Effect of Administration of Oat β-Glucan on Immune Parameters of Healthy and Immunosuppressed Beef Steers

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ABSTRACT

In order to assess the effect of oat β-glucan (OβG) administration on immune parameters of beef steers, 3 experiments were carried out. In experiment 1, the in vitro effect of OβG on the proliferation of blood lymphocytes, with or without the presence of dexamethasone (DXM), was evaluated. In experiment 2, groups of 12 healthy steers were administered OβG or saline solution and immunized with ovalbumin (OVA). Immune parameters studied included IgG antibody levels to OVA, proliferation responses of blood lymphocytes to OVA, and blood leukocyte differential cell counts. For experiment 3, groups of 10 steers were treated with OβG and DXM, DXM only, or saline solution, and immunized with OVA and keyhole limpet hemocyanin (KLH). Serum antibody responses to OVA and KLH, serum IgG concentration levels, blastogenic responses of blood lymphocytes to OVA and KLH, differential blood leukocyte numbers, and iron and zinc concentration in serum were tested to evaluate the effect of OβG to overcome immunosuppression. The in vitro treatment of naive blood lymphocytes with OβG did not increase their ability to proliferate; however, when OβG was added to cultures of DXM-treated lymphocytes, a significant ($P < 0.05$ to $P < 0.001$) reversion of the immunosuppressive effect of DXM occurred. Administration of OβG to clinically healthy steers did not induce significant changes on any of the immune parameters studied. The administration of OβG to DXM-treated steers provoked, on Day 25, a significant increase in IgG anti-OVA ($P < 0.01$) and anti-KLH ($P < 0.05$) responses vs the DXM only group. On Day 25, the specific proliferation responses of lymphocytes, to both OVA and KLH, were significantly increased ($P < 0.05$) in OβG+DXM group compared to DXM group. On Day 4, a significant increase in the number of leukocytes ($P < 0.01$) and neutrophils ($P < 0.001$), and a significant decrease in the number of monocytes ($P < 0.05$) were observed in the group treated with DXM only compared to OβG+DXM group. No significant differences were observed in iron and zinc concentration between OβG+DXM and DXM groups. These results indicated that OβG did not influence immune responses of naive cells in vitro or of healthy steers in vivo; however, when cells or animals were treated with DXM, OβG significantly restored some of the specific and non-specific immune parameters studied.

RÉSUMÉ

Trois expériences ont été menées dans le but d'évaluer l'effet de l'administration du β-glucane de l'avoine (OβG) sur des paramètres évaluant la fonction immunitaire de bouvillons. Dans l'expérience 1, l'effet de l'OβG, en combinaison ou non avec de la dexaméthasone (DXM), sur la prolifération des lymphocytes sanguins a été évalué in vitro. Dans l'expérience 2, des groupes de 12 bouvillons en santé ont reçu de l'OβG ou une solution saline et ont par la suite été immunisés avec de l'ovalbumine (OVA). Les paramètres immunitaires étudiés étaient le niveau d'anticorps de type IgG dirigés contre OVA, la réponse proliférative des lymphocytes du sang à OVA et un comptage différentiel des leucocytes sanguins. Dans l'expérience 3, des groupes de 10 bouvillons reçurent un des traitements suivants : OβG et DXM, DXM uniquement ou solution saline. Par la suite, les animaux furent immunisés avec de l'OVA et de l'hémocyanine de limule (KLH). Dans le but de déterminer si l'OβG permettait de surmonter une immunosupression, les paramètres suivants ont été mesurés : le niveau d'anticorps sériques contre OVA et KLH, la concentration d'IgG sériques, la réponse blastogénique des lymphocytes sanguins suite à une stimulation par OVA et KLH, des comptages leucocytaires différentiels, et les concentrations de fer et de zinc. Le traitement des lymphocytes sanguins naïfs in vitro avec de l'OβG n'a pas augmenté leur capacité à proliférer; par contre, lorsque l'OβG était ajouté aux cultures de lymphocytes traités avec de la DXM, une réversion significative ($P < 0,05$ à $P < 0,001$) de l'effet immunosuppresseur de la DXM fut observée. Le fait d'administrer de l'OβG à des bouvillons cliniquement en santé n'a pas amené de changements significatifs au niveau des paramètres évalués. L'administration d'OβG aux bouvillons ayant reçu de la DXM entraîna chez ces derniers une augmentation significative, au jour 25, des IgG anti-OVA ($P < 0,01$) et anti-KLH ($P < 0,05$) comparativement aux

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animaux n'ayant reçu que la DXM. Au même moment, la réponse proliférative spécifique des lymphocytes suite à une stimulation par OVA et KLH était augmentée de façon significative (*P* < 0,05) chez les animaux du groupe OβG+DXM comparativement au groupe DXM. Au jour 4, une augmentation significative du nombre de leucocytes (*P* < 0,01) et de neutrophiles (*P* < 0,001), de même qu'une diminution significative du nombre de monocytes (*P* < 0,05) furent observées chez les animaux ayant reçu seulement de la DXM comparativement aux animaux du groupe OβG+DXM. Aucune différence significative ne fut observée dans les concentrations de fer et de zinc entre les groupes OβG+DXM et DXM. Les résultats indiquent que l'OβG n'a pas influencé les réponses immunitaires in vitro de cellules naïves, ou in vivo de bouvillons en santé. Toutefois, lorsque des cellules ou des animaux étaient traités avec de la DXM, l'OβG permettait de restaurer certains des paramètres de l'immunité spécifique et non-spécifique.

(Traduit par le docteur Serge Messier)

INTRODUCTION

Adequate resistance of cattle to infectious diseases is dependent on functional immune responses and is especially important at times when the animals are subjected to stress conditions such as transportation. Cattle entering feedlots generally are immunologically compromised, experiencing high levels of stress which can be immunosuppressive and increase the animal's susceptibility to disease. The increased susceptibility is due, in part, to increased endogenous glucocorticosteroid concentration. Glucocorticosteroids are immunosuppressive and affect the acute phase of inflammation by interference with interleukin-1 (IL-1) (1). Stress resulting in increases of endogenous glucocorticosteroids is thought to contribute to the respiratory disease complex of cattle.

For diseases of single etiology, vaccines may offer the most viable manner of disease prevention. However, when a variety of infectious agents and environmental stressors contribute to the pathogenesis of the disease, like the respiratory disease complex of feedlot cattle, non-specific immunostimulators may offer substantial advantages over vaccination programs (2).

The use of immunostimulants, for enhancement of disease resistance to a wide spectrum of pathogens at times of stress, provides a strategy which can improve suboptimal immune function and thus increase resistance to infectious diseases (3). Over the years, many substances have been identified and studied for their immunostimulatory activities. However, most of them cannot be used in domestic food animals due to cost, toxicity, undesirable side effects, or safety concerns for the consumer. Research has shown beneficial effects on the stimulation of the immune system by compounds of microbial or plant origin such as β-glucans, with the potential to minimize the incidence of disease in livestock.

Beta-glucans are major structural components of the cell wall of yeast, fungi and some cereals such as barley and oats. The cell wall β-glucans of yeast and fungi consist of (1→3)-linked glucopyranosyl residues with small numbers of (1→6)-linked branches (4). Beta-glucan with (1→3) and (1→4) linkages are present in the barley and oat endosperm cell walls as polysaccharides of a molecular weight of approximately 2000 kDa (5). Beta-(1→3, 1→6) glucans non-specifically enhance host resistance to bacterial (6,7), viral (8,9), protozoal (10,11) and fungal (12,13) diseases. In vitro and in vivo, β-glucans enhance macrophage functions such as phagocytosis (14), lysosomal enzyme activity (15) and IL-1 production (16). Specific membrane receptors for the (1→3) linkage of β-glucans have been demonstrated in macrophages (17,18).

We have reported previously that β-glucans derived from oats, have an immunostimulant effect on immune cells of mice, both in vitro and in vivo, activating macrophage and lymphocyte functions (19). Also, the administration of oat β-glucan (OβG) enhances non-specific disease resistance to *Staphylococcus aureus* systemic infection in mice (19), and to *Eimeria vermiformis* intestinal infection in immunosuppressed and conventional mice (20,21). This led us to examine the in vitro effect of OβG in bovine lymphocyte proliferative activities and, the in vivo effect of OβG administered to healthy and dexamethasone-immunosuppressed beef steers on immune parameters in blood, which included, antibody responses to specific antigens, lymphocyte proliferation responses to antigens, differential leukocyte numbers and iron and zinc concentration. Results of these experiments provided information about the use of OβG to enhance suboptimal immune function and possibly increase resistance of beef cattle to infectious diseases.

MATERIALS AND METHODS

ANIMALS

To study the in vitro and in vivo effects of OβG on immune parameters, a total of 60 cross-bred steer calves from the Western Beef Development Centre, Saskatoon, Saskatchewan, averaging 250 kg in body weight (BW), were used. Experimental protocols were approved by the Animal Care Committee of the University of Saskatchewan and were performed in accordance with recommendations of the Canadian Council on Animal Care, as specified in the *Guide to the Care and Use of Experimental Animals*.

ANIMAL TREATMENT REAGENTS

We used β-(1→3, 1→4)-linked glucan prepared from oat endosperm (Ceapro Inc., Edmonton, Alberta). The endotoxin contamination of this preparation was less than 10 pg/mg as determined by a *Limulus* amebocyte lysate chromogenic-specific assay (BioWhittaker Inc., Walkersville, Maryland, USA). The preparation consisted of 1- to 3-μm glucan particles, as visualized by light microscopy. The OβG was soluble at a concentration up to 20 mg/mL. Dexamethasone (DXM) was purchased from Austin, division of Vétoquinol Canada Inc., Joliette, Quebec. Ovalbumin (OVA) and keyhole limpet hemocyanin (KLH) were obtained from Sigma Chemical Co., St. Louis, Missouri, USA. Precautions were taken to eliminate endotoxin contamination in the experiments; all glassware was washed and rinsed with

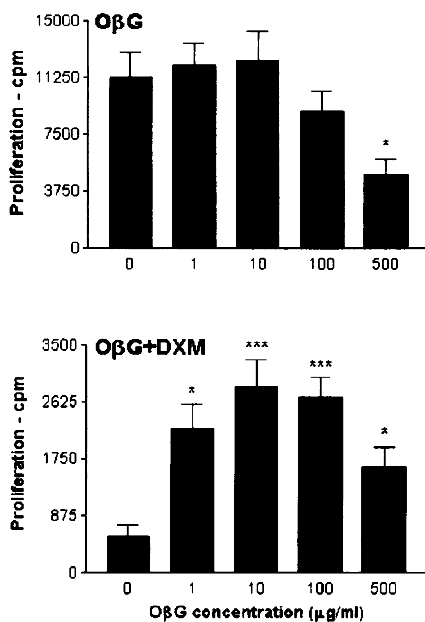

Figure 1. Effects of the administration of OβG on bovine blood lymphocyte proliferation responses. Blood lymphocytes were cultured with OβG only at the concentrations shown or with OβG+DXM, the latter at a concentration of 0.05 μg/mL. Cells were incubated at 37°C in an atmosphere containing 5% CO_2 for 72 h. The samples in each well were then incubated with 0.1 μCi/well of [^{3}H]thymidine for 16 h. After cell harvesting, the radioactivity was measured in a β-scintillation counter. Each bar represents the mean of lymphocyte responses of 6 animals ± SEM of triplicate determinations. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control without OβG.

deionized, double-distilled water and sterilized by heating at 175°C for 3 h. All media and solutions used for inoculations and cell cultures were pyrogen-free and tested by the *Limulus* assay before use.

EXPERIMENTAL PROTOCOLS

In experiment 1, to evaluate the in vitro effects of OβG on lymphocyte proliferative activities, cells were isolated from blood of 6 steers. Lymphocyte blastogenic responses were measured to OβG alone or with the immunosuppressive agent, DXM.

In experiment 2, to evaluate the in vivo effects of OβG on immune parameters of conventional calves, 24 steers were assigned to 2 groups of 12 animals each. The groups were subcutaneously (SC) injected in the left side of the neck with 1 g of OβG in 50 mL suspension or, saline solution, respectively. The treatments were administered 3 times, on Days 0, 7, and 14. Both groups were immunized SC with 2 mg of OVA in the right side of the neck, on Days 0 and 14. Sera were prepared from blood obtained on Days 0, 14, 28, and 42 to measure IgG responses against OVA. Blood samples were collected on Days 0, 3, 7, and 10 to perform leukocyte differential cell counts, and on Days 14, 28, and 42 to determine lymphocyte proliferative responses specific to OVA.

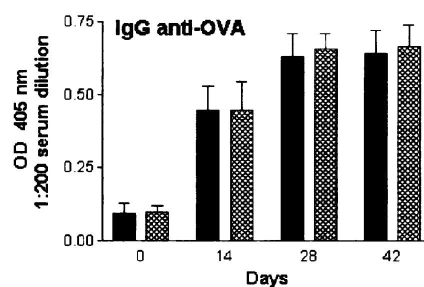

Figure 2. Effects of the administration of OβG (closed bar) or saline (hatched bar) on Days 0, 7, and 14 on IgG anti-OVA responses following immunization on Days 0 and 14 with OVA. IgG anti-OVA levels were determined on Days 0, 14, 28, and 42 by ELISA. The bars represent the mean values of groups of 12 animals ± SEM.

In experiment 3, to evaluate the in vivo effects of OβG on immune parameters of immunosuppressed calves, 30 steers were assigned to 3 groups of 10 animals each. Two groups were treated with 0.1 mg/kg BW of DXM, which was given intramuscularly (IM) to each animal daily for 4 consecutive days (Days 0 to 3). One of these groups was injected SC with 1 g of OβG, in 50 mL suspension, in the right side of the neck, from Days 1 to 4. The third group served as saline-administered control. All groups were immunized with 2 mg of OVA and 1 mg of KLH in saline solution, by SC injection in the left side of the neck, on Days 3 and 18. Serum samples were obtained on Days 4 and 18 for the measurement of iron and zinc concentration and, on Days 11 and 25 to determine non-specific IgG concentration and specific IgG anti-OVA and anti-KLH levels. Blood samples were collected on Days 4, 11, 18, and 25 to perform leukocyte differential cell counts and on Days 11 and 25 to determine specific lymphocyte blastogenic responses to OVA and KLH.

BLOOD LYMPHOCYTE ISOLATION

Blood samples from the jugular vein were collected into heparinized vacuum tubes, centrifuged at 500 × *g* for 35 min and the plasma discarded. For the isolation of lymphocytes, the buffy coat layer was transferred into a 50-mL tube. Thirty-five millilitres of Hank's balanced salt solution (HBSS; Gibco BRL, Life Technologies Inc., Grand Island, New York, USA) were added to the tube and then layered over 15 mL of a Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient in a 50-mL centrifuge tube. The suspension was centrifuged for 45 min at 500 × *g*. The lymphocyte cell band was taken and washed twice with HBSS. The cell isolation procedure yielded lymphocyte populations of 90% purity and > 99% viability, as determined by trypan blue exclusion.

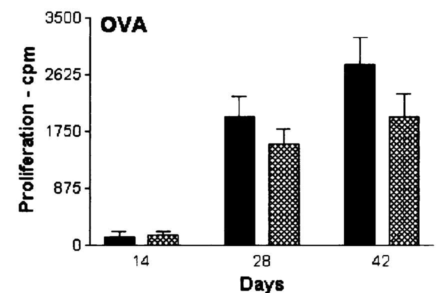

Figure 3. Effects of the administration of OβG (closed bar) or saline (hatched bar) on Days 0, 7, and 14 on specific lymphocyte proliferation responses determined on Days 14, 28, and 42. Blood lymphocytes were cultured with OVA at a concentration of 200 μg/mL. Cells were incubated at 37°C in an atmosphere containing 5% CO_2 for 72 h. The samples in each well were then incubated with 0.1 μCi/well of [^{3}H]thymidine for 16 h. After cell harvesting, the radioactivity was measured in a β-scintillation counter. Each bar represents the mean of groups of 12 animals ± SEM of triplicate determinations.

LYMPHOCYTE PROLIFERATIVE RESPONSES

Isolated lymphocytes were resuspended in RPMI-1640 containing 10% fetal bovine serum (RPMI-FBS; Gibco) and the concentration adjusted to 5 × 10^6 cells/mL. One hundred microlitres of the cell suspension was added to each of the wells of 96-well, round-bottom cell culture plates (Corning Glass Works, Corning, New York, USA). For experiment 1, to evaluate the in vitro effects of OβG on lymphocyte proliferative activities, OβG at 1, 10, 100 and 500 μg/mL concentrations were added to the cells with or without DXM at 0.05 μg/mL. For experiments 2 and 3, to evaluate

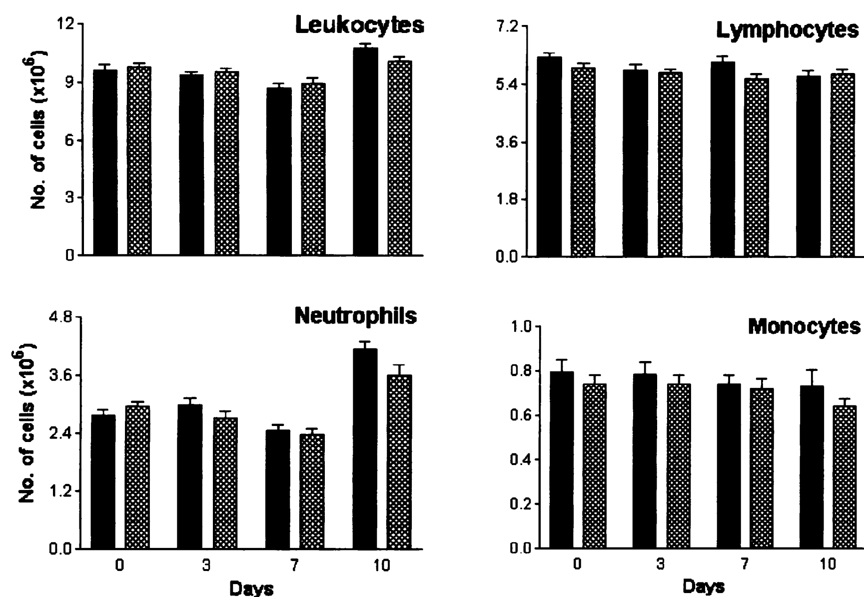

Figure 4. Time dependent effects of the administration of OβG on Days 0, 7, and 14 (closed bar) or saline (hatched bar) on the total number of blood leukocytes, lymphocytes, neutrophils and monocytes. Blood samples were collected on the indicated days and the number of cells counted. Data are expressed as the mean total leukocyte numbers in blood of groups of 12 animals ± SEM.

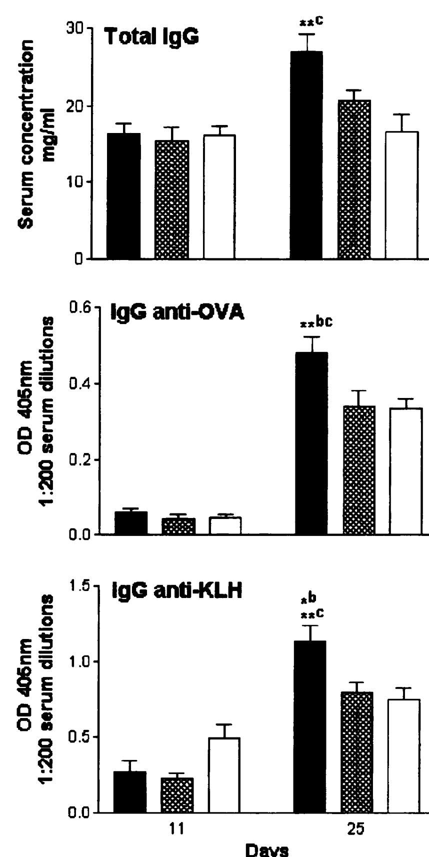

Figure 5. Effects of the administration of OβG+DXM (closed bar[a]), DXM (hatched bar[b]) or saline (open bar[c]) on non-specific serum IgG concentration, IgG anti-OVA and IgG anti-KLH responses following immunization on Days 3 and 18 with OVA and KLH. IgG antibody levels were determined, on Days 11 and 25 by ELISA. The bars represent the mean values of groups of 10 animals ± SEM. Where OβG+DXM, DXM or saline groups are designated as [a,b,c] respectively, means differ vs the group indicated with letter superscript. * $P < 0.05$, ** $P < 0.01$.

the in vivo effects of OβG on specific lymphocyte proliferation of conventional and DXM-treated calves, OVA or KLH at 200 and 50 μg/mL concentrations, respectively, were added to the cultured cells. All the treatments were in triplicate. Cells were also cultured in medium only as negative controls. The plates were incubated for 72 h at 37°C in an atmosphere containing 5% CO_2. The contents of each well were then incubated for 16 h with 0.1 μCi/well of [^{3}H]thymidine (Amersham Canada Ltd., Oakville, Ontario). The cells were harvested using a cell harvester (Skatron, Dolasletta, Norway) and the radioactivity was measured in a β-scintillation counter (LKB, Bromma, Sweden). The proliferation responses were expressed as the mean counts/minute (cpm) of stimulated cells from 3 replicates (experiment 1) or, as the mean cpm minus the mean cpm of unstimulated cells derived from 3 replicates (experiments 2 and 3).

ENZYME-LINKED IMMUNOASSAYS (ELISA)

All sera were clarified by centrifugation and stored at −20°C before analysis by ELISA to measure specific anti-OVA, anti-KLH and non-specific IgG antibodies in serum. The wells of 96-well microtiter plates (Immuno Plate; Nunc, Inter-Med, Denmark) were coated with 20 μg/mL of OVA, 10 μg/mL of KLH, or 10 μg/mL of goat anti-bovine IgG (Kirkegaard and Perry Laboratories, Inc., Mandel Division, Guelph, Ontario) in phosphate-buffered saline (PBS) at 4°C for 18 h. The wells of the plates were washed 3 times with PBS containing 0.05% Tween-20 (PBS-T) and incubated with PBS containing 1% bovine serum albumin (BSA; Sigma Chemical Co.) at 37°C for 30 min, the plates were then washed with PBS-T. One hundred microlitres of duplicate serum samples, diluted 1:200 (for IgG anti-OVA and anti-KLH determination), or from 1:100 to 1:10 000 (for non-specific IgG determination and compared with a standard curve provided by purified bovine IgG (Sigma)), in PBS, were added to the wells and incubated at 37°C for 2 h. The plates were washed 3 times with PBS-T, and 100 μL of alkaline phosphatase-conjugated goat anti-bovine IgG (Kirkegaard and Perry Laboratories) in PBS-T were added and incubated at 37°C for 1 h. After washing with PBS-T, 100 μL of the alkaline phosphatase substrate solution (1 mg/mL of *p*-nitrophenyl phosphate (Sigma) in 1 M diethanolamine buffer, pH 9.8) was added to each well. The absorbance of each well at 405 nm was measured using an automated spectrophotometer (Molecular Devices V_{max} Kinetic microplate reader; Molecular Devices, Menlo Park, California, USA). Immunoglobulin G anti-OVA and anti-KLH levels are given as the optical density (OD) readings, non-specific IgG is reported as the concentration in mg/mL. All results are expressed as the mean ± standard error of the mean (SEM) for each group.

DETERMINATION OF LEUKOCYTE NUMBERS, CELL KINETICS AND DIFFERENTIAL CELL COUNTS IN CIRCULATING BLOOD

The number of white blood cells (leukocytes) per mL of blood was determined using a Coulter Counter (Coulter Electronics, Inc., Hialeah, Florida, USA). Blood smears were made on glass slides, fixed and

stained using Leukostat solution (Fisher Diagnostics, Pittsburgh, Pennsylvania, USA). The slides were examined by light microscopy and differential counts of 100 leukocytes were determined. The cells were differentiated as: total leukocytes, lymphocytes, neutrophils, and monocytes. Leukocyte numbers were calculated by the following formula: No. Leukocyte = (No. of cells per mL) × (% of leukocyte type)/100. The results were expressed as the mean total cell numbers per mL of blood ± SEM.

DETERMINATION OF IRON AND ZINC CONCENTRATION

Serum iron and zinc concentration was measured (ICP-Burgener Nebulizer for Trace Minerals, Thermo Jarrell Ash Corp., Franklin, Massachusetts, USA) and expressed as the mean parts per million (ppm) of iron and zinc per mL of serum.

STATISTICAL ANALYSIS

Results are expressed as means ± standard error of the mean (SEM) and compared between groups by the Student's *t*-test or by analysis of variance (ANOVA) using the Tukey-Kramer multiple comparisons test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

EXPERIMENT 1

The ability of OβG to stimulate lymphocyte proliferation of naive or DXM-treated cells was assessed. Blood lymphocytes were cultured in the presence of OβG or OβG+DXM and the harvested cells were analyzed for proliferation. Figure 1 shows the dose-dependent effects of OβG on lymphocyte proliferation. The OβG alone did not promote proliferation and at the highest dose, 500 μg/mL, it inhibited proliferation ($P < 0.05$). In contrast, when the cells were cultured in the presence of DXM, OβG significantly ($P < 0.05$ to $P < 0.001$) enhanced proliferation.

EXPERIMENT 2

To determine whether there was an immunomodulatory effect by the OβG treatment of conventional, healthy beef steers, serum anti-OVA IgG antibody and blood lymphocyte proliferation in response to OVA and the differential counts of blood leukocytes were evaluated. Animals injected with saline solution were used as controls. As illustrated in Figures 2 to 4, no significant differences were detected between the treatment groups.

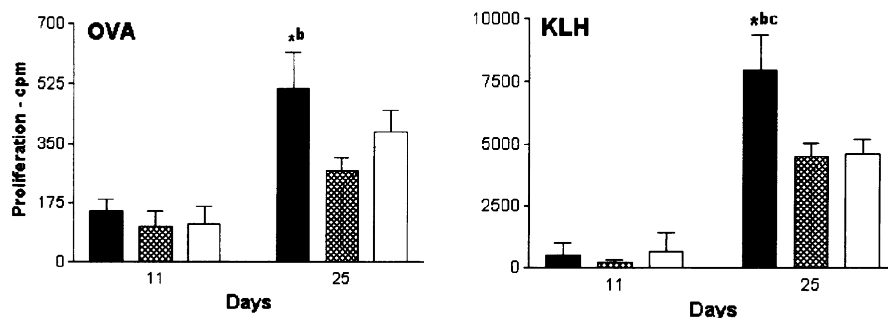

Figure 6. Effects of the administration of OβG+DXM (closed bar[a]), DXM (hatched bar[b]) or saline (open bar[c]) on specific lymphocyte proliferation responses determined on Days 11 and 25. Blood lymphocytes were cultured with OVA or KLH at a concentration of 200 and 50 μg/mL, respectively. Cells were incubated at 37°C in an atmosphere containing 5% CO_2 for 72 h. The samples in each well were then incubated with 0.1 μCi/well of [^{3}H]thymidine for 16 h. After cell harvesting, the radioactivity was measured in a β-scintillation counter. Each bar represents the mean of groups of 10 animals ± SEM of triplicate determinations. Where OβG+DXM, DXM or saline groups are designated as [a,b,c] respectively, means differ vs the group indicated with letter superscript. * $P < 0.05$.

EXPERIMENT 3

The effect of the administration of OβG on serum non-specific IgG concentration and on antigen-specific IgG antibody to OVA and KLH is depicted in Figure 5. On Day 11, there were no differences among groups in the IgG responses studied. On Day 25, OβG+DXM group had significantly increased ($P < 0.01$) serum IgG concentration when compared to the control group. On this day, IgG anti-OVA ($P < 0.01$ vs DXM and control groups) and IgG anti-KLH ($P < 0.05$ vs DXM group; $P < 0.01$ vs control group) were significantly higher than in OβG non-treated groups.

Antigen-specific lymphocyte proliferative responses were determined to assess the overall immunocompetence of DXM-treated calves with or without OβG treatment (Fig. 6). Blood lymphocytes from treated and control animals were cultured in the presence of OVA and KLH. On Day 25, proliferation responses of lymphocytes of OβG+DXM group, to both OVA and KLH, were significantly increased ($P < 0.05$) compared to DXM group, and to KLH ($P < 0.05$) compared to the untreated control group.

To determine whether there was a quantitative change in leukocyte populations after the in vivo treatment with OβG to immunosuppressed steers, the number and type of leukocytes in blood were examined in animals treated with OβG+DXM, DXM, or saline (Fig. 7). Steers injected with saline were used as controls and had no significant changes in differential leukocyte numbers throughout the experiment. The DXM-treated group had significant changes in cell numbers at various time points compared to the control group, while those changes were observed only on Day 4 in the OβG+DXM group. Significant leukocytosis was observed on Day 4 in the group treated with DXM only ($P < 0.01$ vs OβG+DXM group; $P < 0.001$ vs control group). The OβG+DXM and DXM groups presented a lymphopenia on Day 4 ($P < 0.05$ and $P < 0.001$, respectively) in relation to the control group; lymphopenia was still significant on Days 11 and 18 ($P < 0.05$) in DXM group, while in OβG+DXM group the number of lymphocytes were at similar levels as the control group. Neutrophilia occurred on Day 4 in both OβG+DXM ($P < 0.05$) and DXM ($P < 0.001$) groups compared to the control group; however, the DXM group had significantly higher numbers of neutrophils ($P < 0.001$) than OβG+DXM group. An increased number of monocytes was observed on Day 4 in OβG+DXM group ($P < 0.05$) compared to DXM group.

Previous observations have indicated that the administration of DXM to cattle decreased iron and zinc concentrations in serum (22). On this basis, the effect of OβG administration on

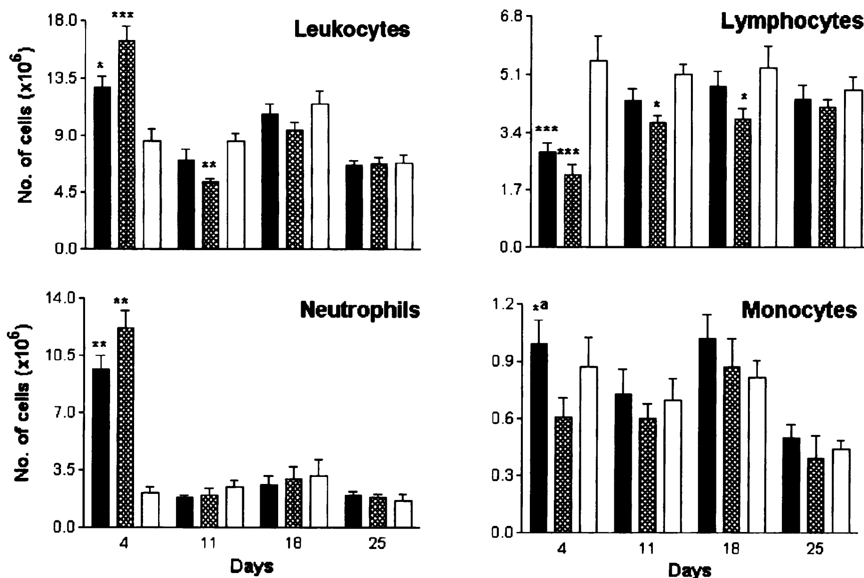

Figure 7. Time-dependent effects of the administration of OβG+DXM (closed bar[a]), DXM (hatched bar[b]) or saline (open bar[c]) on the total number of blood leukocytes, lymphocytes, neutrophils and monocytes. Blood samples were collected on the indicated days and the number of cells were counted. Data are expressed as the mean total leukocyte numbers in blood of groups of 10 animals ± SEM. Where OβG+DXM, DXM or saline groups are designated as [a,b,c] respectively, means differ vs the group indicated with letter superscript. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

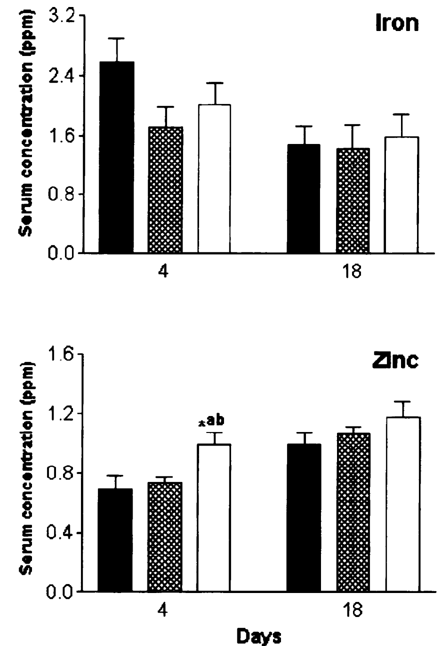

Figure 8. Iron and zinc concentrations in serum of steers administered OβG+DXM (closed bar[a]), DXM (hatched bar[b]) or saline (open bar[c]). Blood samples were collected on Days 4 and 18 and iron and zinc concentrations were tested. The bars represent the mean parts per million (ppm) of iron and zinc per mL of blood serum of groups of 10 animals ± SEM. Where OβG+DXM, DXM or saline groups are designated as [a,b,c] respectively, means differ vs the group indicated with letter superscript. * $P < 0.05$.

serum iron and zinc concentration was assessed. On Day 4, the group OβG+DXM had higher, not quite significant ($P < 0.06$), iron concentration than DXM group; on this day, zinc concentration was significantly lower ($P < 0.05$) in both DXM-treated groups compared to control, non-treated group. On Day 18, serum iron and zinc concentrations of OβG+DXM and DXM groups were parallel to those of the control group (Fig. 8).

DISCUSSION

The purpose of this study was to examine the effect on immune parameters of administration of OβG to beef steers. In vitro, the effect of OβG on the proliferation of lymphocytes with or without the presence of DXM was evaluated. In vivo, 2 experiments were carried out. In the first, healthy beef steers were administered OβG and selected immune parameters on specific and non-specific immune responses were tested. In the second experiment, steers were administered DXM and the effect of OβG to overcome immunosuppression was assessed; also assessed were the induced antibody responses to OVA and KLH and lymphocyte proliferation responses to the same antigens as well as differential blood leukocyte numbers and iron and zinc concentrations in serum.

The suppression of Con A-induced bovine lymphocyte proliferation by DXM has been reported (23). In the present study, the treatment of naive calf lymphocytes with OβG did not increase their ability to proliferate; however, when OβG was added to cultures of DXM-treated lymphocytes, a partial reversion of the inhibitor effect of DXM occurred. The failure of OβG to induce proliferation of naive bovine lymphocytes appears to contradict our previous observation, which demonstrated that OβG added to cultures of mouse spleen cells induced the production of IFN-γ and IL-2 (19). On the other hand, the difference in response to OβG could be explained by the relative differences in number, state of activation, and/or type of accessory cells present in the spleen vs the blood. Accessory cells can mediate the lymphoproliferative response and are likely to be the primary target of OβG effects.

Administration of OβG to clinically healthy steers in experiment 2 did not induce significant changes on the immune parameters studied, which included IgG antibody levels to OVA, specific proliferation responses of lymphocytes to OVA, and leukocyte differential cell counts. This lack of effect is in agreement with our in vitro data and may be explained by homeostatic mechanisms, which, in healthy cattle, may prevent a change in the immune status of the host by immunostimulants. Presence of stress or disease may be required for compounds, such as OβG, to exert immunomodulatory effects, reverting the immune status to homeostasis with competency of immune responses. Other studies in cattle (24–26) have shown variability in the immune responses with immunostimulant administration, depending on the degree of stress to which the animal was subjected.

It is well recognized that stress occurs in cattle subjected to handling for examination or transport (27). Stress is associated with increased susceptibility to infectious diseases (3). This increased susceptibility is due, in part, to increased endogenous glucocorticosteroid secretion (23).

Glucocorticosteroids are immunosuppressive and are considered to contribute to the respiratory disease complex of cattle (28). Dexamethasone is a potent synthetic glucocorticosteroid and DXM-induced immunosuppression has been used as a model for evaluating several potential immunostimulants in cattle (24,29,30).

It is well established, for cattle, that glucocorticosteroids induce an alteration of leukocyte profiles with leukocytosis, lymphopenia, neutrophilia and monocytopenia (26,27). These changes were observed on Day 4 in the DXM-treated group, while in the OβG+DXM group, lymphopenia and neutrophilia were partially reversed and monocytopenia was completely reversed. Significant lymphopenia ($P < 0.05$) was still present in the DXM group, but not in the OβG+DXM group, on Days 11 and 18. The leukocyte profiles confirmed the immunosuppressive effect of glucocorticosteroids and clearly indicated an immunomodulating effect provided by OβG treatment.

Evidence of DXM-induced immunosuppression was observed only for blood leukocyte profiles. Non-specific serum IgG, as well as primary, secondary IgG and lymphocyte proliferative responses to OVA and KLH were not affected by DXM treatment. However, the co-administration of OβG and DXM significantly increased non-specific serum IgG concentration and augmented secondary antibody and proliferative responses to OVA and KLH, such that these responses were greater than those observed in the DXM-only group and the saline-treated control group. The responses in excess of control in the OβG+DXM group were not expected, nor is an explanation readily apparent.

Increased serum non-specific and antigen-specific IgG responses following OβG treatment is in agreement with previous work in DXM-immunosuppressed mice (20) and may be attributed to macrophage stimulation (19). Ovalbumin and KLH are considered to be T cell-dependent antigens and, thus, macrophages are necessary for the induction of antibody responses to those antigens in cattle (31). Treatment with OβG induced a positive effect in blood monocyte numbers and this effect may also indicate a general improvement in macrophage function (19).

Significant serum non-specific IgG, antigen-specific IgG, and lymphoproliferative responses to OβG treatment were observed only on Day 25 following the second vaccination, and not on Day 11 following the primary vaccination, the time at which the OβG treatment was administered. As expected, the primary antigen-specific immune responses were low; however, close examination indicates that the OβG+DXM group demonstrated numerically higher responses than the DXM group. It appears that relatively small differences in primary immune responses can have a significant impact on the magnitude of the responses following secondary exposure to antigens (32). Alternatively, blood lymphocyte counts indicate that the immunomodulatory effects of OβG persisted until Day 18 when the secondary vaccination was administered. The high molecular weight and β-linked structure of OβG is consistent with the suggestion that OβG is cleared relatively slowly (33) and counteracted the immunosuppressive effects of DXM on both primary and secondary responses.

Glucocorticoids such as DXM appear to impair cell-mediated immunity by decreasing antigen presentation, lymphocyte numbers, proliferative responses and cytokine production (26,34). Humoral immunity is most likely influenced by decreases in T cell and macrophage functions (35). Dexamethasone-immunosuppressed steers treated with OβG had higher serum antibody levels and increased specific lymphocyte proliferative responses than DXM-only, untreated animals. There is possibility that the immunomodulating effects of OβG in cattle are directed toward the up-regulation of cellular immunity.

The administration of DXM to steers caused a slight decline of iron concentration in the serum of OβG-untreated animals, an effect that was apparently reversed by OβG treatment. There was no OβG effect on the decreased serum zinc concentration. The administration of DXM produced a decreased concentration of both minerals in cattle in another study (22).

From the results of this study, immunosuppression appears to be essential for the immuno-modulatory effects provided by OβG. This was particularly evident in experiment 2, in which the steers were not treated with DXM and no significant effects of OβG treatment were observed in any of the immune parameters studied.

A challenge infectious disease model was not performed in this research because our aim was to evaluate the effect of the administration of OβG to normal and mildly immunosuppressed animals, in order to better understand the mechanism of action of the immunostimulant prior to its evaluation in challenge models.

The results indicated that the administration of OβG to DXM-immunosuppressed steers may exert an immunomodulatory effect, as observed on non-specific IgG antibody concentration, IgG responses in response to OVA and KLH, specific lymphocyte proliferation responses to OVA and KLH, and leukocyte cell counts. These data suggest that immune functions suppressed by DXM may be partially restored by OβG, and that these restored immune functions may play important roles in providing resistance to infectious diseases. In light of the potential benefit to beef industry, further studies using larger number of animals, clinically relevant antigens, and infectious disease challenges are required to confirm present results and extend them to practical applications.

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