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The Effect of Dexamethasone on Some Immunological Parameters in Cattle

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ABSTRACT

Immunosuppression as a consequence of acute and chronic stress can increase the susceptibility of cattle to a range of infectious diseases. In order to develop a panel of immune function assays for investigating the effects of potential stressors on immune competence in cattle, the effect of treatment with short- and long-acting preparations of the synthetic glucocorticoid dexamethasone was examined. Short-acting dexamethasone (dexamethasone sodium phosphate 0.08 mg/kg) followed 37 h later by long-acting dexamethasone (dexamethasone-21 isonicotinate 0.25 mg/kg) was injected intramuscularly and blood was collected to assess immune functions at intervals over the subsequent 11 days from 6 treated and 6 control Hereford steers. Dexamethasone induced leukocytosis (neutrophilia, eosinopenia, lymphopenia, monocytosis), an increased neutrophil:lymphocyte ratio, an elevated percentage of $CD4^+$ lymphocytes, a decreased total $CD8^+$ lymphocyte count, decreased total and percentage $WC1^+$ lymphocytes, an elevated percentage of IL-2 receptor alpha $(IL\text{-}2R\alpha)^+$ lymphocytes, and an elevated percentage of B lymphocytes. *In vitro* chemotaxis of peripheral blood neutrophils to human C5a and ovine IL-8 was increased by dexamethasone treatment. Lymphocyte proliferation in the presence of phytohaemagglutinin, and serum concentrations of IgM, but not IgA or IgG1, were suppressed by dexamethasone treatment, whereas mitogen-induced production of interferon-γ (IFN-γ), neutrophil expression of CD18, neutrophil myeloperoxidase activity and natural killer (NK) cell activity were not influenced by dexamethasone treatment. The results indicate the potential for haematology and immune function assays to reflect elevated activity of the hypothalamic–pituitary–adrenocortical axis in cattle. Immunological parameters may thus provide a useful adjunct to cortisol and behavioural observations for assessing the impact of stress on the welfare of cattle.

Keywords: cattle, dexamethasone, IgM, immune competence, lymphocytes, neutrophils, stress, welfare

Abbreviations: ACD, acid citrate dextrose; ANOVA, analysis of variance; CD, cluster designation; cpm, counts per minute; EBSS, Earle's balanced salt solution; EDTA, ethylenediaminetetraacetic acid; EMAI, Elizabeth Macarthur Agricultural Institute; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HBSS, Hanks' balanced salt solution; IFN, interferon; Ig, immunoglobulin; IL, interleukin; i.m., intramuscular; MEM, minimum essential medium; IBR, infectious bovine rhinotracheitis; NK, natural killer; PBS, phosphate-buffered saline; PBS-G, phosphate-buffered saline containing 0.01% thiomersal and 0.25% gelatin; PBS-T, phosphate-buffered saline containing 0.05% Tween-20; PE, phycoerythrin; PHA, phytohaemagglutinin; SDS, sodium dodecyl sulphate; SI, stimulation index; WC, workshop cluster

INTRODUCTION

Husbandry procedures and intensive production systems have the potential to stress cattle and thus to compromise animal welfare through the induction of behavioural stereotypies and stress-related diseases. The association between stress and increased susceptibility to disease is well recognized for many species. In cattle, for example, transport is associated with an increased risk of bovine respiratory disease (Hails, 1978), and the hormonal and metabolic changes occurring at parturition increase the risk of bacterial mastitis in dairy cows (Van Kampen and Mallard, 1998; Detilleux *et al.*, 1995). Recently, the measurement of immunological functions has been proposed as an adjunct to the measurement of behaviour and adrenocortical homones for assessing the welfare of cattle (Amadori *et al.*, 1997). To this end, a range of parameters of innate and acquired immunity was assessed in Hereford steers to examine the effect of dexamethasone, which pharmacologically mimics some of the effects of the elevated activity of the hypothalamic–pituitary–adrenocortical axis that accompanies stress.

MATERIALS AND METHODS

Experimental design

Year-old Hereford steers from the breeding herd at CSIRO Pastoral Research Laboratory Armidale were run in a 4-hectare holding paddock adjacent to the cattle yards, with free access to phalaris and white clover pasture. No stock had been introduced to the herd since it had tested seronegative for infectious bovine rhinotracheitis (IBR) virus at the Elizabeth Macarthur Agricultural Institute (EMAI), Camden, NSW, Australia. Six steers received short-acting dexamethasone (dexamethasone sodium phosphate 0.08 mg/kg, i.m.) at 1700 h on day 1 followed 37 h later by long-acting dexamethasone (dexamethasone-21 isonicotinate 0.25 mg/kg, i.m., at 0630 h on day 3), while six steers served as controls and concurrently received equal volumes of sterile saline. Using sterile procedures, 40 ml blood was collected by jugular venepuncture into 10 ml acid citrate–dextrose anticoagulant contained in 50-ml disposable syringes (Terumo Medical Corporation, Elkton, MD, USA). Ten ml of blood and 2×5 ml of blood were collected into plain vacutainers (Becton Dickinson, Lane Cove, NSW, Australia) for serum and into ethylenediaminetetraacetic acid anticoagulant, respectively, at 0630 h on days –2, 2, 3, 4, 6, 9 and 12. At the same sampling times, several ml of saliva was collected from the buccal cavity using a 10-ml displacement pipette with wide-mouth polypropylene tips.

Haematology

The total leukocyte count, red cell count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and platelet count were determined on EDTA blood with a Coulter Counter (model S880).

Flow cytometry

A two-colour staining reaction using monoclonal antibody to CD45 (leukocyte common antigen) to identify leukocytes and a second antibody to a panel of cell surface markers was used. Immunoglobulin isotype-specific antibodies coupled to FITC or PE were used to report the staining reaction. Aliquots (100 ml) of EDTA blood were placed in 4.5-ml polystyrene tubes (Falcon, Becton Dickinson) and incubated with 50 μl of a solution of primary antibody (Table I) at 4°C for 20 min in the dark. The cells were washed twice (5 min, 350*g*, 4°C) with 2 ml of phosphate-buffered saline (PBS) and then incubated with 50 μl fluorochrome-conjugated secondary antibodies for 20 min at 4°C in the dark. To lyse the majority of the red blood cells, 100 μl 8% formalin in PBS was added, then 1 min later 1 ml of water at 37°C was added. The tubes were incubated at 37°C for 3 min, then 2 ml of PBS (4°C) was added and the tubes were centrifuged (5 min, 350*g*, 4°C). The cells were washed a second time with 2 ml PBS and centrifuged (5 min, 350*g*, 4°C). 1% Paraformaldehyde (200 μl) in PBS was added and the tubes were kept in the dark until acquisition of data on a FACS Vantage flow cytometer (Becton Dickinson) within 24 h of staining. Data were collected from 10 000 $CD45^+$ events (leukocytes) and later analysed with CellQuest software (Becton Dickinson). Gates set on forward scatter versus side-scatter plots were used to identify lymphocytes or neutrophils for determining the percentage of positive-staining cells and the mean fluorescent intensity (CD18). Differential leukocyte counts were calculated by plotting CD14 staining versus side-scatter. Monocytes, lymphocytes, neutrophils and eosinophils were evident as discrete clusters on this plot.

TABLE I
Monoclonal antibodies used to identify cell surface markers on leukocytes

Monoclonal antibody	Specificity	Isotype	Dilution	Source
CC30	CD4	IgG1	1:100	Serotec[a]
CC17	CD5	IgG1	1:100	Serotec
CC63	CD8	IgG2a	1:100	Serotec
BAQ30A	CD18	IgG1	1:50	VMRD[b]
19.19	WC1	IgG1	Neat	CAB[c]
BAQ44A	B cells	IgM	1:50	VMRD
28.1	MHC class II	IgG1	1:10	CAB
CACT26A	ACT2	IgG1	1:50	VMRD
CACT116A	IL2Rα	IgG1	1:50	VMRD
1.28	CD45	IgG2a	1:5	CAB
1.11.32	CD45	IgG1	1:5	CAB

[a]Serotec, Kidlington, Oxford, UK

[b]Veterinary Medical Research and Development, Pullman, WA, USA

[c]Centre for Animal Biotechnology, Victoria, Australia

Preparation of cells

Neutrophils: Neutrophils were isolated by the method of Roth and Kaeberle (1981), modified as follows: ACD blood was centrifuged in 50-ml polypropylene Falcon tubes at 1000*g* for 20 min at 4°C. Plasma was discarded, but the buffy coat was retained as a source of peripheral blood mononuclear cells. The red blood cells were then lysed by addition of 2 volumes of cold 0.0132 mol/L phosphate buffer, pH 7.2, followed by mixing and rotating for 45 s, after which the isotonicity was restored with 1 volume of the above buffer containing 2.7% NaCl. The lysate was centrifuged at 600*g* for 10 min at 4°C, the supernatant was discarded, and the red blood cells remaining in the pellet were lysed by the addition of 10 ml ice-cold Tris–ammonium chloride buffer, pH 7.2, (0.017 mol/L Tris, 0.14 mol/L NH_4Cl). The second lysate was immediately centrifuged at 400*g* for 10 min and cells were then washed twice with cold Hanks' balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+} and resuspended in 1 ml HBSS. Twenty μl was removed and diluted for counting in a Coulter Counter and the cell concentration was adjusted to 5×10^7 viable cells/ml for the myeloperoxidase assay or 3×10^6 viable cells/ml for the chemotaxis assay.

Mononuclear cells: The buffy coat was made up to 6 ml with a cell wash medium that consisted of PBS, pH 7.2, supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml) and 2% heat-inactivated fetal bovine serum. Mononuclear cells were isolated by density gradient centrifugation on Ficoll-Paque (AMRAD Pharmacia Biotech, Melbourne, Victoria, Australia). Contaminating red blood cells were removed by Tris–ammonium chloride lysis before washing and suspension in 10 ml RPMI 1640 medium, supplemented with Hepes buffer (20 mmol/L), L-glutamine (2 mmol/L), 10% FBS and 2-mercaptoethanol (5×10^{-5} mol/L). The cells were incubated overnight in 25-cm^2 tissue culture flasks at 37°C with 5% CO_2 in air atmosphere and $>95\%$ humidity to deplete the suspension of macrophages, which can also effect target cell killing and interfere with NK cell assays. Non-adherent cells were removed and washed once with RPMI 1640 and the cell concentration was adjusted to 5×10^6 viable cells/ml in RPMI 1640 without 2-mercaptoethanol (effector cells for NK cell assay), or 1×10^6 cells/ml in RPMI 1640 with 2-mercaptoethanol (IFN-γ and lymphocyte stimulation assays).

Neutrophil chemotaxis

Migration of cells towards $10^{-8.5}$ mol/L ovine IL-8 (Seow *et al.*, 1994) and $10^{-8.5}$ mol/L human C5a (Sigma, St Louis, MO, USA) was assessed in microchemotaxis chambers (Neuro Probe Inc., Bethesda, MD, USA) with 3-μm polycarbonate filters (Neuclepore, Costar Corp., Cambridge, MA, USA) as described by Mulder and Colditz (1993). Neutrophil migration was assessed with the aid of an image analyser (Quantimet 500, Leica, Lane Cove, NSW, Australia) and expressed as the percentage of the total area that was covered by migrated cells.

Neutrophil myeloperoxidase activity

Pre-opsonization of zymosan and the myeloperoxidase assay were carried out by the methods described by Roth and Kaeberle (1981). The results were expressed as an iodination rate, which is the amount of organic iodide consumed by 10^7 neutrophils in 1 h (nmol NaI/10^7 neutrophils per h).

Lymphocyte proliferation assay

Aliquots (200 μl) of the 1×10^6/ml cell suspensions were added to the wells of Falcon 96-well flat-bottom tissue culture plates. Volumes of 20 μl of mitogens, concanavalin A (5 μg/ml) and phytohaemagglutinin (50 μg/ml) diluted in Earle's balanced salt solution (EBSS) were added to the cells in quadruplicate; control cells received 20 μl EBSS. Mitogen concentrations just below the optimum stimulation plateaux were chosen from dose–response curves in order to identify subtle treatment differences that might otherwise have been masked. Plates were incubated for 72 h as described, and then the cells were pulsed with 20 μl tritiated thymidine (25 μCi $6H_3T$/ml EBSS, ICN Biomedicals, Seven Hills, NSW, Australia). Sixteen hours later, the cells were harvested on Titertek filter mats (ICN) using a Skatron cell harvester (model AS) and the mats were oven-dried at 50°C. Cell disks were punched from the filter mats into 6-ml Poly-Q scintillation vials (Beckman Instruments, Brisbane, Qld, Australia), 2 ml of liquid scintillation cocktail was added (CytoScint ES, ICN) and 2-min counts were carried out in a Beckman beta counter (model LS 3801). Preliminary assays indicated that macrophage depletion did not interfere with the lymphocyte proliferative responses when intact and depleted cultures were compared.

IFN-γ production

Aliquots (200 μl) of the 1×10^6/ml cell suspensions were added in triplicate to the wells of Falcon 96-well flat-bottom tissue culture plates. The plates were incubated as described and, approximately 20 h later, 100 μl of the supernatant was removed from each well and stored at –20°C. Interferon-γ was measured quantitatively using an enzyme-linked-immunosorbent assay (Rothel *et al.*, 1990).

Natural killer cell assay

Bovine NK cell activity was measured by a ^{51}Cr-release assay in which effector cells (bovine peripheral blood mononuclear cells) were incubated with ^{51}Cr-labelled, virus infected, xenogeneic target cells (Cook and Splitter, 1989; Cook *et al.*, 1989).

Target cells: Canine osteosarcoma cell line D17 (ATCC CCL183) and human lung carcinoma cell line A549 (ATCC CCL185) (American Type Culture Collection,

Rockville, MD, USA) were maintained in continuous culture as monolayers in 75-cm^2 vented flasks in MEM (with Earle's salts), supplemented and incubated as described for effector cells. Adherent cells were collected by first washing the monolayers with warm (37°C), modified EBSS (without Ca^{2+} and Mg^{2+}), followed by incubation in warm cell-dissociation solution containing trypsin–EDTA (Sigma-Aldrich, Castle Hill, NSW, Australia). The cells were washed in MEM, the cell concentration was adjusted to 1×10^5 viable cells/ml MEM, and 100 μl of the cells was added to the wells of 96-well U-bottom tissue culture plates (Falcon), giving a target cell concentration of 1×10^4 cells/well. The cells were labelled overnight with 3 μCi $Na_2{}^{51}CrO_4$/well (Amersham, Castle Hill, NSW, Australia) and then infected for 2 h with IBR virus strain Y535 (EMAI) at a multiple of infection of 10. The plates were then washed three times with warm (37°C) Earle's balanced salt solution before the addition of 100 μl MEM.

Assay: Aliquots of 100 μl of effector cells were added to target cells in quadruplicate, giving an effector cell:target cell ratio of 50:1. This suboptimal ratio was chosen from dose–response curves for reasons outlined in the lymphocyte stimulation assay, as well as to ensure economical use of effector cells combined with adequate cytotoxicity. Spontaneous release controls received 100 μl RPMI 1640 and maximum-release cells received 100 μl 10% SDS. Plates were centrifuged for 1 min at 100*g* and then incubated as described for 20 h. Following incubation, the plates were centrifuged for 1 min at 100*g* and 100 μl supernatant samples were transferred to microtubes, which were counted for 1 min in a gamma counter (1282 Compugamma, Wallac, Linbrook Pty Ltd, Thornleigh, NSW, Australia). Cytotoxicity was calculated according to the equation:

$$\%\ \text{Cytotoxicity} = \frac{(\text{CPM mean sample release} - \text{CPM mean spontaneous release})}{(\text{CPM mean maximum release} - \text{CPM mean spontaneous release})} \times 100$$

Immunoglobulin determinations

Immunoglobulin standards were quantified using radial immunodiffusion kits specific for each bovine immunoglobulin class (VMRD, Pullman, WA, USA) and the concentrations were adjusted to 10 mg/ml (IgG1 and IgM) or 2 mg/ml (IgA) in PBS, pH 7.2, containing 0.01% thiomersal and 0.25% gelatin (PBS-G). Aliquots of 200 μl were prepared and stored at –80°C. Pooled bovine serum and bovine colostral whey stored at –80°C were used as positive controls and negative controls were prepared by depleting pooled bovine serum of the relevant antibody class using an appropriate immunoabsorbant.

Serum or saliva immunoglobulins were measured using enzyme-linked immunosorbent assays. Immulon 2, 96-well, flat-bottom microtitre plates (Dynex Technologies, Chantilly, Virginia, USA) were coated with 100 μl per well of mouse monoclonal anti-bovine immunoglobulin capture antibody (VMRD) diluted to 5 μg/ml (anti-bovine IgG1 and anti-bovine IgM) or 4 μg/ml (anti-bovine IgA) in PBS. The plates were sealed

with plastic wrap and incubated overnight, humidified at 4°C. Capture antibody was emptied from the wells and the plates were washed by filling the wells twice with PBS containing 0.05% (v/v) Tween-20 and then once more with PBS, allowing 3 min soak time between washes. Remaining active sites were blocked by the addition of 200 μl per well of 1% gelatin in PBS, followed by sealing the plates with plastic wrap and incubating for 2 h, humidified at 37°C. After incubation, the plates were washed as before, then sealed with plastic wrap and stored at –20°C. A range of standards from 0.5 to 60 mg/dl (IgG1), 0.1 to 50 μg/dl (IgA) or 1 to 200 μg/dl (IgM) were prepared by diluting stock immunoglobulins in PBS-G. Samples and controls were diluted 1:400 (serum IgG1), 1:4000 (serum IgA), 1:10 000 (saliva IgA) or 1:10 000 (serum IgM) in PBS-G and 100 μl volumes of samples, standards and controls were added in duplicate or triplicate to the wells of the coated microplates; blank wells received PBS-G. The plates were sealed with plastic film and incubated overnight, humidified at 4°C; the next day, the plates were emptied and washed three times with PBS-T, allowing 3 min soak between washes.

IgG1: Horseradish peroxidase-conjugated rabbit anti-bovine IgG1 heavy- and light-chain-specific (Zymed Laboratories Inc., San Francisco, CA), 100 μl diluted 1:3000 in PBS-G, was added to all the wells. The plates were sealed and incubated overnight, humidified at 4°C. The following day, the plates were emptied and washed as described, before the addition of 100 μl of substrate consisting of 0.4 mg/ml *o*-phenylenediamine dihydrochloride and 0.014% hydrogen peroxide in 0.05 mol/L phosphate-citrate buffer, pH 5.0. Colour was allowed to develop for 30 min in the dark at room temperature, when the reaction was stopped by the addition of 50 μl 12.5% sulphuric acid. Absorbances were read at 492 nm in a microplate reader (Molecular Devices Emax, Beckman Instruments) and standard curves and values of unknowns were obtained by plotting the absorbance against a $\log_{10}$ concentration of standards using Softmax software (Molecular Devices).

IgA: Rabbit anti-bovine IgA heavy- and light-chain-specific (ICN), 100 μl diluted 1:50 000 in PBS-G, was added to all the wells. The plates were sealed and incubated for 1 h, humidified at 37°C, then emptied and washed as described for IgG1. The incubation and washing procedure was repeated twice more, first following the addition of 100 μl of biotinylated goat anti-rabbit IgG heavy- and light-chain-specific (Zymed Laboratories), diluted 1:10 000, and then after the addition of 100 μl horse-radish peroxidase-conjugated streptavidin (Zymed Laboratories), diluted 1:10 000. Substrate was added and the plates were read as for the IgG1 assay.

IgM: Monoclonal biotinylated anti-bovine IgM (Sigma-Aldrich), 100 μl, diluted 1:40 000 in PBS-G, was added to all the wells. The plates were sealed and incubated for 1 h, humidified at 37°C, then emptied and washed as described for IgG1. The ensuing steps, the addition of enzyme conjugate and substrate were identical to the IgA assay.

Statistical analyses

Where necessary, data were transformed to stabilize variances, and were analysed for the effects of time, treatment and their interaction by ANOVA for repeated measures in the statistical package Systat. Values of $p < 0.05$ were considered significant.

RESULTS

Haematology

At the first blood sample, taken 13.5 h after treatment with dexamethasone, the total leukocyte count was elevated owing to neutrophilia (Figure 1). Lymphocyte and eosinophil percentages were depressed and the monocyte percentage tended to be higher in the treated animals than in the controls. Similar changes were evident 24 h later when the long-acting dexamethasone preparation was given. The trends observed during treatment with the short-acting dexamethasone continued over the 9 days following treatment with the long-acting dexamethasone, except that the numbers of monocytes increased significantly in the treated animals. During this time, a subset of control animals displayed an increase in neutrophil and monocyte counts, suggesting that repeated mustering and sampling of these animals was provoking a mild stress response (Figure 1).

A slight decrease in mean corpuscular volume on days 4, 6 and 9 was the only red cell parameter affected by dexamethasone treatment. Platelet counts were also unaffected by dexamethasone treatment.

Flow cytometry

There were marked effects of dexamethasone on the subpopulations of lymphocytes (Figure 2). The percentage of $CD4^+$ lymphocytes was elevated during the acute and chronic phases of dexamethasone treatment, whereas $CD8^+$ lymphocytes were transiently depressed following initial dexamethasone treatment (Figure 2). These changes in CD4 and CD8 percentages led to a transient increase in the ratio of $CD4{:}CD8^+$ lymphocytes following dexamethasone treatment. $WC1^+$ lymphocytes were profoundly depressed throughout the treatment period, while IL-$2R\alpha^+$ lymphocytes were elevated for 3 days after dexamethasone treatment (Figure 2). $CD5^+$, $MHCII^+$, $ACT2^+$ and B lymphocytes did not differ between groups. CD18 expression by neutrophils and the mean fluorescent intensity of staining were not affected by dexamethasone treatment.

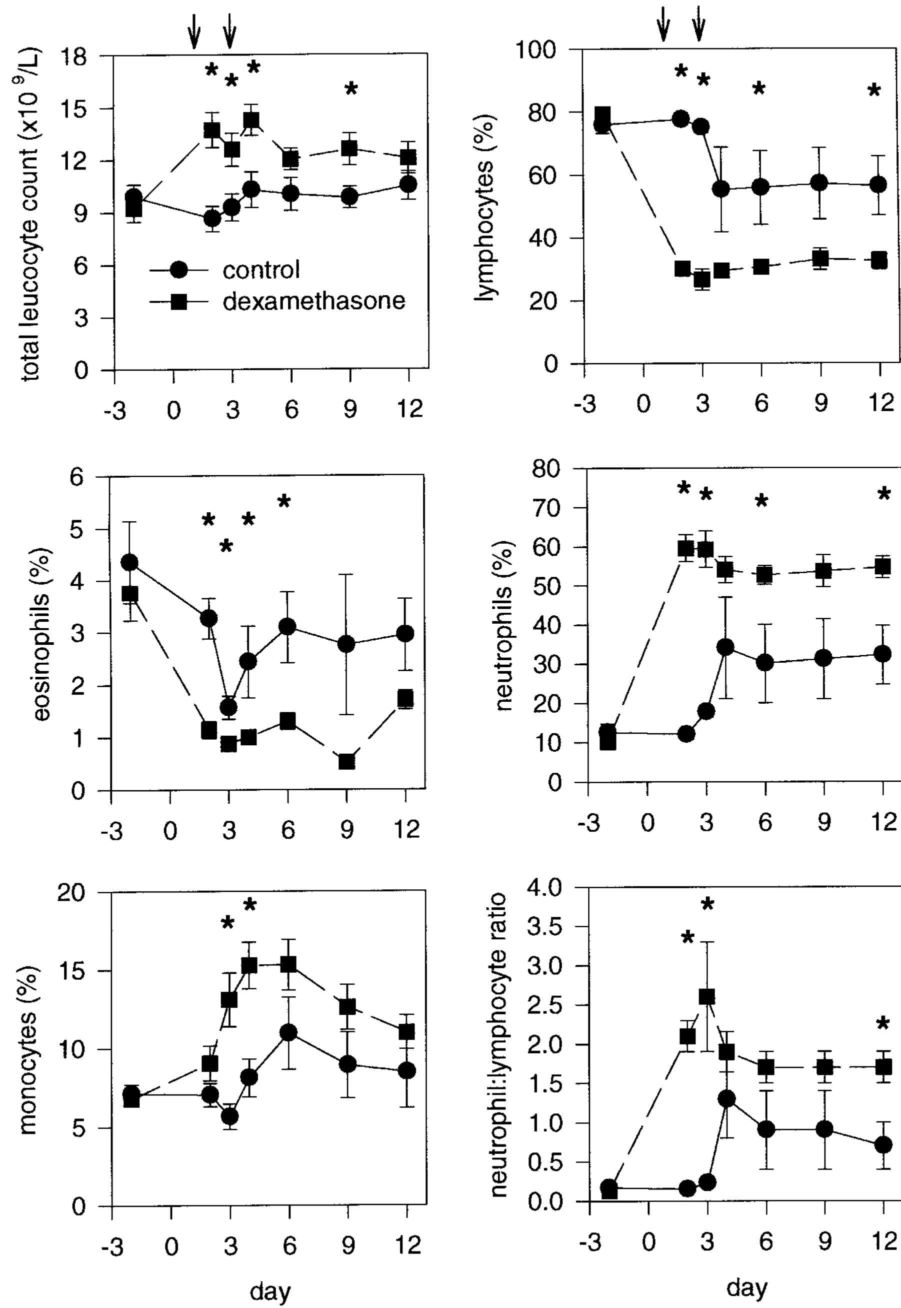

Figure 1. Leukocyte counts (mean ± SEM) in peripheral blood. Arrows indicate the times of treatment with dexamethasone. *Significant difference between the control and treated groups

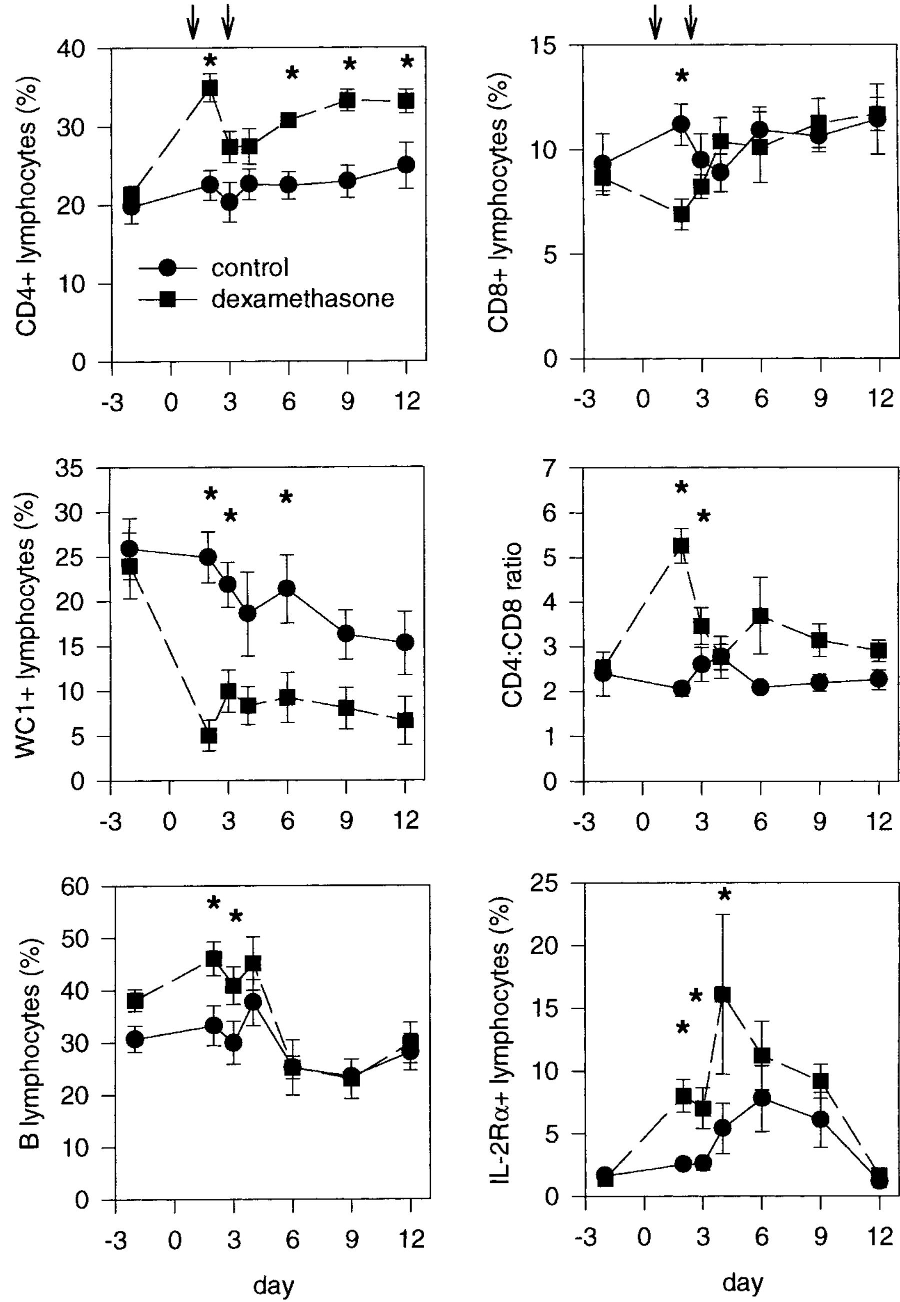

Figure 2. Lymphocyte phenotypes (mean ± SEM) as determined by flow cytometry. Arrows indicate the times of treatment with dexamethasone. *Significant difference between the control and treated groups

Neutrophil chemotaxis

Migration of neutrophils towards both C5a and IL-8 was elevated throughout the sampling period following dexamethasone treatment, although random migration of cells in the absence of chemoattractant did not differ between the groups.

Neutrophil myeloperoxidase

There was significant day-to-day variation in iodine incorporation in the myeloperoxidase assay, ranging from 4 to 25 nmol NaI/10^7 neutrophils per h. On day 6 following dexamethasone treatment, there was a significantly lower myeloperoxidase activity by neutrophils from the dexamethasone-treated steers.

NK cell assay

There was a small day-to-day fluctuation in values ranging from 19% to 27% in the percentage lysis of D17 targets by NK cells. Lysis of A549 targets showed more day-to-day variation, ranging from 4% to 25% of targets lysed. Dexamethasone treatment did not influence lysis of either target.

Lymphocyte proliferation

The cpm from tritiated thymidine in ConA- or PHA-stimulated lymphocyte cultures did not differ between groups. When the responses were expressed as stimulation indices, there was a trend to lower SI for ConA-stimulated lymphocytes and a significantly lower SI for PHA-stimulated lymphocytes at intervals during both the acute and chronic phases for the dexamethasone-treated group (Figure 3).

IFN-γ production

IFN-γ titres in lymphocyte culture supernatants ranged from 0.6 to 0.95 ng/ml culture supernatant but did not differ between the groups.

IgG1, IgA and IgM

Serum and salivary IgA and IgG1 did not differ between the treated and control groups. In contrast, serum IgM was suppressed in the dexamethasone group from day 6 onwards (Figure 4).

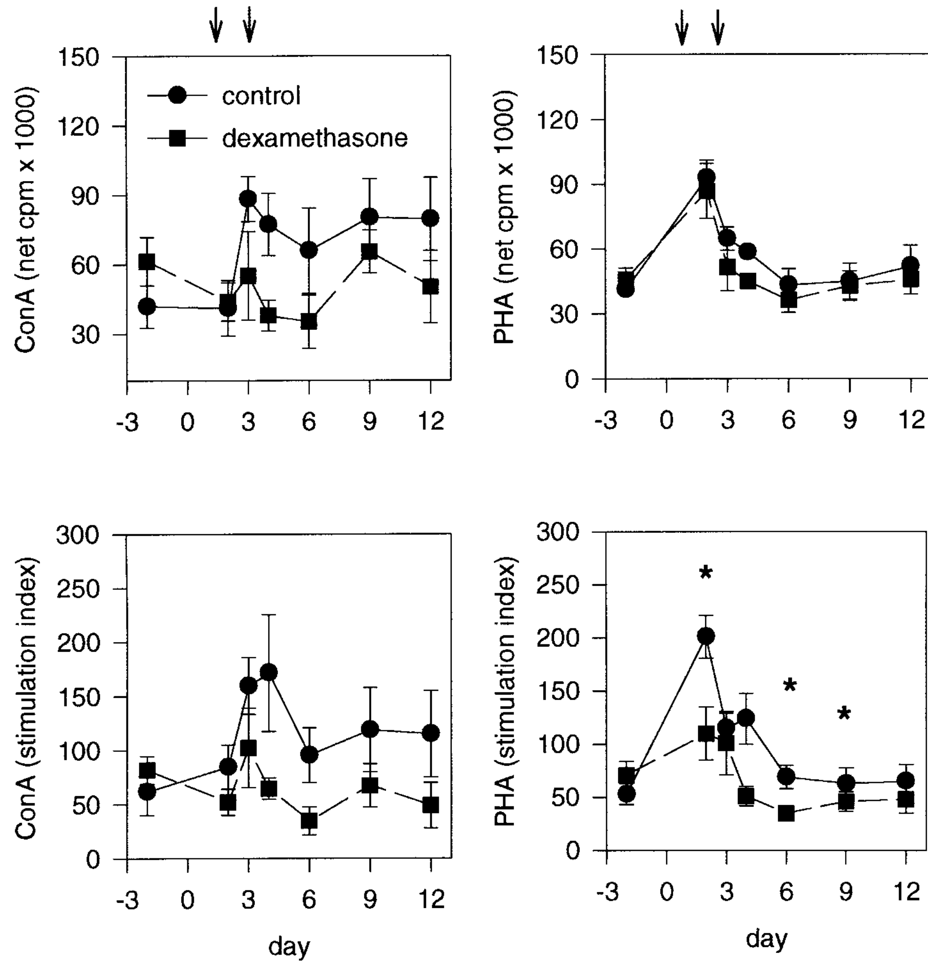

Figure 3. Lymphocyte proliferation (mean ± SEM) in response to PHA and ConA. Arrows indicate the times of treatment with dexamethasone. *Significant difference between the control and treated groups

DISCUSSION

Dexamethasone is commonly employed to pharmacologically mimic the effects of adrenal glucocorticoids. In the current experiment, an established immunosuppressive dose (Burton and Kehrli, 1996) was used in an attempt to induce effects that reflect the physiological response of cattle to stress. The dose falls within the therapeutic range recommended by the product manufacturers, although larger doses are sometimes used to treat inflammation, shock, or allergies.

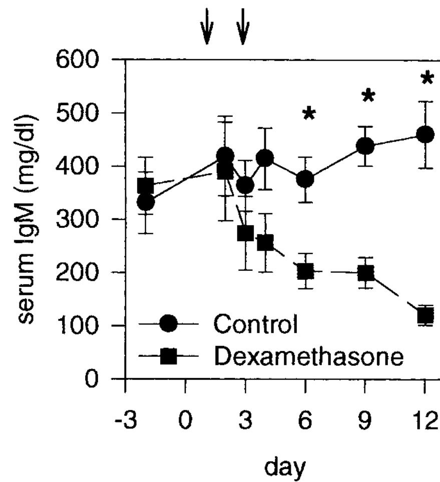

Figure 4. IgM concentrations (mean ± SEM) in serum. Arrows indicate the times of treatment with dexamethasone. *Significant difference between the control and treated groups

The pronounced leukocytosis seen in the current experiment is characteristic of the response to dexamethasone (Lan *et al.*, 1998). A rapid neutrophilia was paralleled by a marked lymphopenia that led to a dramatic alteration in the ratio of neutrophils to lymphocytes. Lymphopenia was accompanied by a large decrease in $WC1^+$ lymphocytes, as reported by Burton and Kerhli (1996). In contrast to their findings, however, there was a significant increase in $CD4^+$ lymphocytes and a transient decrease in $CD8^+$ lymphocytes that resulted in a transient increase in the CD4:CD8 ratio in the treated steers. This differs from the response to glucocorticoid treatment in humans, where a decrease in $CD4^+$ lymphocytes leads to a decreased CD4:CD8 ratio (Cohen, 1992). The sensitivity of $CD8^+$ rather than $CD4^+$ lymphocytes to stressors such as impending parturition has previously been noted in cattle (Van Kampen and Mallard, 1998). Together, these findings suggest that CD4:CD8 ratios in cattle may not have the utility seen in humans as a marker of immunosuppression associated with stress.

The functional responses of leukocytes were less sensitive to the effects of dexamethasone treatment than the haematological parameters and cell surface markers. Lymphocyte proliferation was moderately decreased in dexamethasone-treated steers, despite a moderate increase in the expression of the IL-2 receptor, which accompanies lymphocyte activation. Suppression of lymphocyte proliferation was in accord with the findings of Lan and colleagues (1998), who, however, noted a parallel decrease in IL-2Rα^+ lymphocytes in dexamethasone-treated calves. Surprisingly, NK cell activity, which is suppressed in humans by corticosteroids (Hseuh *et al.*, 1994) and also by a variety of stressors (Jonsdottir *et al.*, 1997), was not influenced by dexamethasone treatment in the current experiment. In a similar fashion, dexametha-

sone had little effect on myeloperoxidase activity in the present experiment. The phagocytic and nitroblue tetrazolium activities of neutrophils can be suppressed by stress (Murata *et al.*, 1985) or ACTH treatment (Paape *et al.*, 1981) in cattle, so it is likely that the failure to see such effects in the current experiments is related to the low dose of dexamethasone used. Elevated *in vitro* chemotaxis of neutrophils from the dexamethasone-treated steers was not in accord with the effects of dexamethasone on neutrophil migration *in vivo* (Clarke *et al.*, 1994). It has previously been noted, however, that dexamethasone promotes chemotaxis of bovine neutrophils *in vitro* (Jayappa and Loken, 1983; Zwahlen and Roth, 1990). It is therefore likely that the adhesion events affected by dexamethasone *in vivo* (Burton *et al.*, 1995), which contribute to the reduced accumulation of neutrophils in tissues following dexamethasone treatment, are not important in the membrane assays used to measure neutrophil migration *in vitro*.

Dexamethasone induced a pronounced depression of IgM concentrations in the blood during the chronic phase of the current experiments. *In vitro* suppression of IgM production by mononuclear cells from dexamethasone-treated bulls has been noted by Nonnecke and colleagues (1997) and depression of IgA responses to foot and mouth disease virus by dexamethasone has been noted *in vivo* (Ilott *et al.*, 1997). These findings suggest that serum IgM concentrations may be a useful marker of the effects of chronic stress in cattle.

In conclusion, the results indicate that haematological parameters, especially the ratio of neutrophils to lymphocytes, lymphocyte phenotypes and IgM concentration, are particularly sensitive to the effects of acute and chronic dexamethasone treatment in cattle. Further studies are needed to determine the utility of these parameters as markers of the effects of stress on the welfare of cattle.

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