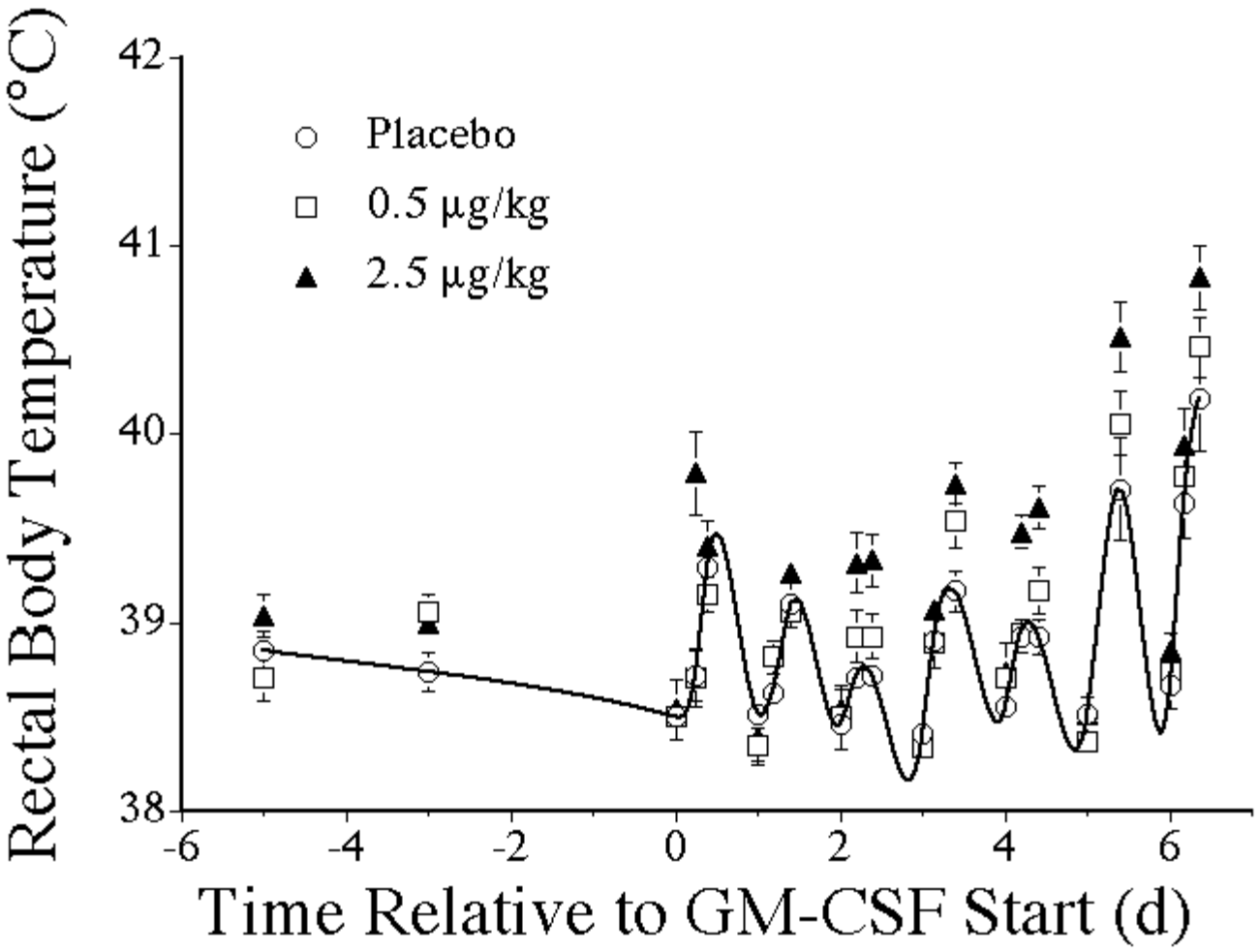
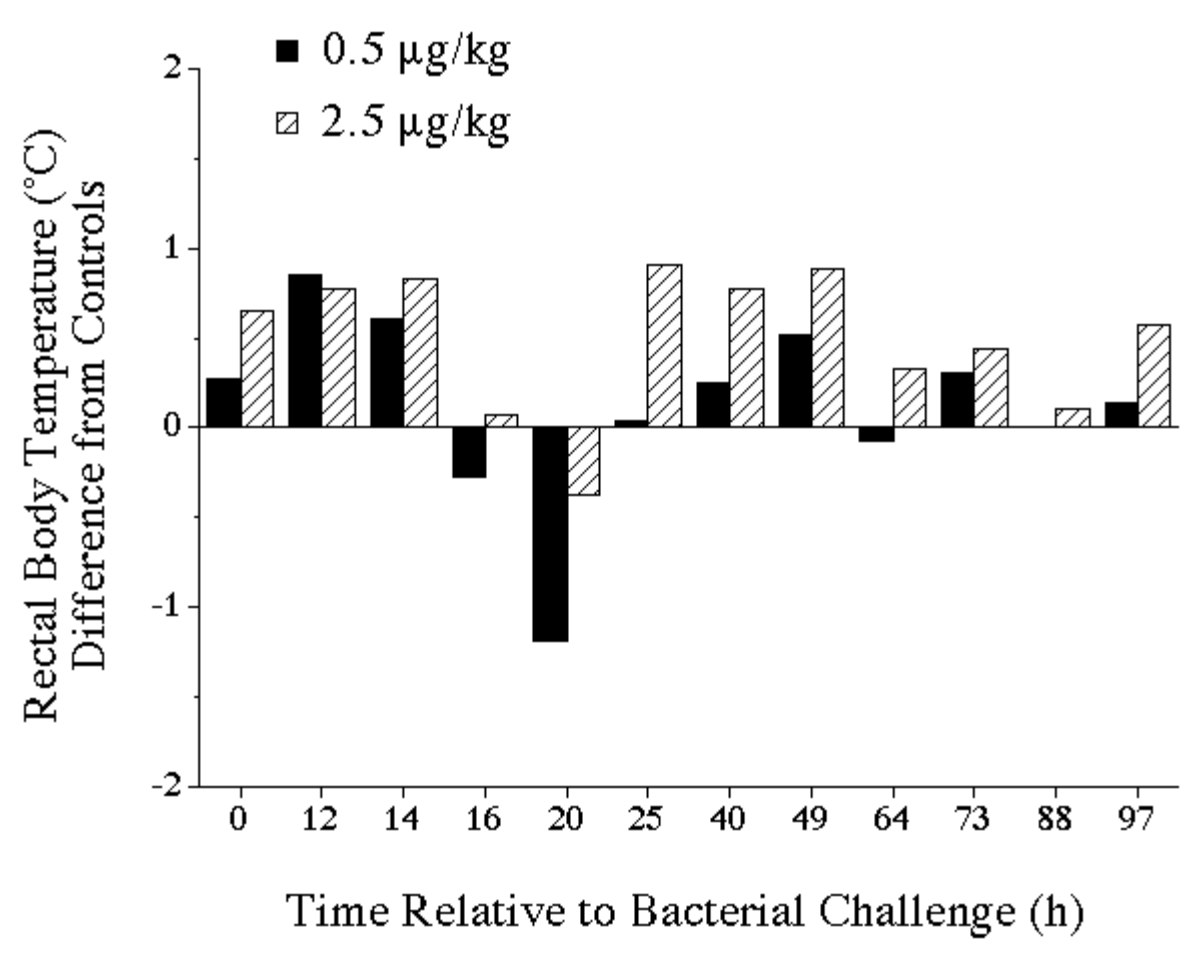
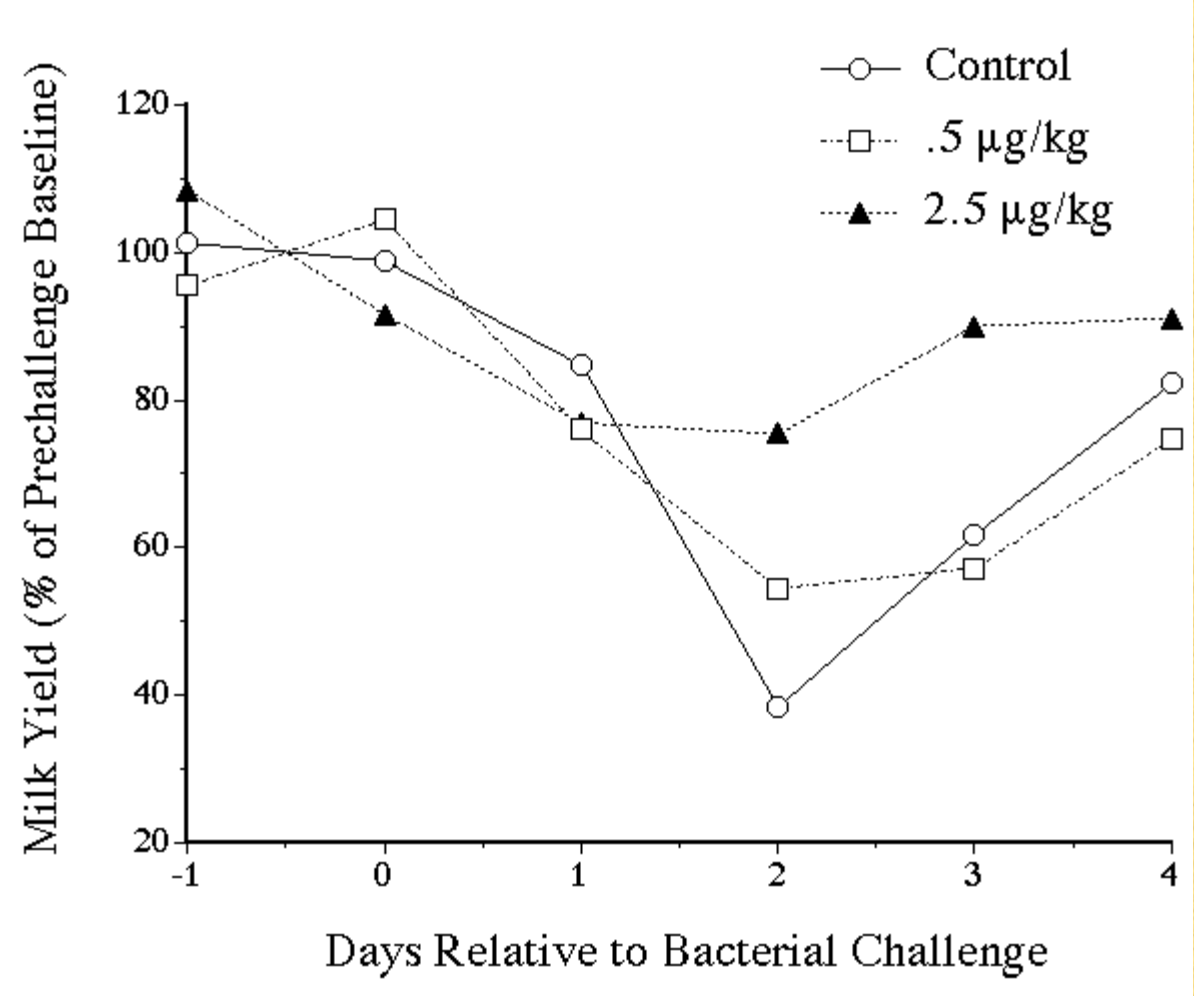
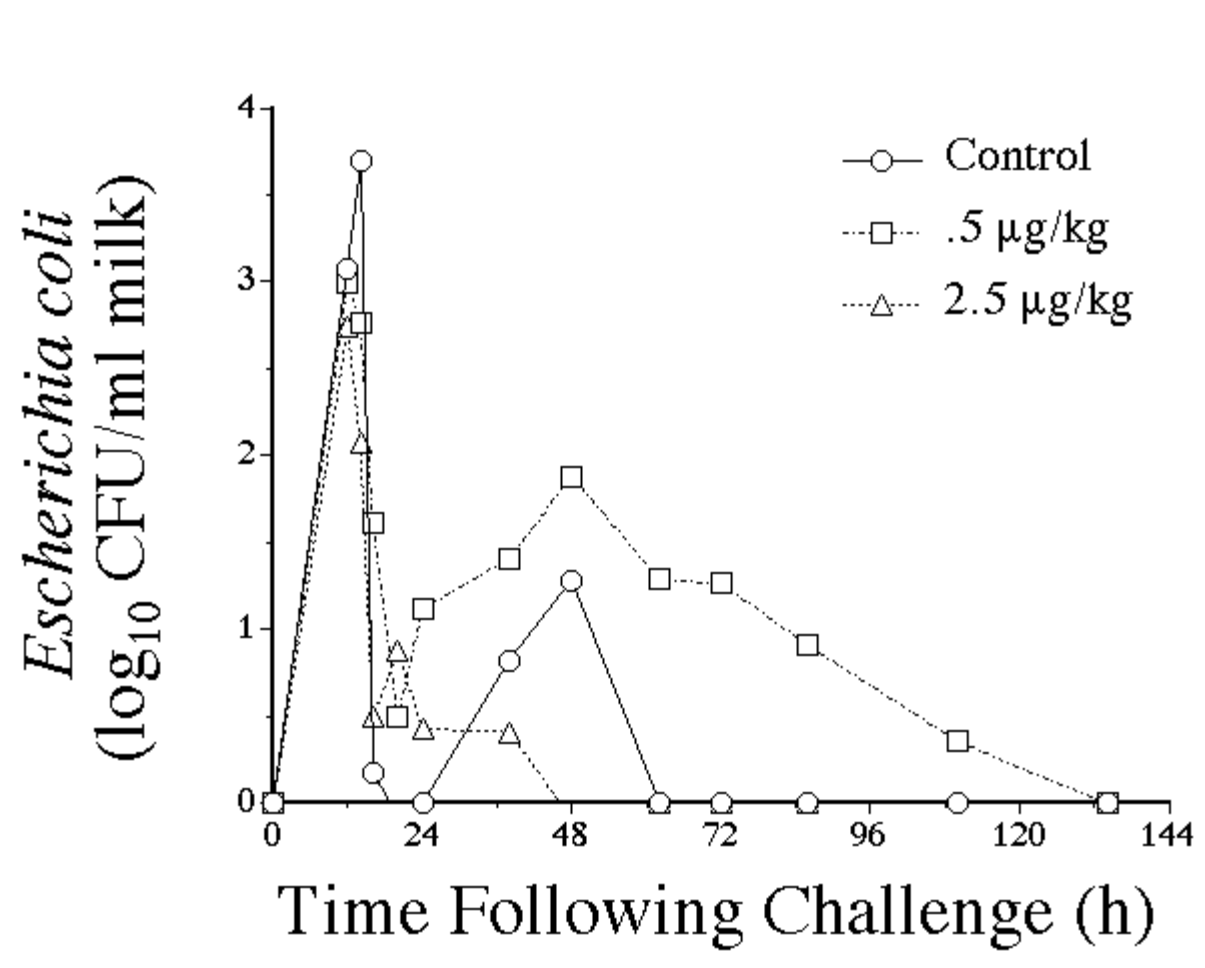
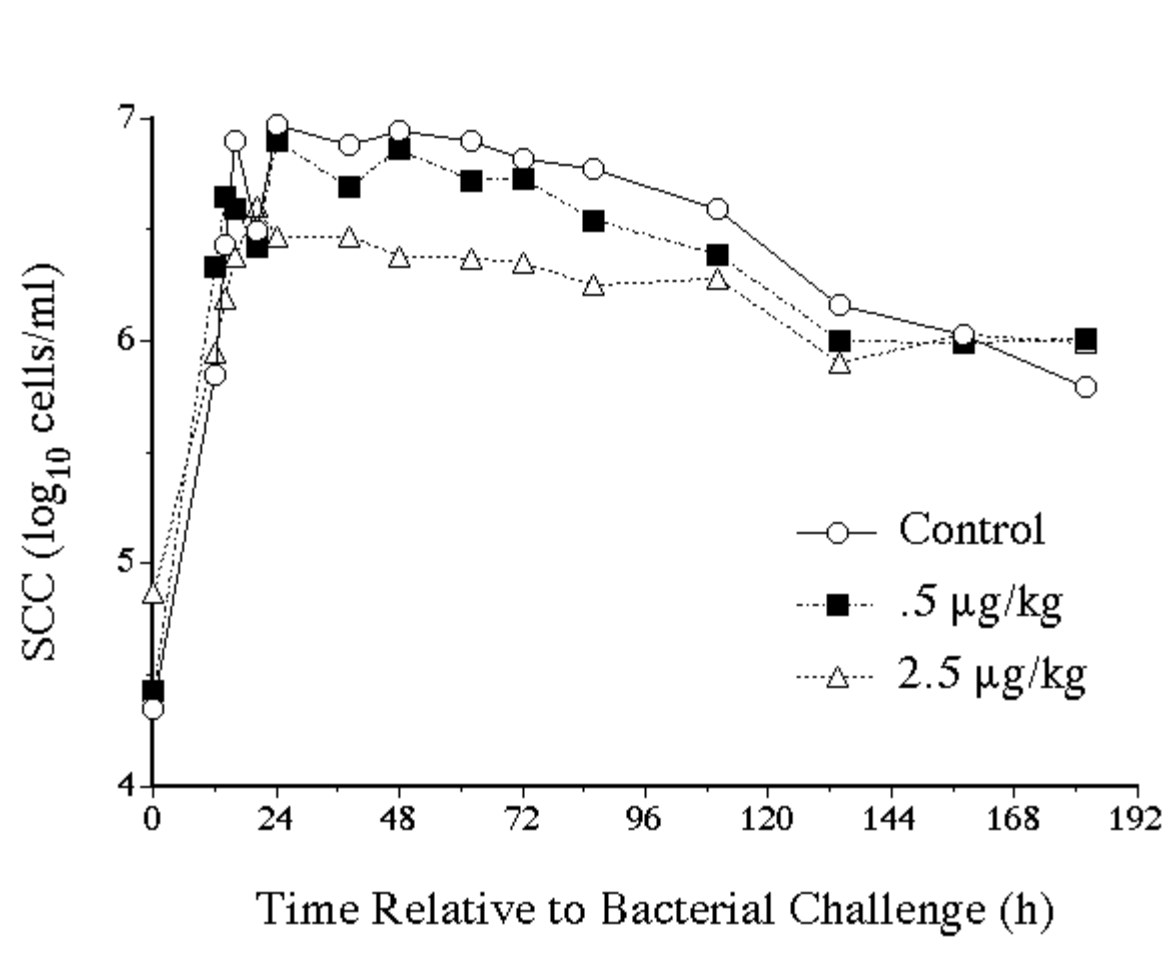

Rectal Body Temperature (°C)
42
41
40
39
38
Placebo
0.5 µg/kg
2.5 µg/kg
-6
-4
-2
0
2
4
6
Time Relative to GM-CSF Start (d)


0.5 µg/kg
2.5 µg/kg
Rectal Body Temperature (°C)
Difference from Controls
2
1
0
-1
-2
0
12
14
16
20
25
40
49
64
73
88
97
Time Relative to Bacterial Challenge (h)


Control
.5 µg/kg
2.5 µg/kg
Milk Yield (% of Prechallenge Baseline)
120
100
80
60
40
20
-1
0
1
2
3
4
Days Relative to Bacterial Challenge


Escherichia coli
(log10 CFU/ml milk)
4
3
2
1
0
0
24
48
72
96
120
144
Time Following Challenge (h)
Control
.5 µg/kg
2.5 µg/kg


SCC (log_{10} cells/ml)
7
6
5
4
0
24
48
72
96
120
144
168
192
Time Relative to Bacterial Challenge (h)
Control
.5 µg/kg
2.5 µg/kg

G00044

Effects of recombinant bovine granulocyte-macrophage-colony stimulatory factor administration on the bovine immune system and resistance to intramammary challenge with *Escherichia coli*.

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Abstract:
An experiment was conducted to determine the immunomodulatory activity of recombinant bovine granulocyte-macrophage colony stimulatory factor (rbGM-CSF) in lactating dairy cows. Administration of rbGM-CSF was via single daily subcutaneous injection on alternating sides of the lateral cervical region at 0, 0.5, or 2.5 micrograms/kg body weight for eight consecutive days. Control cows received a placebo saline injection. Cows were challenged in one mammary quarter with 41 colony forming units of *Escherichia coli* after receiving seven daily injections of rbGM-CSF to measure any effects on experimentally-induced mastitis. A dose-dependent febrile response developed upon repeated administration of rbGM-CSF. Increases in rectal temperatures in response to rbGM-CSF administration after the first day averaged 0.37° C greater than control cows within 5 to 6 h. By 9 to 10 h after rbGM-CSF injection, increases in rectal temperatures averaged 0.59° C over control cows. Rectal temperatures returned to control levels by 24 h after each injection. Associated with the febrile responses were increases in respiration and heart rates. No enhancement of neutrophil or lymphocyte functional activities was detected. The only consistent immunomodulatory effect was that of suppression of lymphocyte blastogenesis induced by concanavalin A and phytohemagglutinin P. The rbGM-CSF used did not appear to possess any in vivo colony stimulating activity since no leukocytosis was observed (a neutrophilia and/or monocytosis was expected). Paradoxically, cows administered rbGM-CSF appeared to have less severe mastitis reactions at 20 h following intramammary challenge with *E. coli* based upon a 1°C lower febrile response to *E. coli*.

Introduction:
Bovine mastitis is one of the most economically important diseases to the beef and dairy cattle industries. The pathogenesis is highly complex and involves many factors including various microbial etiologies, stress, management and environmental hygiene. There is considerable evidence of impaired neutrophil and lymphocyte function in periparturient dairy cows which coincides with the high incidence of new intramammary infections (IMI) 2 weeks prepartum and clinical mastitis in early lactation.
Recent developments in recombinant DNA technologies have resulted in the cloning, sequencing and expression of several recombinant bovine cytokines, including granulocyte-macrophage colony stimulatory factor (rbGM-CSF)[9, 10]. Many studies in other animal species have indicated that GM-CSF is effective in treating immunocompromised individuals [1, 6, 11, 12, 18, 22]. Recent in vitro studies have found rbGM-CSF enhances bovine neutrophil functions [15]. Other research using a related cytokine, rbG-CSF, have also found potentially beneficial immunomodulatory properties with in vivo administration [3-5, 7, 14, 19].

Materials and Methods:
Recombinant Bovine Granulocyte/Macrophage-Colony Stimulatory Factor--Recombinant bovine GM-CSF (Lot #2090-41) was supplied by American Cyanamid at 1.43 mg/ml of 100 *mM* Tris buffer at pH 8. This lot of rbGMCSF

had been tested in a neonatal calf bone marrow proliferation assay at American Cyanamid and found to have a colony stimulating activity (CSA) of 3.8 x 10^7 Units/mg. Recombinant bGM-CSF was diluted with PBSS such that each cow received an injection volume of 2 ml/d.

Experimental Design and Animals used--Experimental groups were blocked by body weight and milk production to avoid pre-existing group differences. Administration of rbGM-CSF was via single daily (s.i.d.) subcutaneous injection on alternating sides of the lateral cervical region at 0, 0.5, or 2.5 micrograms/kg body weight for eight consecutive days at 0930 h. Control cows received a placebo saline carrier. After seven rbGM-CSF or placebo injections, all cows were challenged with 41 colony forming units (cfu) of *Escherichia coli* in the right front mammary gland after the evening milking (~1830 h).

Neutrophil function assays--In vitro evaluations of neutrophil FcR-mediated ingestion of *Staphylococcus aureus* were performed as described [16]. Assessments of zymosan phagocytosis-associated native (nonluminol-dependent) chemiluminescence activity [8], myeloperoxidase catalyzed iodination [8], and superoxide anion generation [2] were performed using described procedures. A neutrophil bactericidal assay was performed as described [21]. Random and directed neutrophil migration under agarose were assessed as described [13]. Antibody-dependent neutrophil-mediated cytotoxicity (ADNC) and antibody-independent neutrophil-mediated cytotoxicity (AINC) were evaluated, using standard procedures [17]

Lymphocyte blastogenesis and in vitro IgM production --Lymphocyte blastogenesis elicited by various mitogens was assessed in a tritiated thymidine uptake assay. Polyclonal-driven production of antibody (ng IgM/ml) in mononuclear cell culture supernatants was also determined [20]. Cultures were either nonstimulated or stimulated with 0.1 micrograms PWM per ml tissue culture medium. Tissue culture media was replenished on day 4 of culture after centrifugation. Supernatants were collected on d 12 and stored at -70°C until analyzed by ELISA.

Results:

Clinical Effects of rbGM-CSF Administration--Cows receiving rbGM-CSF at 2.5 micrograms/kg exhibited a febrile response (increase of 1.1° C) within 5.5 h after the first injection. This response was transient (Figure 1) and not observed at all doses. After the second and subsequent injections, a recurrent pattern of a dose dependent febrile response developed upon repeated administration of rbGM-CSF. Associated with the febrile responses were increases in respiratory rates and pulse rates.

Clinical Effects of Intramammary Bacterial Challenge--Cows receiving rbGM-CSF had less severe inflammation ~16 h after *E. coli* challenge and recovered more quickly than control cows. All control cows had abnormal milk secretions and palpably hard, distended and hot right front quarters. No detectable febrile response followed *E. coli* challenge and the mastitic episode. However, at 16 to 20 hours postchallenge there appeared to be lower rectal temperatures in cows receiving rbGM-CSF than controls. Increased ambient temperatures made this difficult to see and therefore, differences in rectal temperatures from controls were calculated and presented (Figure 2). A mild tachycardia occurred 12 to 24 h postchallenge in all treatment groups. Milk production declined in all treatment groups in response to *E. coli* infection (Figure 3).

Bacterial Shedding of E. coli into Lacteal Secretions--All control cows shed many *E. coli* into milk from challenged quarters. Peak bacterial numbers ranged from 235 to 64,000 cfu/ml (Figure 4). Overall, *E. coli* numbers remained lower, and bacteria were eliminated sooner in cows receiving 2.5 micrograms/kg rbGM-CSF ($P < .05$ at 48 h post challenge).

Leukocyte recruitment into milk--Initial milk SCC in all challenged quarters before infection averaged less than 60 x 10^3 cells/ml (Figure 5). Average SCC postchallenge in the high dose cows was roughly 40% ($P < .05$) and 35% ($P < .10$) lower than in control and low dose cows, respectively.

Effects on Neutrophil Function--No prolonged beneficial effects were observed on neutrophil functions.

Effects on Lymphocyte Function--No beneficial effects were observed on lymphocyte blastogenesis or in vitro IgM production. Lymphocyte blastogenesis induced by ConA and PHAP was suppressed by 20% to 70% during the administration period compared to controls for both rbGM-CSF treatment doses (P<.05). No significant effects on PWM-induced lymphocyte blastogenesis were observed.

Discussion and Conclusions:

The primary role of colony stimulatory factors is to induce expansion and differentiation of precursor stem cells in the bone marrow. Therefore, expansion and differentiation of myeloid precursor cells should be stimulated by rbGM-CSF and this should result in a leukocytosis attributable to a granulocytosis and monocytosis. No colony stimulating activity attributable to the form of rbGM-CSF administered to cows in this experiment was ever detected. Furthermore, in contrast to a vast number of reports in other species, no enhancement of neutrophil effector functions was observed. Our dose of rbGM-CSF was chosen based upon the amount of rbGM-CSF available, its demonstrated in vitro colony stimulating activity of 3.8 x 10^7 Units/mg in a calf bone marrow proliferation assay, and previous experience with rbG-CSF of known in vitro activity (1 x 10^8 Units/mg) administered at 1, 3, 5 and 10 micrograms/kg in vivo [5, 7, 19]. We therefore administered less colony stimulating activity than previous studies with G-CSF in cattle. However, considering the febrile response to rbGM-CSF administration, higher doses of rbGM-CSF do not seem advisable.

Another hypothesis tested in this experiment was that rbGM-CSF administration would improve a cow's resistance to bacterial challenge. Although no immunomodulatory effects of rbGM-CSF administration were detected, subjective measures (e.g. abnormal secretions, edema, swelling and pain) of the clinical course of *E. coli* mastitis suggested that cows receiving rbGM-CSF experienced a less severe form of coliform mastitis at ~16 h after challenge. Supporting evidence for this can be drawn from milk production and SCC data. Further support comes from bacterial shedding in milk where infections appeared to be cleared more rapidly in cows that received high dose rbGM-CSF.

Administration of a biologically inactive form of rbGM-CSF could possibly induce a beneficial response following intramammary bacterial challenge. This effect could be envisioned if a mild, local inflammatory response was induced with rbGM-CSF administration which in turn primed the capacity of the immune system to respond to subsequent bacterial challenge. The rbGM-CSF administration in this experiment also may have resulted in a priming effect on the bone marrow without any resultant leukocytosis.

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Comments:

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Questions and Answers