CELL-MEDIATED IMMUNE FUNCTION IN LAMBS
CHRONICALLY TREATED WITH DEXAMETHASONE

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ABSTRACT

Crossbred ewe and wether lambs were individually stanchioned in environmentally controlled rooms at 20°C. On d 0, lambs were treated with 0.04 mg of dexamethasone (DEX; n = 10)/kg of BW or given an equal volume of saline vehicle (SAL; n = 10). Treatment was repeated every 48 h for 14 d. Samples of blood were obtained by puncture of the jugular vein on d 0 (before treatment), 2, 4, 7, 10, and 14. Total and differential leukocyte numbers, lymphocyte blastogenic responses to mitogens, and in vitro production of interleukin-2 (IL-2) were determined. No treatment × day interaction was noted for any of the experimental end points (P > .10); therefore, within-day comparisons between DEX- and SAL-treated lambs were not made. However, over all 14 d, DEX-treated lambs had increased (P < .05) numbers of lymphocytes (6.5 ± 0.4 vs 5.1 ± 0.4 x 10³ cells/μl for SAL) and monocytes (0.8 ± 0.1 vs 0.6 ± 0.1 x 10³ cells/μl for SAL), and these increases contributed to an increase (P < .01) in total leukocytes (11.2 ± 0.5 vs 9.1 ± 0.5 x 10³ cells/μl for SAL). Lymphocyte blastogenic responses to mitogens were not affected by DEX treatment. Production of IL-2 was reduced (P < .05) for DEX- (.90 ± 0.12 units/ml) compared with SAL-treated lambs (1.27 ± 0.13 units/ml). The data suggest that continued treatment of lambs with DEX may result in a modest reduction in production of IL-2, but mitogen-stimulated blastogenic responses of lymphocytes are not reduced by DEX treatment.

Key Words: Dexamethasone, Immune Response, Sheep


Introduction

Dexamethasone (DEX) is a potent, synthetic glucocorticoid that has been used as a model treatment to study the effects of elevated adrenal glucocorticoids on the function of the immune system of cattle (Roth, 1985; Roth and Flaming, 1990). The immunological responses to DEX have been generalized to represent responses to stressor-induced elevations in endogenous glucocorticoids because application of stressors elevates serum concentrations of cortisol and can reduce some measures of immunological function (Blecha et al., 1984; Klemcke et al., 1990; Coppinger et al., 1991). Indeed, cell-mediated immune functions of cattle are reduced by treatment with DEX (Roth and Kaeberle, 1985). However, immunological responses of swine to DEX are not influenced at doses that are immunosuppressive in cattle (Flaming et al., 1989). In fact, extremely high doses of DEX are required to reduce lectin-induced lymphocyte blastogenesis of pigs (Flaming et al., 1989; Saulnier et al., 1991). Therefore, DEX treatment may not necessarily represent an acceptable, generalized model for studying stressor-induced changes in endocrine and immune functions of domestic farm animals.

Treatment of lambs at 48-h intervals for 14 d with doses of DEX that suppress the immune system of cattle (Roth, 1985) reduced ACTH and cortisol, even at the conclusion of the
treatment period (Minton et al., 1991). In that experiment, lambs given DEX had reduced antibody production against ovalbumin, but lymphocyte production of interleukin-2 (IL-2) in vitro was not affected by treatment. This finding raised the question whether cell-mediated functions of lymphocytes from lambs were relatively resistant to DEX (as in swine) or whether cell-mediated functions were acutely reduced by DEX but gradually lost their sensitivity upon repeated treatment. Thus, by implication, the acceptability of DEX treatment as a model system for studying stress-associated endocrine and immune function of lambs was also at issue, because 3 d of repeated restraint stress reduced cell-mediated immune functions of lambs (Coppinger et al., 1991), whereas 14 d of DEX treatment did not do so (Minton et al., 1991).

The current study was designed to evaluate changes in measures of cell-mediated immune function in lambs chronically treated with DEX.

Materials and Methods

Crossbred ewe and wether lambs (approximately 6 to 7 mo of age) were housed in individual stanchions in two temperature-controlled rooms at 20°C. Lights were illuminated continuously in one room, and a 12 h light, 12 h dark photoperiod was used in the other room. Different photoperiods were used because this experiment was conducted concurrently with another experiment in which photoperiod was an environmental variable of interest (Minton et al., 1991). The photoperiod did not affect any of the experimental end points evaluated (detailed below). Water was available continuously, and lambs were fed 1 kg of a 50% whole corn, 50% dehydrated alfalfa pellet diet daily (.5 kg in each of two feedings).

Beginning on d 0 of the experiment, lambs (n = 10) were injected intramuscularly with .04 mg of DEX/kg of body weight every 48 h for 14 d. This dose and treatment protocol was chosen because it had been effective in reducing cell-mediated immune functions of cattle (Roth and Kaebrole, 1985) and had remained effective at reducing plasma ACTH in lambs after 14 d of treatment (Minton et al., 1991). Control lambs (n = 10) were treated with volumes of sterile saline equal to the volumes used to deliver DEX.

Samples of whole blood were collected into sterile heparinized vacuum tubes by jugular vein puncture on d 0 (before treatment), 2, 4, 7, 10, and 14 of treatment. Total and differential leukocyte numbers (Blecha et al., 1984), lymphocyte proliferation in response to mitogens (Blecha et al., 1984), and in vitro production of IL-2 by lymphocytes (Blecha and Baker, 1986) were determined.

Lambs were blocked by environmental room, sex, and BW and assigned randomly to treatments within blocks so that equal numbers of DEX- and SAL-treated lambs were housed in each room and similar numbers and weights of ewe and wether lamb were assigned to each treatment. The data were analyzed as a split-plot experiment with repeated measures (Gill and Hafs, 1971). Initially, sources of variation in the main plot were DEX, photoperiod (environmental room), and the interaction of these two sources of variation; sources of variation in the subplot were days of the experiment and the two-way interactions of DEX and photoperiod with days. Using this approach, no significant (P > .10) effects of photoperiod or interactions with photoperiod were noted. Therefore, the final model used for analysis was simplified to include only DEX in the main plot (tested by the lamb-within-treatment variance) and day of the experiment and the treatment × day interaction in the subplot. Comparisons between DEX and SAL treatments within days were made only if a significant (P < .05) F-test for the treatment × day interaction was detected. Total and differential leukocyte numbers also were analyzed using days of treatment as a continuous independent variable. The profiles of leukocytes over time were fit to polynomial equations and the models were tested for parallelism. We have described this approach in detail for analysis of changes in hormonal concentration over time (Minton and Blecha, 1990).

Results and Discussion

Evaluation of changes in total and differential leukocyte numbers has been used as an index of stressor- (or glucocorticoid-) induced effects on that population of cells. Likewise, we evaluated changes in leukocytes in lambs during treatment with DEX (Figure 1). The fitted-time trends of total leukocytes and of the four individual cell types enumerated did not
Dexamethasone and Immune Function in Lambs

Dexamethasone and immune function in lambs differ significantly ($P > .10$) over time, indicating that the changes across the course of the experiment were essentially parallel in DEX and SAL lambs. This observation was corroborated by a separate analysis in which the day $\times$ treatment interaction also was not to be significant ($P > .10$). Therefore, comparisons between treatments on individual bleeding days were not made. However, over all days of the experiment, total leukocytes ($P < .01$), lymphocytes ($P < .05$), and monocytes ($P < .05$) were increased in DEX lambs. The increase of total leukocytes in DEX-treated lambs is generally consistent with the leukocytosis associated with treatment of lambs with cortisol (Collins and Suarez-Guemes, 1985) or exposure to restraint stress (Coppinger et al., 1991). However, the leukocytosis is usually accompanied by elevated numbers of neutrophils (primarily) and decreased lymphocytes (Collins and Suarez-Guemes, 1985). In the current experiment, neutrophils were not affected by treatment. The increase in total leukocytes observed here is largely explainable by increased lymphocytes in DEX lambs. The reason for this effect is unclear. However, close inspection of the lymphocyte profiles suggests that this effect, rather than being treatment-induced, may have been more related to the chance occurrence that lambs assigned to the DEX treatment already had greater numbers of lymphocytes. The magnitude of the difference between SAL- and DEX-treated lambs seemed to be as great on d 0, before any treatment, as on any other day of the study.

We were similarly surprised by our observation that, on average, monocytes were increased in DEX lambs. This, too, is difficult to reconcile, because such an effect was not observed in lambs treated previously with an identical protocol (Minton et al., 1991). Furthermore, the effect of DEX on numbers of monocytes has not been evaluated specifically.

Figure 1. Total leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils in lambs treated every 48 h with dexamethasone (DEX) or saline (SAL). Each point represents the mean cell number $\pm$ SEM of 10 animals in each treatment. Over all days, there was an increase in leukocytes ($P < .01$), lymphocytes ($P < .05$), and monocytes ($P < .05$) in lambs treated with dexamethasone.
in other domestic farm animals. Although the effect we observed seemed to be the result of greatly elevated concentrations on only 2 d (d 7 and 10), the physiological implications of such a change remain unclear. Finally, it should also be noted that DEX produces an eosinopenia in cattle (Roth, 1985), another DEX-induced characteristic that was not seen in the current study. However, stressors that also elevated cortisol markedly in lambs failed to produce eosinopenia (Minton and Blecha, 1990).

Stressors can reduce the ability of lymphocytes from lambs to proliferate in response to mitogens (Coppinger et al., 1991), an effect also seen in cattle (Blecha et al., 1984) and pigs (Klemcke et al., 1990). In general, reduced proliferative responses of lymphocytes from stressed animals is thought to be the result of the suppressive action of glucocorticoids. Treatment of lambs with cortisol (albeit very high doses) also markedly reduced proliferative responses of lymphocytes (Collins and Suarez-Guemes, 1985). Therefore, because DEX also reduced cell-mediated immune function (Roth, 1985), treatment with DEX is frequently used as a model for evaluation of stress-associated endocrinological and immunological changes. However, the immunosuppressive action of DEX may not be as ubiquitous, at least among farm animals, as was generally assumed in the past. Evidence in support of this assertion is that DEX, at doses that were clearly suppressive to lymphocyte function in cattle, failed to produce such a result in pigs (Flaming et al., 1989). Here, we also report that mitogen-stimulated proliferative responses of lymphocytes from lambs were unaffected by treatment with DEX (Figure 2). Admittedly, we cannot rule out the possibility that transient suppression of lymphocyte proliferation might have been seen had it been evaluated more frequently (for example at 12 or 24 h after the first few DEX injections).

The IL-2 production by lymphocytes from SAL and DEX lambs is depicted in Figure 3. As was the case with leukocytes and lymphocyte blastogenic responses, no significant treatment x day interaction was noted in the analysis, so individual treatment comparisons within days were not made. However, over all days, production of IL-2 was reduced (P < .05)

![Figure 2. Lymphocyte blastogenic responses to phytohemagglutinin (PHA; top panel), concanavalin A (Con A; middle panel), and pokeweed mitogen (PWM; bottom panel) in lambs treated with saline (SAL) or dexamethasone (DEX) every 48 h for 14 d. Net CPM are mitogen-stimulated CPM minus background CPM. Each bar represents the mean ± SEM of 10 animals in each treatment.](image)

![Figure 3. Production of interleukin-2 (IL-2) by lymphocytes of lambs treated with saline (SAL) or dexamethasone (DEX) every 48 h for 14 d. Each bar represents the mean ± SEM of 10 animals in each treatment. Asterisk denotes a reduction (P < .05) in production of IL-2 in DEX-treated lambs over all days of the experiment.](image)
in DEX lambs. In agreement with this observation, restraint stress also decreased production of IL-2 by lymphocytes from lambs (Coppinger et al., 1991). Also, treatment with ACTH decreased IL-2 production in cattle (Blecha and Baker, 1986) and pigs (Klemcke et al., 1990). In contrast, identical doses of DEX did not reduce production of IL-2 by lambs in our previous study (Minton et al., 1991). However, in that study, IL-2 production was evaluated only at the conclusion of the 14-d treatment with DEX. The question arose as to whether DEX might have reduced production of IL-2 early in the study, but this effect diminished with repeated administration of DEX. The results of this experiment do not support the conclusion that DEX had a strongly suppressive action on IL-2 production early on and later lost this ability.

We should emphasize that, although DEX did not reduce proliferative responses of lymphocytes to mitogens in lambs, it is not completely without effect on these cells. As mentioned above, production of IL-2 was reduced by DEX, although this effect is apparently not always seen (Minton et al., 1991). Furthermore, an identical dose of DEX given to lambs repeatedly reduced production of antibodies to ovalbumin (Minton et al., 1991). Such inconsistencies may reflect differential sensitivities of subpopulations of lymphocytes to pharmacological actions of DEX, but also may indicate that DEX is not always an appropriate model treatment for studying stressor-affected immunomodulation.

Implications

The results of the current study suggest that repeated treatment of lambs with dexamethasone does not affect total and differential leukocyte numbers or the proliferative responses of lymphocytes to mitogens in the same manner that stressors do. Furthermore, the tendency for dexamethasone to reduce production of interleukin-2 in the current study, but not in other experiments, suggests that the treatment is not consistently effective in this regard. Therefore, treatment with dexamethasone, which is widely used to study stressor-induced immunological function in some species, may not be appropriate for such applications in sheep.

Literature Cited


