A Glucocorticoid Model to Study Stress-Induced Immunosuppression in Red Deer.

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Abstract

Our recent studies in deer indicate that stress is a major effector in the pathophysiology of infectious disease. In the present study we developed an in vivo model of immunosuppression to examine the effects of glucocorticoids on the immune response in deer. Red deer were treated with varying doses of dexamethasone for three weeks and either vaccinated with a live attenuated strain of Mycobacterium paratuberculosis or challenged with a low dose of live virulent Mycobacterium bovis. Measurements of haematological and immunological parameters were made, along with an evaluation of both vaccine response and severity of disease. Comparisons were made with untreated controls to confirm that any effects observed were due to the steroid treatment. Total numbers of white blood cells increased with steroid treatment, accounted for by a significant increase in the number of circulating neutrophils. Numbers of mononuclear leucocytes, consisting of lymphocytes and monocytes, were significantly reduced in the peripheral blood of steroid treated animals, as were the levels of eosinophils. The functional capacity of peripheral blood lymphocytes was evaluated using a nonspecific, mitogen driven, lymphocyte transformation assay and was shown to be significantly suppressed during the period of steroid treatment The animals treated with dexamethasone showed a reduced response to vaccination with Mycobacterium paratuberculosis as measured by specific lymphocyte transformation driven by Johnin PPD. Greater disease severity was observed in animals treated with dexamethasone when compared to untreated controls that received the same infectious dose of Mycobacterium bovis. The results from this study indicate that the administration of dexamethasone to red deer represents an appropriate model for stress-induced immunosuppression in this species.

Key Words

Stress, Immunosuppression, Glucocorticoids, Tuberculosis, Deer.

Introduction

Stress has for centuries been associated with the onset of medical illness. It is increasingly considered to be a major effector in the pathophysiology of infectious disease. Both physical and emotional stressors stimulate a variety of endocrine changes including activation of the hypothalamic-pituitary-adrenal (HPA) system.[1] Hyperactivity of the HPA system is accompanied by increased secretion of corticotropin-releasing hormone (CRH). This stimulates the release of adrenocorticotropic hormone (ACTH) by the pituitary gland. ACTH binds to receptors on the adrenal gland and causes an increase in the production and release of corticosteroid hormones.[2]

Corticosteroid hormones secreted in response to stress, are known to increase the susceptibility of animals to infectious disease, by exerting profoundly suppressive effects on the immune system.[3,4] The mechanisms involved in this immunosuppression have been well characterised. Alterations in both leucocyte traffic and function, along with decreases in the production of cytokines and certain mediators of inflammation, are among the main immunosuppressive effects of glucocorticoids.[5]

Previous studies in our laboratory have established a link between stress and immunosuppression, in deer.[6,7] To further examine this relationship we have developed a glucocorticoid model for stress-induced immunosuppression.

Glucocorticoid models of immunosuppression offer a number of advantages over the more traditional methods of achieving stress-induced suppression of immune function. The exposure of animals to a stressful situation, such as aversive handling,[8] transport,[9] extreme climate,[10] or weaning,[11] introduces an array of uncontrollable variables into the experiment. These include genetic differences, pretreatment status and individual variation in the perception of threat.[12]

A more reliable approach is to short circuit the stress response and deliver the end product of HPA activation, the glucocorticoid, directly to the animal. This allows one to predictably suppress immune function in a dose dependent manner, alleviating some of the problems associated with the physical stress paradigms.

The glucocorticoid model used most extensively in ruminants is dexamethasone administration to cattle.[12] Dexamethasone is a glucocorticoid with approximately 25 times the anti-inflammatory potency of cortisol.[27] Advantages associated with the use of the dexamethasone model include: the ability to induce a sustained level of immunosuppression when administered every 24 hours; the capacity to reliably reproduce a dose dependent level of immunosuppression; the ability to suppress a wide range of host defence mechanisms and the ethically sound nature of the treatment as dexamethasone causes very little distress to the animals.[12]

In this paper we report on the development of the dexamethasone model for immunosuppression in deer.

The Model

Two dose levels with distinct delivery profiles were evaluated in two separate experiments. In the first trial twenty-one red deer hinds, 8-12 months old, were randomly allocated into three groups and treated according to the protocol in Table 1.

Table 1. The experimental protocol for the first trial.

Group (n=7)	Dexamethasone	Vaccination	Blood sample
I	Yes	Yes	Yes
II	No	Yes	Yes
III	No	No	Yes

Group I received dexamethasone at 0.1mg/kg of body weight for 14 days. Following this treatment, groups I and II were vaccinated with live attenuated

Mycobacterium paratuberculosis. Group III was an untreated, unvaccinated control. This dose and release profile of dexamethasone proved too severe as three of the animals died subsequent to treatment.

Analysis of total and differential white blood cell (WBC) counts showed significant alterations in the leucocyte profiles of dexamethasone treated deer. There was a significant increase in the total number of circulating WBC in Group I, for the duration of the treatment ($p<0.05$). This was accounted for by a significant elevation in circulating neutrophils ($p<0.005$). In contrast, a significant decrease in both mononuclear cells (consisting of lymphocytes and monocytes) and eosinophils occurred during treatment ($p<0.001$ & $p<0.001$, respectively).

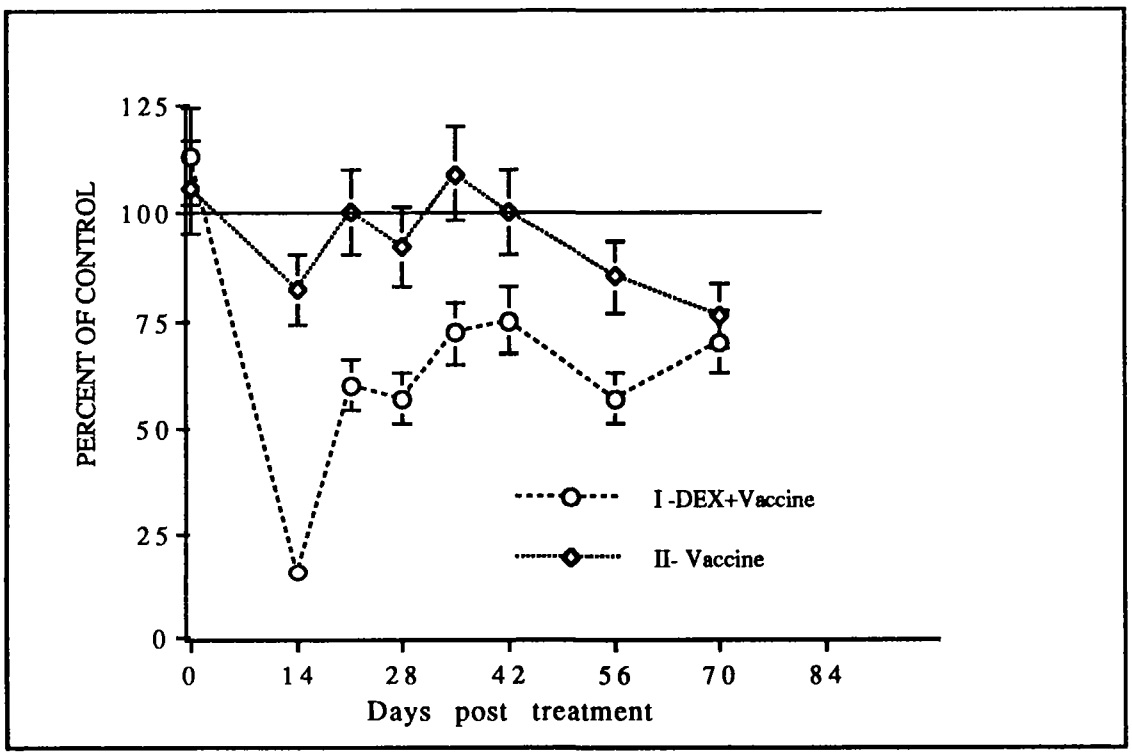

Figure 1. The influence of dexamethasone on lymphocyte function expressed as a mean (±SEM) percentage of the values for control animals.

Lymphocyte transformation assays were performed to assess the effect of dexamethasone on nonspecific, mitogen driven, lymphocyte function (Fig 1). Dexamethasone significantly suppressed the functional capacity of lymphocytes by up to 85% during the course of treatment ($p<0.05$).

The specific immune response to *Mycobacterium paratuberculosis* was evaluated by performing antigen specific, lymphocyte transformation assays, driven by Johnin PPD (purified protein derivative from *M.paratuberculosis*). This was shown to be suppressed by up to 60% in dexamethasone treated animals (Fig 2).

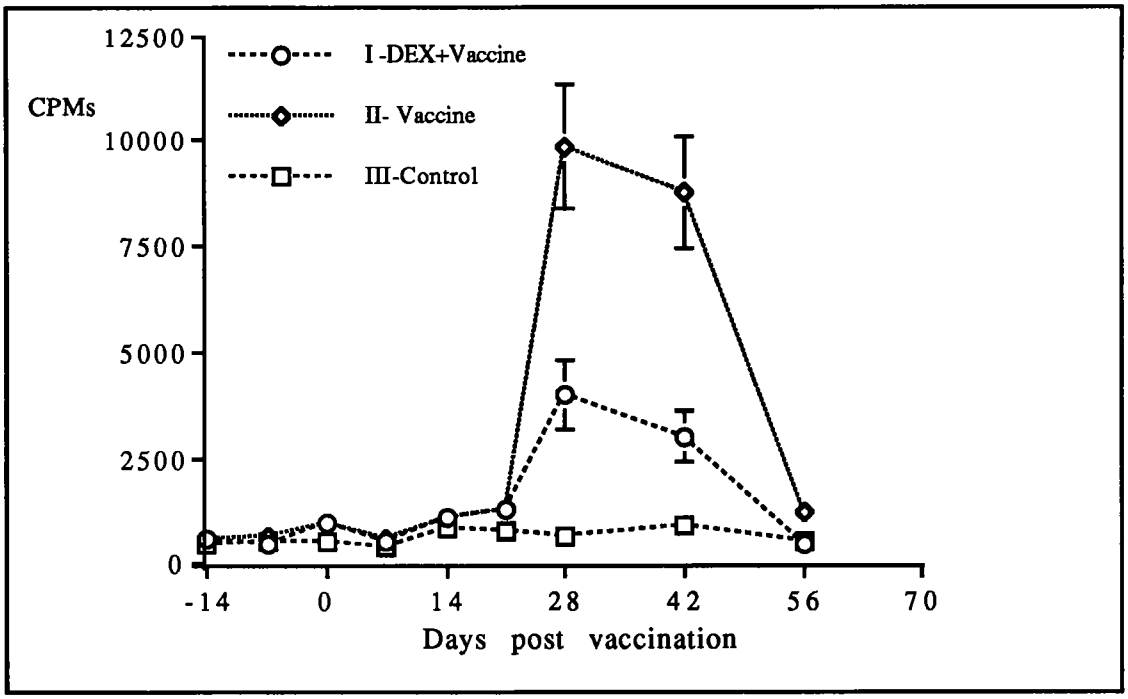

Figure 2. The specific lymphocyte response to Johnin PPD for each treatment group, expressed as the mean (±SEM).

In the subsequent experiment the dose of dexamethasone was reduced and the release profile modified in an attempt to alleviate the severity problems encountered in the first trial. In this experiment the effect of dexamethasone on the pathophysiology of disease was evaluated. Twenty-four red deer hinds, 8-12 months of age, were randomly allocated into three groups and treated according to the following protocol in Table 2.

Table 2. The experimental protocol for the second trial.

Group(n=8)	Dexamethasone	Challenge	Blood sample
A	Yes	Yes	Yes
B	No	Yes	Yes
C	No	No	Yes

Group A received dexamethasone at a peak dose of 0.075mg/kg of body weight which reduced daily, in a non-linear profile, over the next 28 days. During this treatment, groups A and B were challenged with a low dose of live virulent *Mycobacterium bovis*. Group C was an untreated, unchallenged control.

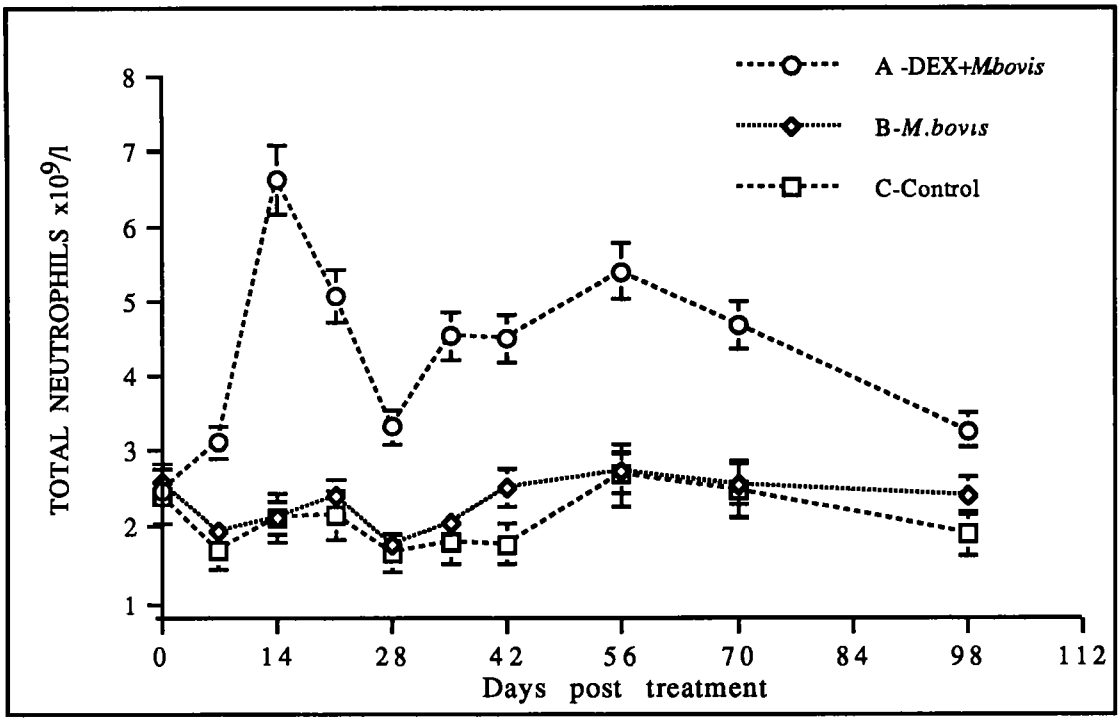

Figure 3. The effects of dexamethasone on the mean (±SEM) total number of circulating neutrophils in deer.

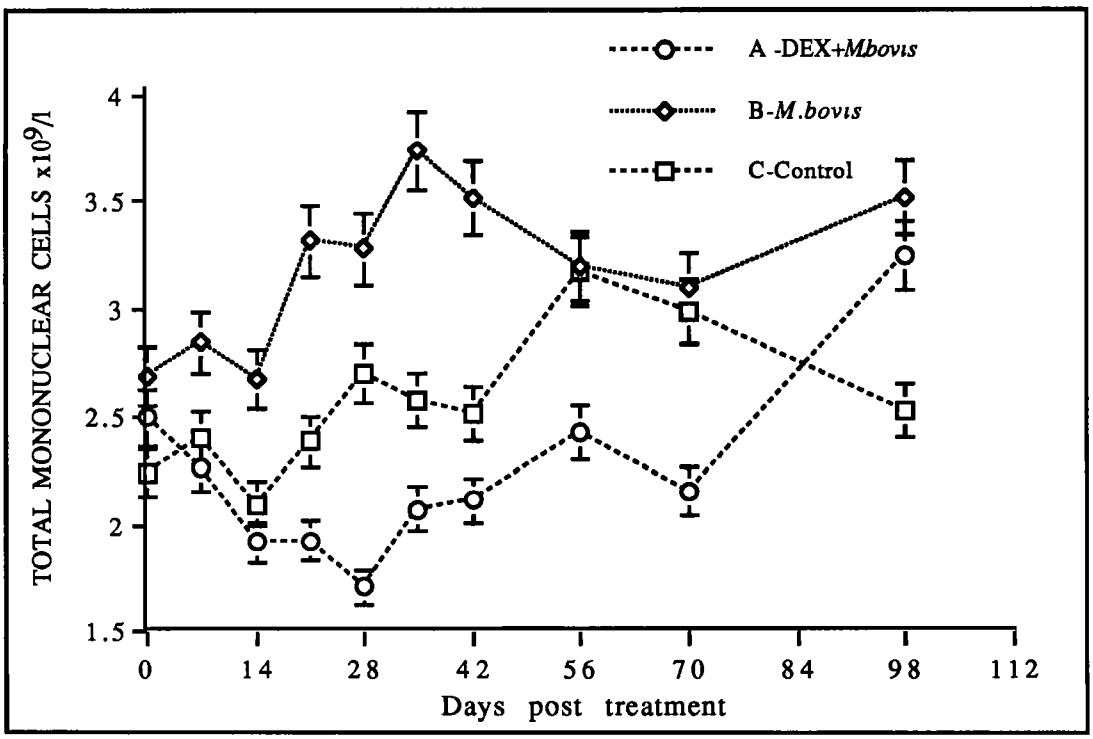

Figure 4. The effects of dexamethasone on the mean (±SEM) total number of circulating mononuclear leucocytes in deer.

Haematological parameters were assessed during the course of the treatment and showed similar patterns of alteration to the first experiment. There was a significant neutrophilia ($p<0.05$), with circulating numbers of neutrophils increasing by up to 300% when compared with the untreated controls (Fig 3). This was accompanied by a significant reduction in the number of mononuclear leucocytes ($p<0.05$) in the peripheral blood (Fig 4). Eosinophils were reduced significantly ($p<0.05$) to almost undetectable levels in the peripheral blood of treated animals (Fig 5).

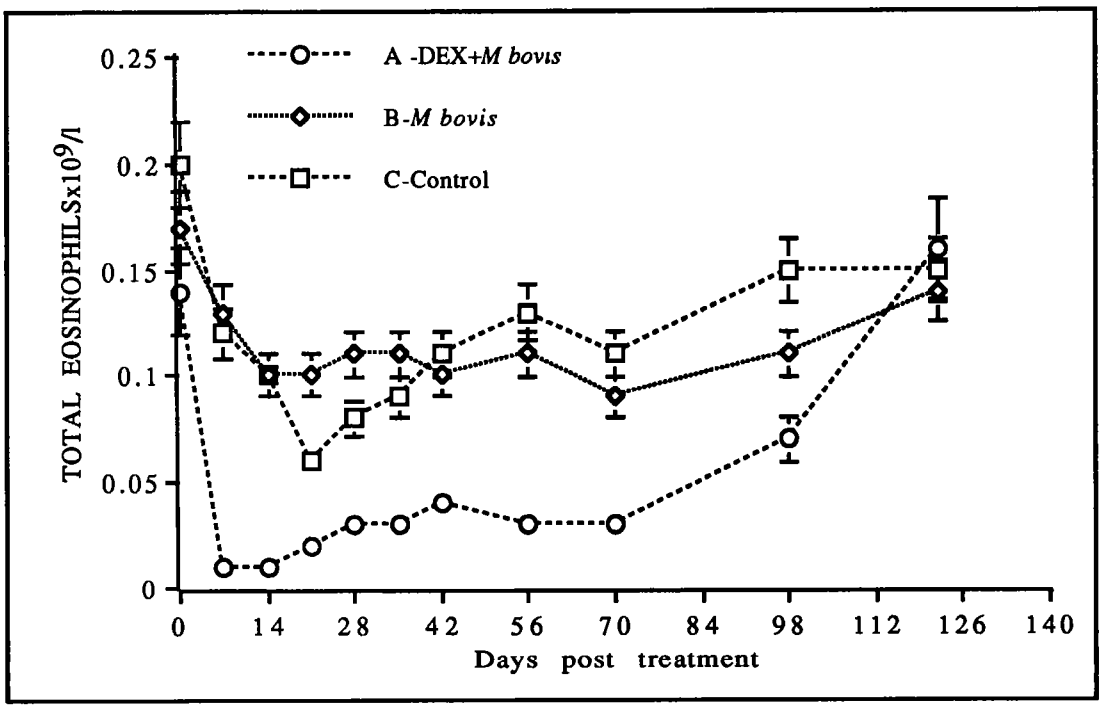

Figure 5. The effects of dexamethasone on the mean (±SEM) total number of circulating eosinophils in deer.

Lymphocyte function was assessed using the nonspecific, mitogen driven, lymphocyte transformation assay (Fig 6). Dexamethasone significantly suppressed the functional capacity of lymphocytes by more than 75% during the course of treatment ($p<0.05$).

The severity of disease was ranked using autopsy and histopathological data, accounting for number, location, size, consistency and bacterial content of the lesions found. The pathology exhibited by animals treated with dexamethasone was greatly increased when compared to the controls receiving the same infectious dose of *Mycobacterium bovis*, as shown in Table 3.

One of the animals treated with dexamethasone died from yersiniosis, 4 weeks after inoculation with *M.bovis*, although in the context of the trial this was unfortunate, it does serve as an indication of the immunosuppressive effect that the treatment had.

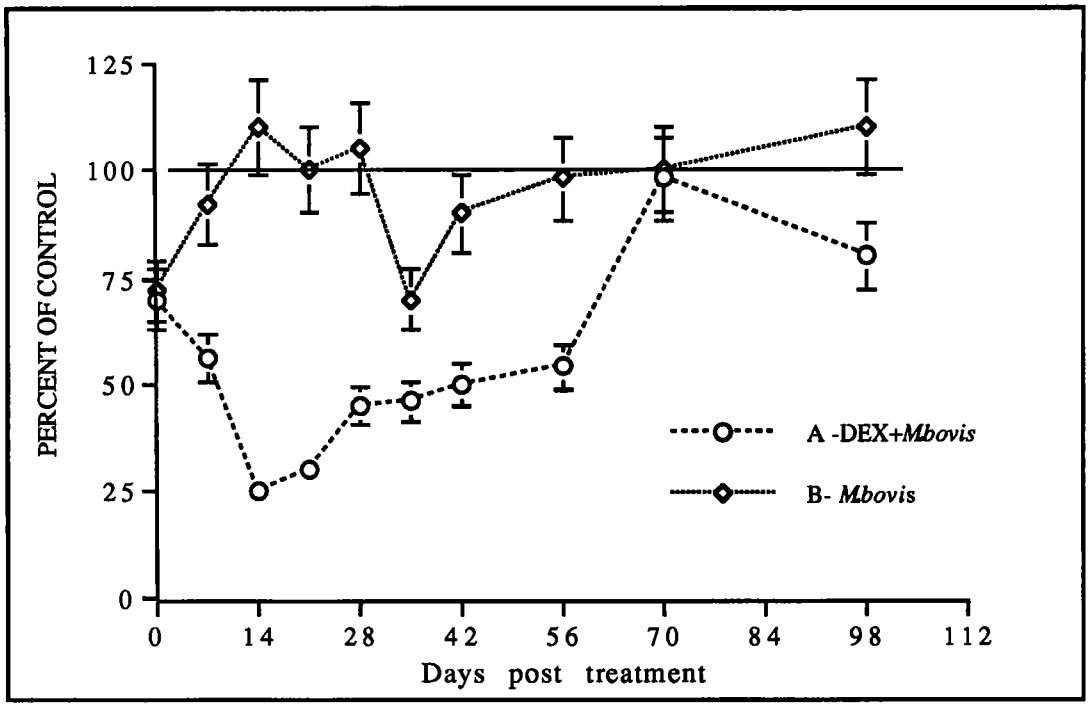

Figure 6. The influence of dexamethasone on lymphocyte function expressed as a mean (±SEM) percentage of the values for control animals.

Table 3. The effect of dexamethasone on the severity of tuberculosis in deer experimentally infected with *M. bovis.*

GROUP	NVL	MILD	MODERATE	SEVERE
A (n=7) DEX+*M bovis*	28%	14%	14%	43%
B (n=8) *M bovis*	50%	25%	25%	0%
C (n=8) Control	87.5%	0%	12.5%	0%

Discussion

The ability to maintain homeostasis is inherent to the survival of all living organisms. This immensely dynamic and harmonious equilibrium is constantly challenged by intrinsic and extrinsic stressors, which in this context, can be defined as forces which threaten homeostasis or cause disharmony.[2] Both physical and psychological stressors elicit responses developed to preserve homeostasis.[28] These adaptational responses attempt to counteract the effects of stressors and reestablish the equilibrium.[2] In general the 'stress response' functions

beneficially at an acute level, with few adverse consequences, on the other hand, if it is activated chronically, without appropriate adaptation, suppression of vital immune function may result.[2]

In this paper, we describe a method for studying the immunological effects, of chronic activation of the 'stress response' in deer.

The haematological changes reported here are in agreement with other studies where *in vivo* administration of high pharmacological concentrations of glucocorticoids result in a significant increase in circulating neutrophils and a decrease in the numbers of lymphocytes, monocytes and eosinophils in the peripheral blood.[1,3] We observed a significant elevation in neutrophil numbers in the peripheral blood, in animals receiving two different doses of dexamethasone. The increase in neutrophil numbers in the circulation can be attributed to two distinct effects. In humans glucocorticoids have been shown to decrease the margination of neutrophils and their exit from the blood into the tissues.[1 3] Administration of glucocorticoids to cattle, causes a decrease in the egress of neutrophils from the circulation, along with an influx of these cells into the blood, from the bone marrow storage pool.[14] These alterations have the nett immunological effect of reducing the ability of neutrophils to arrive at the site of inflammation.

Dexamethasone administration had a negative effect on the mononuclear leucocyte population in the treated deer, causing significant reductions in the peripheral blood levels of these cells. In human studies corticosteroids have been shown to reduce the numbers of both lymphocytes and monocytes in the blood, this is due to a redistribution of these cells from the blood into the tissues.[15] This is also reported to be the case in cattle.[16] The numbers of eosinophils were reduced to almost undetectable levels in the dexamethasone treated animals. In most other species glucocorticoids have been reported to rapidly induce eosinopaenia.[17] In rats this is reportedly due to redistribution of eosinophils into the peripheral lymphoid tissue.[18]

The functional capacity of the remaining lymphocytes in the circulation was assessed by a nonspecific, mitogen driven, lymphocyte transformation assay and was significantly reduced in dexamethasone treated animals. This is in agreement with studies in humans[23] and in rats.[24] It is likely that this is due in part to a decrease in the production of the necessary co-factors or cytokines required for lymphocyte proliferation. Glucocorticoids have been shown to inhibit lymphocyte blastogenic capacity in response to mitogens in cattle[20,22] and this decrease was associated with a reduction in the synthesis of interleukin-2(IL-2),[20] by lymphocytes. In deer, *in vitro* administration of dexamethasone reduces lymphocyte proliferation in response to mitogens and this inhibition can be partially restored by the addition of IL-2 (unpublished data).

The major consequence of an impaired immune system is the increased susceptibility to infectious disease. There exist few studies, which directly tested whether activation of the HPA system suppressed immune function and resulted in an increase in susceptibility to infectious disease.[8] Dexamethasone administration suppressed the immune response to *Mycobacterium paratuberculosis* as measured by the specific lymphocyte transformation to Johnin PPD (purified protein derivative from *Mycobacterium paratuberculosis*) This provides evidence that along with alterations to individual immune parameters, the immune system's capacity to function *in vivo*, in its entirety, was compromised. Studies in cattle have shown that administration of dexamethasone increases the severity of pneumonia caused by *Haemophilus somnus* [21] This is in agreement with studies where activation of the HPA system, was shown to increase the susceptibility of mice to *in vivo* mycobacterial growth.[25] In humans,

susceptibility to mycobacterial disease has been shown to be associated with stress.[26] By assessing the impact that glucocorticoids have on disease resistance, the question of whether or not a model for immunosuppression is valid, can be addressed. We have shown that along with suppression of immune function, dexamethasone administration does influence the susceptibility of deer to infectious disease.

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