mouse mammary gland stroma that is independent of TNF-α or mast cells and suggest caution in the use of 10,12-CLA for breast cancer chemoprevention. J. Nutr. 137: 1200–1207, 2007.

Introduction

The mammary gland is a complex tissue composed of epithelium embedded in a stromal matrix containing fibroblasts, adipocytes, blood vessels, and lymphatics, as well as a variety of infiltrating leukocytes (1,2). Although most cancers of the breast arise in the epithelium, the stroma has potential to alter both the progression or suppression of epithelial cancers [reviewed in (3)]. The local stromal environment has been found to influence mammary epithelial morphogenesis (4–8), hormonal responsiveness (9), and functional differentiation (6) in vivo. Stromal cell populations undergo changes in abundance and phenotype during the extensive postnatal development that occurs in the mammary gland during pregnancy and lactation (10,11). The hormone-dependent stromal plasticity of the adult mammary gland may reflect a potential readiness of the stroma to be modified, in a positive fashion, by chemopreventive agents.

In addition to adipocytes and fibroblasts, other cells have the potential to modify the epithelium directly, or indirectly, through the stroma. Peripheral blood-derived leukocytes (PBL),⁶ including lymphocytes, monocytes, and polymorphonuclear leukocytes (PMN), which include eosinophils, neutrophils, and granulocytes, can infiltrate both the adipose stroma (the fat tissue occupying the regions between ducts and lobules) and the parenchymal stroma (the sheath of fibrocellular stroma that surrounds the ductal and lobular epithelium). Tissue resident leukocytes, such as macrophages and mast cells, may also play a pivotal part in augmenting this infiltration by secreting cytokines, such as tumor necrosis factor (TNF)-α, which recruit PBL (12). Cooperation between tissue resident leukocytes (mast cells and macrophages) and PBL (PMN and lymphocytes) in mammary gland remodeling has been previously demonstrated; macrophages and eosinophils are required for the terminal branching that is essential for full epithelial development (13). Like macrophages, mast cells are abundant mammary gland

¹ Supported by NCI CA61763 (M.I.), AI/CR-02A112-REN (M.I), and by the shared resources of the NCI Cancer Center Support Grant CA 16056 to Roswell Park Cancer Institute.

² Author disclosures: J. S. Russell, no conflicts of interest; S. O. McGee, no conflicts of interest; M. M. Ip, no conflicts of interest; D. Kuhlmann, no conflicts of interest; and P. A. Masso-Welch, no conflicts of interest.

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⁶ Abbreviations used: BAT, brown adipose tissue; CLA, conjugated linoleic acid; 9,11-CLA, cis-9, trans-11-CLA; 10,12-CLA, trans-10, cis-12-CLA; PBL, peripheral blood-derived leukocytes; PMN, polymorphonuclear leukocyte; TNF, tumor necrosis factor; WAT, white adipose tissue.
resident cells. However, unlike macrophages, mast cells are proliferation competent and contain large abundant granules of concentrated bioactive compounds, including leukocyte recruiting cytokines (eotaxin, tryptase) (14,15), vascular remodeling and vascular permeability factors (vascular endothelial growth factor, heparin, TNF-α, histamine) that stimulate fibroblast proliferation and extracellular matrix deposition (16), and enzymes such as matrix metalloproteinases. All of these can potentially contribute to mammary stromal remodeling (17).

Stromal remodeling has been documented to occur in the presence of several chemopreventive compounds, including conjugated linoleic acid (CLA) [reviewed in (3)]. CLA is a family of fatty acids containing 18 carbons with 2 double bonds conjugated linoleic acid (CLA) [reviewed in (3)]. CLA is a potentially contribute to mammary stromal remodeling (17).

Materials and Methods

Animals and diets
Expt. 1: Effect of mixed CLA isomers on mammary gland remodeling. Six-wk-old CD2F1/Cr female mice (National Cancer Institute, Frederick Cancer Research Facility, Biological Testing Branch, Frederick, MD) were fed semisynthetic AIN-76A diets containing 5 g/100 g corn oil, without or with supplementation with 1 or 2 g/100 g CLA, as described previously (22,23). CLA was obtained from Nu-Chek Prep., and contained 85–88% 9,11- and 10,12-CLA at an 1:1 ratio, as well as trace amounts of other isomers. The mice were killed by cervical dislocation 1, 3, 5, or 7 wk after initiation of diet and the mammary glands were fixed in formalin for paraffin embedding. A total of 120 mice were used, with 10 mice per diet and time point.

Expt. 2: Effect of purified CLA isomers on mammary gland remodeling. A second group of 6-wk-old female CD2F1/Cr mice was used to determine isomer-specific effects of CLA. Eight mice per group were fed AIN 76A diets, supplemented with 0, 0.5, or 1 g/100 g of either 9,11-CLA or 10,12-CLA. The purified CLA isomers were obtained from Natural ASA and were of >90% purity. Mice were killed by cervical dislocation at 3 d, 7 d, or 7 wk after initiation of diet.

Expt. 3: Comparison of effects of dietary 10,12-CLA on mammary gland remodeling in TNF-α null and wild-type mice. To determine the TNF-α requirement for mammary gland remodeling, we used 7th generation C57BL/6-background TNF-α null mice and their 7th generation TNF-α wild-type controls. These mice were originally obtained from Dr. George Kollias (Alexandros Fleming Biomedical Sciences Research Center, Vari, Greece) at the C57BL/6 sixth generation and were bred in a conjugated configuration. Of the potential isomers, family of fatty acids containing 18 carbons with 2 double bonds conjugated linoleic acid (CLA) [reviewed in (3)]. CLA is a potentially contribute to mammary stromal remodeling (17). All of these can potentially contribute to mammary stromal remodeling (17). Enzymes such as matrix metalloproteinases. All of these can potentially contribute to mammary stromal remodeling (17).

Leukocyte infiltration of the WAT
The cellular infiltration of the WAT was quantified by assessing the number of nuclei per 40× (Fig. 2, Table 6) or 20× objective field (Table 5). The maximum vertical and horizontal diameters of 15–20 randomly selected adipocytes per field (each completely contained within the photographic field) were measured. The results were then averaged to obtain the mean value of adipocyte diameter.

Masson’s trichrome staining
Collagen was visualized in paraffin sections using the revised Masson’s trichrome staining protocol, with Light Green dye rather than Aniline Blue dye (24). Using this procedure, we could distinguish multiple cellular and tissue structures, with collagen and reticulin staining greenish-blue [blue using the original aniline blue protocol (24)], nuclei staining black, and cytoplasm, keratin, muscle fibers, and intercellular fibers staining from red to pink.

Adipocyte diameter
Hematoxylin and eosin-stained paraffin sections were prepared from the number 4 mammary glands of mice killed 7 d after dietary intervention. Nine fields of unilocular fat per mouse were photographed under 20× objective magnification under bright field illumination using a Hitachi KPD-50 camera connected to an Olympus BX40 microscope. The maximum vertical and horizontal diameters of 15–20 randomly selected adipocytes per field (each completely contained within the photographic field) were measured. The results were then averaged to obtain the mean value of adipocyte diameter.

Mast cell staining and analysis
To analyze mast cell recruitment, we stained paraffin sections (5 µm thick) using the May-Grünwald Giemsa staining protocol, which causes the cell nuclei to stain blue, the cytoplasm to stain pink, and the mast cells to stain purple with magenta-stained granules. Mast cell abundance was evaluated under a microscope using a 20× field of view (200× total magnification). The following items were analyzed: 1) mast cells per duct; 2) mast cells per lobule; 3) ductules per lobule; and 4) mast cells per lobular ductule. At least 10 ducts per mammary gland were analyzed.
Expt. 3, 18–24 fields from 3–4 mice per group were quantified and, in Expt. 4, 18–27 fields from 3 mice per group were quantified.

Analysis of BAT abundance
The evaluation of percentage fields containing BAT was performed as described (23). Briefly, mammary BAT was defined as regions of multilocular fat cells with a central nucleus, supported by an abundant capillary network. All adipose stromal fields present in the paraffin section were evaluated for the presence or absence of BAT under a 20× objective. The number of fields examined were as follows. Expt. 3: n = 155, n = 125, n = 220, n = 175 fields analyzed for TNF+/+ control, TNF−/+ 10,12-CLA, TNF−/− control, TNF−/− 10,12-CLA, respectively (3 mice per group); Expt. 4: n = 174, n = 130, n = 178, n = 129 fields analyzed for control wild-type, control 10,12-CLA, Steel control, and Steel 10,12-CLA groups, respectively (n = 3 mice per group, except for Steel 10,12-CLA, where there were n = 4 mice per group).

Statistics
Means for each mouse were calculated for statistical analysis. Data were analyzed using Minitab software as follows. For single variables, we performed 1-way ANOVA, followed by Dunnett's test; for mast cell number, leukocyte infiltration, % BAT, and Expt. 1, for weights and mast cell numbers, compared with control, performed 1-way ANOVA, followed by the following post hoc tests: analyzed using Minitab software as follows. For single variables, we

Means for each mouse were calculated for statistical analysis. Data were analyzed using Minitab software as follows. For single variables, we performed 1-way ANOVA, followed by Dunnett's test; for mast cell number, leukocyte infiltration, % BAT, and Expt. 1, for weights and mast cell numbers, compared with control, performed 1-way ANOVA, followed by the following post hoc tests: analyzed using Minitab software as follows. For single variables, we

Within and adjacent to this region of collagen deposition, cellular infiltration occurred in the adipose compartments (Fig. 1E,F, arrowheads). The WAT is normally a paucicellular environment, consisting of adipocytes and stromal vascular stem cells interlaced with blood vessels, and lymphatics. The cellular infiltrate consisted largely of PMN, which are defined morphologically as small round cells with condensed chromatin and a multi-lobed nucleus.

10,12-CLA, but not 9,11-CLA, induces PMN infiltration and collagen deposition in the WAT. To determine which isomer of CLA was responsible for collagen deposition and PMN recruitment to the mammary fat pad, we fed CD2F1/Cr mice purified isomers of CLA and the mice were analyzed for changes in the fat pad at early (d 7) and later (wk 7) time points after CLA supplementation was initiated (Expt. 2). No alterations in body weight occurred except at wk 7, and then only in the group receiving the highest dose of 10,12-CLA (1 g/100 g), which showed a modest but significant decrease (23). CLA induced deposition of fibril collagen in the white fat as early as 7 d after diet initiation in mice fed 0.5% 10,12-CLA (Fig. 2C, arrow), but not in those fed the control diet (Fig. 2A), or the diet supplemented with 9,11-CLA (Fig. 2B). These differences were maintained at wk 7 (Fig. 2D–F).

To quantify the changes in collagen deposition, we examined trichrome-stained serial paraffin sections. We evaluated WAT staining only (and ignored the interstitial protein clearly associated with trabeclae, large blood vessels, or surrounding the epithelium), and found the percentage of trichrome-positive WAT fields was 21.5 ± 2.9% (SEM), 16.5 ± 2.6% and 79.5 ± 2.9% in the control, 9,11-CLA, and 10,12-CLA groups. Both the 9,11-CLA group and the 10,12-CLA group differed from the control group (P < 0.05, n = 4 mice per group).

Isomer-specific effects of 10,12-CLA were also seen in the induction of PMN recruitment to the mammary WAT (Fig. 2C and F, arrowheads). 10,12-CLA supplementation significantly

### TABLE 1

<table>
<thead>
<tr>
<th>Time</th>
<th>CLA, g/100 g</th>
<th>g</th>
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<tr>
<td>wk</td>
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</tr>
<tr>
<td>0</td>
<td>19.8 ± 0.3</td>
<td>19.6 ± 0.2</td>
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<tr>
<td>1</td>
<td>21.1 ± 0.4</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>21.1 ± 0.2</td>
<td>20.2 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>22.4 ± 0.4b</td>
<td>20.8 ± 0.6b</td>
</tr>
<tr>
<td>7</td>
<td>23.5 ± 0.7b</td>
<td>21.2 ± 0.4b</td>
</tr>
</tbody>
</table>

*Values are means ± SEM (n = 10, except 0 wk: n = 40). Means in a row with superscripts without a common letter differ, P < 0.05.*
and dose-dependently increased leukocyte recruitment as early as d 7 (Fig. 2G). Leukocyte recruitment in the mice fed the 9,11-CLA supplemented diet was similar to that of the control group. CLA feeding rapidly induces an increase in mast cells associated with the mammary parenchymal stroma. Feeding mixed CLA isomers for 7 wk (Expt. 1) specifically increased mast cell staining in the parenchymal-associated stroma rather than the adipose stroma, with that in the fibrocellular stroma surrounding mammary ducts and lobules (Fig. 3, Tables 2 and 3) significantly greater than that in mice fed the control diets (Fig. 3A). As early as 1 wk after initiating dietary supplementation, the number of mast cells in the fibrocellular stroma surrounding each duct was greater in mice fed either the 1 g/100 g or 2 g/100 g mixed isomer CLA-supplemented diets than in those fed the control diet (Table 2). Although this CLA-dependent increase was maintained over the 7 wk feeding, a dose-dependent effect of CLA occurred only at wk 5 (Table 2, and Fig. 3).

Independent of diet, there were time-dependent increases in the background numbers of mast cells associated with the fibrocellular stroma (Table 2, first column). The number of mast cells per duct increased from wk 1 to wk 3 of diet treatment (P < 0.05) and, in fact, was almost doubled in the control dietary group, a time period which corresponds to an age of 7–9 wk. A smaller increase occurred in mice at later time points, suggesting that the greatest age-dependent increase in mast cells is associated with ductal growth during the peripubertal period. There was no interaction between diet and time.

To determine whether mast cell changes were also present in the lobular parenchymal stroma, we assessed the number of mast cells associated with the lobular stroma and expressed data

### TABLE 2
**Effect of mixed CLA isomers on the number of mast cells in the parenchymal stroma surrounding ducts in CD2F1/Cr mouse mammary glands (Expt. 1)**

<table>
<thead>
<tr>
<th>Time</th>
<th>CLA, g/100 g</th>
<th>0</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>wk</td>
<td>n/40x duct-containing field</td>
<td>n/40x duct-containing field</td>
<td>n/40x duct-containing field</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.20 ± 0.05</td>
<td>0.46 ± 0.09</td>
<td>0.64 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.44 ± 0.14*</td>
<td>1.19 ± 0.30h</td>
<td>1.82 ± 0.52b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.45 ± 0.10**</td>
<td>1.67 ± 0.29b</td>
<td>1.72 ± 0.18b</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.97 ± 0.25**</td>
<td>1.33 ± 0.23k</td>
<td>2.06 ± 0.44b</td>
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1 Values are means ± SEM (n = 10, except values with *: n = 9). Means in a row with superscripts without a common letter differ, P < 0.05.
per lobular duct to compensate for the different degrees of lobular development during the estrous cycle (25). Similar to its effects on the ductal stroma, CLA significantly increased mast cell number in the lobular stroma (Table 3).

10,12-CLA is the isomer responsible for the recruitment of mast cells. The ability of CLA to recruit mast cells into the ductal stroma was isomer specific (Table 4, Expt. 2). No change occurred in the mammary glands of mice fed 9,11-CLA at any time point examined. However, ductal mast cell number in the parenchymal stroma surrounding lobules in CD2F1/Cr mouse mammary glands (Expt. 1)1

| Table 3 | Effect of mixed CLA isomers on the number of mast cells in the parenchymal stroma surrounding lobules in CD2F1/Cr mouse mammary glands (Expt. 1)1
<table>
<thead>
<tr>
<th></th>
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<tr>
<td>CLA, g/100 g</td>
<td>n/lobular ductule in a 40× field</td>
</tr>
<tr>
<td>wk</td>
<td>Time</td>
</tr>
<tr>
<td>1</td>
<td>0.19 ± 0.05</td>
</tr>
<tr>
<td>3</td>
<td>0.32 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.26 ± 0.10</td>
</tr>
<tr>
<td>7</td>
<td>0.60 ± 0.25</td>
</tr>
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</table>

1 Values are means ± SEM (n = 10). Means in a row with superscripts without a common letter differ, P < 0.05.

Is TNF-α required for CLA-induced stromal remodeling? To determine whether TNFα, an abundant cytokine in mast cells, was required for CLA-induced leukocyte recruitment and subsequent mammary gland remodeling, we fed TNF null and wild-type mice the control or 0.5 g/100 g 10,12-CLA-supplemented diets for 7 d (Expt. 3). TNF deficiency did not alter adipocyte leukocyte recruitment (Table 4), protein deposition in the interstitial matrix of the mammary fat pad, or mast cell infiltration (data not shown) in mice fed 10,12-CLA. However, loss of brown fat in the mammary gland was incomplete when compared with TNF wild-type mice, which showed a total loss of mammary BAT during the same time period (Table 5).

Does mammary gland remodeling require mast cells? To determine whether an increase in mast cells was required for the stromal remodeling induced by 10,12-CLA, mast cell-negative Steel mice (WBB6F1/J-kit1−/kit2−) and wild-type (WBB6F1/J) mice were fed control or 0.5 g/100 g 10,12-CLA-supplemented diets for 7 d. The mammary glands were subsequently observed for stromal remodeling (Expt. 4). Similar to CD2F1/Cr mice, 10,12-CLA induced protein deposition in the WAT (Fig. 4, arrows) and leukocyte infiltration in the mammary glands (Fig. 4, arrowheads) of both wild-type and Steel mice. Interestingly, the baseline number of infiltrating cells was significantly greater and the starting adipocyte size significantly smaller in Steel mice than in control mice. Regardless, both Steel mice and their wild-type controls showed an increased leukocyte infiltration and a decrease in mean white adipocyte diameter in response to 10,12-CLA (Table 5). Feeding 10,12-CLA did not completely ablate BAT in the wild-type or Steel mice, but it did significantly decrease BAT in the wild-type mice (Table 5).

Discussion

Breast cancer incidence varies widely in different countries. One possible factor is societal differences in consumption of foods containing cancer-protective compounds such as CLA. However, epidemiological studies vary in their conclusions about the effects of consumption of CLA-rich foods, or of levels of serum or tissue CLA, on breast cancer risk. Although a case-control study demonstrated a decreased risk of breast cancer in women with a high intake of dairy products rich in CLA (26), other studies have not confirmed this (27–29); this is probably due to the small range of CLA intake. Despite the varied conclusions of

| Table 5 | 10,12-CLA induces PMN recruitment and decreases BAT in C57BL/6 TNF null mice (Expt. 3)1
<table>
<thead>
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<tbody>
<tr>
<td>Variable</td>
<td>Genotype</td>
</tr>
<tr>
<td>n/20× WAT field</td>
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</tr>
<tr>
<td>Nuclei, %</td>
<td>C57BL/6-TNF−/−</td>
</tr>
<tr>
<td>Fields with BAT, %</td>
<td>C57BL/6-TNF−/−</td>
</tr>
<tr>
<td>C57BL/6-TNF−/−</td>
<td>33.0 ± 9.4</td>
</tr>
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</table>

1 Values are means ± SEM (n = 3, except *: n = 4). Means for a variable without a common letter differ, P < 0.05.

FIGURE 4 The effect of 10,12-CLA on extracellular matrix deposition and PMN recruitment in the mammary fat pad of mast-cell negative Steel mice (WBB6F1/J-kit1−/kit2−) and their wild-type controls (WBB6F1/J) (Expt. 4). Analysis of mammary glands from mast-cell negative Steel mice after short-term CLA feeding (7 d with 0.5 g/100 g 10,12-CLA) revealed that interstitial matrix protein deposition and PMN recruitment occur in the absence of functional mast cells. Arrows indicate sites of protein accumulation; arrowheads show PMN.

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TABLE 6

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotype</th>
<th>Control</th>
<th>10,12-CLA</th>
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<tbody>
<tr>
<td>Nuclei, n/40&lt; WAT field</td>
<td>WBB6F1/J</td>
<td>19.4 ± 1.4a</td>
<td>66.8 ± 12.9f</td>
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<td>WBB6F1/J-kit&lt;kit-</td>
<td>32.9 ± 3.1b</td>
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<tr>
<td>WAT adipocyte diameter, μm</td>
<td>WBB6F1/J</td>
<td>86.8 ± 3.0a</td>
<td>79.4 ± 3.4f</td>
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<tr>
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<td>WBB6F1/J-kit&lt;kit-</td>
<td>79.3 ± 0.4b</td>
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<tr>
<td>Fields with BAT, %</td>
<td>WBB6F1/J</td>
<td>34.5 ± 4.7a</td>
<td>20.5 ± 6.9b</td>
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<td></td>
<td>WBB6F1/J-kit&lt;kit-</td>
<td>36.0 ± 4.3a</td>
<td>30.1 ± 2.8a</td>
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1 Values are means ± SEM (n = 3). Means for a variable without a common superscript letter differ, P < 0.05.

Although both wild-type and Steel mice retained BAT after 7 d of feeding, CLA-fed Steel mice showed an attenuation of BAT ablation compared with their wild-type (WBB6F1/J) counterparts, suggesting that mast cells contribute to the diet-induced remodeling of BAT that occurs in the mouse mammary gland. It is also evident that strain background plays a role in the remodeling, because ablation of BAT (and its supporting vasculature) in response to 10,12-CLA is normally brisk and virtually complete within 7 d in other mouse models, including C57BL/6 TNF-control mice (in this study) and in a larger study with CD2F1/Cr mice (23).

The isomer-specific PMN recruitment and collagen deposition in the mammary fat pad was associated with additional collagen deposition in the sheath of interstitial matrix surrounding the epithelial compartment and recruitment of mast cells to both the lobular and ductal parenchymal stroma. Parenchymal stroma-associated mast cells can act to recruit PMN to the adjacent adipose compartment, via the secretion of a number of biological effectors, including cytokines such as IL-4, IL-5, and TNF, eicosanoids, platelet activating factors, proteoglycans such as heparin, and proteases (36). The ability of 10,12-CLA to induce some mammary adipose remodeling (PMN recruitment and collagen I deposition) in mast cell–deficient Steel mice suggests that mast cells do not play a critical role in decreased adipocyte size or PMN recruitment to the mammary fat pad.

The function of the increased mast cells seen in response to 10,12-CLA is not known. Although 10,12-CLA has been associated with decreased angiogenesis and apoptosis of the adipose vasculature (23), mast cells are usually associated with increased angiogenesis (37,38). The overall role of mast cells as an accelerator or inhibitor of breast cancer is still unknown. Their association with breast cancer induction is suggested by several studies, including increased incidence of breast cancer in women with high histamine levels (39,40) and growth inhibition by mast cell stabilizers (41). Furthermore, mast cells accumulate at sites of hyperplasia (42,43), and at the tumor-host interface (17,44), and this accumulation has been associated with increased risk of metastasis (45,46).

In contrast, an increased number of mast cells and increased histamine levels were associated with a favorable prognosis and enhanced survival in a human breast cancer study of 187 women (47). It is noteworthy that this study showed no correlation between mast cell number and inflammatory infiltrates (47), suggesting that the presence of mast cells is not sufficient to indicate their functional activation state. Moreover, in a study of 483 women, tumor-free lymph nodes were found to have higher numbers of mast cells than lymph nodes containing breast cancer metastases (48). However, a study with 424 patients found no significant difference in 10-y disease-free survival in patients with high vs. low mast cell numbers associated with their breast tumors (49).

The conflicting literature regarding the ability of mast cells to act in a chemoprotective or cancer-promoting manner in the breast during cancer induction and/or progression suggests that the effects of the tissue-resident mast cells may depend on other factors in the local breast microenvironment regulating their activation status, similar to the ability of macrophages to act in a chemoprotective or cancer-promoting manner [reviewed in (50)]. The observation that 10,12-CLA induces a seemingly inflammatory mammary phenotype, complete with leukocyte recruitment and fibrosis, is surprising, given the generally...
protective effects of this isomer in mammary and other tumor models. These results may suggest that caution is warranted against the ingestion of this specific isomer at the high doses that are potentially attainable through supplementation with synthetic (rather than dietary) sources.

Acknowledgments
We thank Mary Vaughan for technical assistance and John Fischer for critical reading of this manuscript.

Literature Cited


