Influence of Tumor Necrosis Factor-a on Insulin-like Growth Factor-I Binding Sites in Thecal Cells

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Authors:

L.J. Spicer

Story in Brief

The effect of bovine tumor necrosis factor-a (TNFa) on insulin-like growth factor-I (IGF-I) binding sites in follicular thecal cells of cattle was evaluated. Thecal cells were obtained from large (³ 8 mm) ovarian follicles collected from cattle and cultured for 3 to 4 d. During the last 2 d of culture, cells were treated with recombinant bovine TNFa and specific binding of ^{125}I-IGF-I was determined. Two-day treatment with 10 and 30 ng/mL of TNFa decreased specific binding of ^{125}I-IGF-I to thecal cells. TNFa did not compete for ^{125}I-IGF-I binding to thecal cells whereas unlabeled IGF-I suppressed ^{125}I-IGF-I binding.

(Key Words: Tumor Necrosis Factor-a , Insulin-like Growth Factor-I, Thecal Cells, Cattle.)

Introduction

Tumor necrosis factor-a (TNFa) is a cytokine produced by activated macrophages, and in cattle systemic levels of TNFa increase acutely during infections (Kenison et al., 1990; Elsasser et al., 1995). It has been hypothesized that this increased level of TNFa may cause ovarian pathology such as ovarian cysts because TNFa directly inhibits granulosa and thecal cell steroidogenesis in cattle (Spicer and Alpizar, 1994; Spicer et al., 1995; Spicer, 1998). Alone, TNFa has little effect on ovarian steroidogenesis but in the presence of insulin-like growth factor-I (IGF-I), TNFa has dramatic inhibitory effects on steroidogenesis (Spicer, 1998). How TNFa accomplishes its inhibitory effect on steroidogenesis is unknown. Therefore, we set out to determine if the inhibitory effect of TNFa was due to either 1) a reduction in the number of IGF-I binding sites or 2) direct competition for IGF-I binding sites on bovine thecal cells.

Materials and Methods

Ovaries of beef and dairy cattle obtained at slaughter from a nearby abattoir were brought to the laboratory on ice and processed as previously described for obtaining thecal cells (Stewart et al., 1995). The medium was a 1:1 mixture of Dulbecco's modified Eagles medium and Ham's F12 containing gentamicin, glutamine and sodium bicarbonate. Approximately 2 x 10^5 viable cells were seeded in each plastic multiwell containing 1 ml of medium. Cultures were kept at 38.5° C in a 95% air – 5% CO_2 atmosphere, and medium was changed every 24 h. Cells were maintained in the presence of 10% fetal calf serum (FCS) for the first 2 d of culture, washed and treated for an additional 2 d with various doses (0, 3, 10 or 30 ng/ml) of recombinant bovine TNFa [3] in serum-free medium for Experiment 1. For Experiment 2, cells were maintained in 10% FCS for a total of 3 d. At the end of the culture period, ^{125}I-IGF-I binding assays were conducted as previously described (Spicer and Stewart, 1996). In parallel cultures, numbers of cells were determined using a Coulter counter. Experimental data are presented as means ±SE of measurement from replicated experiments. Each experiment was replicated at least three times. Binding of ^{125}I-IGF-I to thecal cells was expressed as cpm per 10^5 cells.

Results

In Experiment 1, 2-d treatment of thecal cells with 3 ng/mL of TNFa had no effect (P>.10) on specific binding of ^{125}I-IGF-I to thecal cells (Figure 1). However, 10 and 30 ng/ml of TNFa decreased (P<.05) specific binding of ^{125}I-IGF-I to thecal cells by 20% and 25%, respectively (Figure 1).

In Experiment 2, 10 and 100 ng/well of TNFa did not inhibit binding of ^{125}I-IGF-I to thecal cells, whereas both 10 and 100 ng/well of IGF-I inhibited binding of ^{125}I-IGF-I (Figure 2).

Discussion

Previous studies have shown that, in cattle, TNFa inhibits insulin- and IGF-I-induced estradiol production by granulosa cells and androstenedione production by thecal cells (Spicer and Alpizar, 1994; Spicer, 1998). Results of the present experiments indicate that TNFa may directly decrease the number of thecal cell IGF-I binding sites but that TNFa can not compete for IGF-I binding sites on thecal cells. Thus, the inhibitory effect of TNFa on IGF-I-induced thecal steroidogenesis may involve a decrease in the number of IGF-I receptors. However, the TNFa -induced decrease in IGF-I receptor numbers is much smaller (i.e., 20-25% decrease) than the TNFa -induced decrease in androstenedione production (i.e., 50% decrease) previously reported (Spicer, 1998) indicating that the inhibitory effect of TNFa on steroidogenesis may not be solely due to a TNFa effect on IGF-I receptor numbers. Additional studies will be required to clarify this possibility.

Previous studies have also shown that bovine thecal cells have high affinity, specific TNFa binding sites (Spicer, 1998). Thus, TNFa likely inhibits thecal steroidogenesis via signal transduction from its own receptor. The precise mechanism of action of TNFa on bovine thecal cell steroidogenesis awaits further elucidation. It has been suggested that TNFa may play an endocrine role and inhibit ovarian function during infection and disease (Spicer, 1998) and this may be why metritis increases the risk of abnormal estrus and cystic ovaries in lactating dairy cattle (Erb et al., 1985; Klerx and Smolders, 1997). However, this suggestion awaits verification.

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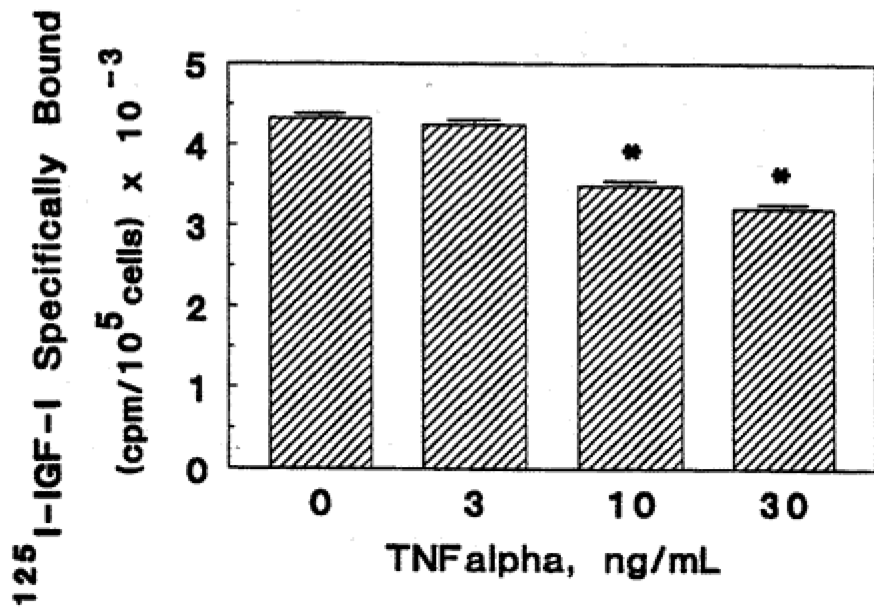

Figure 1. Effect of 2-d treatment of bovine TNFa on specific ^{125}I-IGF-I binding sites in bovine thecal cells (Experiment 1). After the 2-d treatments, cells were washed and then incubated with 100,000 cpm of ^{125}I-IGF-I. Non-specific binding was determined in the presence of 500 ng/well of recombinant human IGF-I. Values are means ± SE of four separate experiments. *Mean differs (P<.05) from control (0 ng/ml).

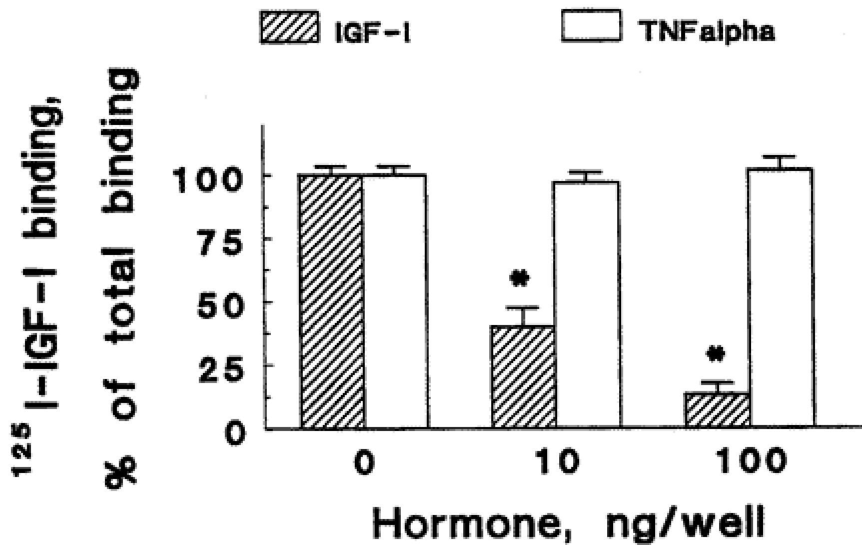

Figure 2. Comparison of TNFa and IGF-I on competing for IGF-I binding by thecal cells (Experiment 2). Thecal cells were cultured for 3 days in the presence of 10% FCS, and then cells were washed and incubated with 50,000 cpm of ^{125}I-IGF-I in the absence or presence of 0, 10 or 100 ng/well of TNFa or IGF-I. Values are means of three separate experiments and are expressed as a percentage of control total ^{125}I-IGF-I binding. *Mean

differs (P<.05) from control (0 ng/well).

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