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Suppression of bovine lymphocyte responses to mitogens following in vivo and in vitro treatment with dexamethasone

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ABSTRACT

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Gnotobiotic calves given intramuscular injections of dexamethasone (DM, 0.5 mg kg^{-1} day^{-1}) showed marked changes in haematological parameters including a neutrophilia and a lymphopaenia. Not only was there a reduction in the numbers of circulating mononuclear cells, but there was also a significant ($P<0.01$) decrease in the in vitro responsiveness of the remaining circulating peripheral blood lymphocytes to the mitogens, phytohaemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM). Responses to all three mitogens were suppressed to a similar degree. Analysis of the circulating mononuclear cell sub-populations before and during DM treatment demonstrated a selective depletion of B cells; the T lymphocyte sub-population that expresses the gamma/delta form of T cell receptor, are $CD2^-$, $CD5^+$, $CD8^-$, $CD4^-$ and constitute a major population in peripheral blood of calves.

In vitro studies in gnotobiotic and conventional calves confirmed that DM was highly inhibitory for PHA responses but, in contrast to the in vivo findings, showed little effect of DM on ConA responses. Expression of surface antigens after 72 h in vitro culture in the presence of DM were little affected with the exception of BoCD8 and MHC II, which showed increased and decreased expression, respectively. These observations would suggest that distinct mechanisms are involved in glucocorticosteroid suppression of the responses to these two mitogens.

INTRODUCTION

The administration of glucocorticosteroids (GC) is widely used as an experimental means of mimicking the immunosuppressive effects of a stress-induced rise in blood cortisol. Glucocorticosteroid-induced immunosuppression is also used in the therapy of inflammatory and immune-mediated diseases (Cupps and Fauci, 1982) and in the study of immune responses to infectious agents (Davies and Carmichael, 1973; Crouch et al., 1985). The result

of GC treatment on the immune system varies considerably between species giving rise to designations of a species being either "cortisone-resistant" or "cortisone-sensitive" (Claman et al., 1971). However, not only is there inter-species variation but the effects of GC treatment are dependent on other parameters such as the age of the animal (Yang and Schultz, 1986) and the dose of GC used (Roth and Kaeberle, 1982).

Although cattle are regarded as a "corticosteroid-resistant" species (Wilkie et al., 1979), suppression of proliferative responses of cattle peripheral blood lymphocytes to mitogens has been reported following both in vivo administration of GC (Muscoplat et al., 1975; Roth et al., 1984; Pruett et al., 1987) and in vitro treatment of lymphocytes with GC (Ojo-Amaize et al., 1988). However, there has been no comparison in cattle of in vivo and in vitro effects of dexamethasone (DM) either on responses to the three mitogens most commonly used, i.e. phytohaemagglutinin (PHA), concanavalin A (ConA) and pokeweed mitogen (PWM), or on different sub-populations of T cells.

In cattle, as in other animal species, the equivalents of the T helper/inducer ($CD4^+$, $CD8^-$, $CD2^+$, $CD5^+$) and T cytotoxic/suppressor ($CD4^-$, $CD8^+$, $CD2^+$, $CD5^+$) cells represent two major sub-populations in the peripheral blood. However, a third numerically large sub-population is also present in cattle. These are $CD2^-$, $CD5^+$, $CD4^-$, $CD8^-$ (Clevers et al., 1990) and express the gamma/delta (γ/δ) form of T cell receptor (TCR). They can be identified with monoclonal antibodies (mAbs) directed against a surface molecule with a molecular mass (M_r) of 215–300 kDa, called the BoWC1 antigen, that has no known human CD homologue (Clevers, 1990; Howard and Morrison, 1991). These cells are called $BoWC1^+$ for convenience here, in accordance with the recommendation of the recent workshop on bovine leukocyte differentiation antigens (Howard and Morrison, 1991).

In this paper we compare the effects of both in vivo and in vitro treatment with dexamethasone (DM) on the responsiveness of calf lymphocytes to PHA, ConA and PWM. Also, we relate these observations to changes in lymphocyte sub-populations in the peripheral blood occurring during DM treatment.

MATERIALS AND METHODS

Animals and experimental design

Ten gnotobiotic (nine male, one female) calves, from 7 to 43 days of age were used in the in vivo experiments. These animals were produced and reared on a milk-based diet (Dennis et al., 1976; Hoare et al., 1976).

Calves were randomly assigned to one of two groups ($n=5$), Group A remained untreated, whilst Group B received daily injections of DM ('Dexadreson', Intervet, Cambridge, UK; intra-muscular, 0.5 mg kg^{-1} body weight) for 20 days starting at between 11 and 15 days of age.

Blood was collected regularly, at the same time of day and shortly before the injection of DM for haematological analysis and to prepare mononuclear cells to assess proliferative responses to mitogens (see below). Mononuclear cells were obtained from two calves in Group B prior to and during DM treatment and were used for the determination of cell sub-populations by FACS analysis (see below).

Blood was taken from six male gnotobiotic and seven male conventional calves aged between 17 and 260 days for in vitro experiments. Mesenteric lymph nodes and spleens were taken from two of these animals at slaughter.

Haematology

Blood was collected in vessels containing heparin (20 units ml^{-1}). Total white blood cell counts were made and differential counts were performed on Leishman stained blood smears.

Lymphocyte proliferation assays to mitogens

Peripheral blood mononuclear cells were isolated by centrifugation of heparinised blood over Histopaque 1083 (Sigma Chemical Co., Poole, UK) at $900 \times g$ for 40 min. Suspensions of mesenteric lymph node and spleen cells in phosphate-buffered saline (PBS, 0.15 M, pH 7.2) were obtained from tissues disrupted by pressing through a wire mesh before centrifugation over Histopaque as above. The cells from the interface were washed three times with PBS and resuspended in tissue culture medium at a concentration of 2×10^6 cells ml^{-1}. The tissue culture medium used was RPMI1640 (Gibco, Paisley, UK) supplemented with heat-inactivated (56°C, 30 min) 10% FCS (Flow Laboratories, Irvine, UK), 2 mM glutamine, 100 IU ml^{-1} penicillin, 0.2 mg ml^{-1} streptomycin, 20 mM HEPES and 27 mM sodium bicarbonate.

Mononuclear cell preparations (0.2 ml, 2×10^6 cells ml^{-1}) were cultured in flat-bottomed microplates (Nunclon Delta, Nunc, Roskilde, Denmark) with or without the addition of mitogens. Optimal concentrations of the mitogens PHA, ConA and PWM (all mitogens purchased from Sigma Chemical Co., Poole, UK) were determined in previous experiments and were 45 μg ml^{-1}, 5 μg ml^{-1} and 1:100 of stock, respectively. All cultures were set up in triplicate and the mean used in subsequent calculations. After culture for 72 h in a humidified atmosphere of 5% CO_2, 95% air, 1 μCi [^{3}H]thymidine ([^{3}H]TdR, 5 Ci $mmol^{-1}$, Radiochemical Centre, Amersham, UK) was added to each well. After a further 5 h culture, the cells were harvested onto glass-fibre filters with a semi-automated harvester. Incorporation of [^{3}H]TdR into cellular DNA was determined by liquid scintillation counting and the results expressed as counts per minute (cpm).

In vitro effects of DM on proliferative responses

Lymphocyte proliferation was assayed with cells from untreated control calves as described above. Tenfold dilutions of DM were made in tissue culture medium and added to some of the cultures to give a range of final concentrations from 5.1×10^{-4} M to 5.1×10^{-10} M. Dexamethasone was added at the start of lymphocyte culture and proliferation was assessed by incorporation of [^{3}H]TdR and the results expressed as the percentage of the control culture.

$$\text{Proliferation, \% of control} = \frac{\text{cpm culture with DM}}{\text{cpm culture without DM}} \times 100$$

Effect of interleukin 2 on DM treated cultures

In three experiments, recombinant human interleukin 2 (IL2, Boehringer Mannheim, Lewes, UK) was added to the cells over a range of concentrations at the commencement of culture. Proliferation was assessed as above and the results expressed as the percentage of the control culture.

$$\text{Proliferation, \% of control} = \frac{\text{cpm culture with DM} + \text{IL2}}{\text{cpm culture without DM} + \text{IL2}} \times 100$$

In vitro effects of DM on cell surface antigen expression

Peripheral blood lymphocytes (10 ml; 2×10^6 cells ml^{-1}) were cultured in 25-cm^2 flasks (Nunc) to study the in vitro effect of DM on cell surface markers. They were cultured in the presence or absence of the mitogens, PHA or ConA, added at optimal concentrations, and without DM or with DM added at two concentrations, 10^{-9} M or 10^{-6} M. After 72 h culture, the cells in the flasks were transferred into plastic Universals (Flow) and washed twice with PBS prior to staining for surface antigens (see below).

Immunofluorescent staining

The mAbs used for immunofluorescent staining of cell suspensions together with their specificities are listed in Table 1. Monoclonal antibody IL-A51 is considered to recognise the BoCD8 antigen (Ellis et al., 1986; Howard and Morrison, 1991). Monoclonal antibody IL-A21 recognises a non-polymorphic MHC class II determinant in cattle (kindly provided by the International Laboratory for Research on Animal Disease, Kenya). Monoclonal

TABLE 1

Monoclonal antibodies (mAbs) used for fluorescent staining of surface antigens

mAb	Bovine antigen detected	Human homologue (or specificity)	Reference
CC42	BoCD2	CD2	Howard et al., 1989
IL-A11	BoCD4	CD4	Baldwin et al., 1986
CC8	BoCD4	CD4	Howard et al., 1989
CC29	BoCD5	CD5	Howard et al., 1988
IL-A27	BoCD6	CD6	Baldwin et al., 1988
Il-A51	BoCD8	CD8	-
CC15	BoWC1	($CD4^-$ $CD8^-$ γ/δ T cells)	Clevers et al., 1990
PIg 45A	IgM	(IgM)	Davis et al., 1988
CC21	B-145 kDa	(B cells)	Naessens et al., 1990
IL-A24		(monocytes)	Ellis et al., 1988
IL-A21		(MHC II)	

antibody CC15 is directed against the BoWC1 antigen present on the $BoCD2^-$, $BoCD5^+$, $BoCD4^-$, $BoCD8^-$, T lymphocyte sub-population in bovine peripheral blood and recognises the equivalent population to those cells expressing SBU-19 in sheep (MacKay, 1988; Clevers et al., 1990). Monoclonal antibody CC21 is directed against an antigen with an approximate molecular mass of 145 kDa that is present on peripheral bovine B cells (Naessens et al., 1990).

Suspensions of cells were stained for surface antigen expression as described previously (Howard et al., 1989) for examination on a FACS Analyser or FACScan (Becton Dickinson, Sunnyvale, CA) except that FITC-conjugated anti-mouse Ig (Southern Biotechnology Associates Inc., Birmingham, AL, USA) was utilised for analysis on a FACScan.

Statistical analysis

Student's *t*-test was used to compare the differences between mean proliferative responses.

Analyses comparing the differences between responses by control and DM-treated calves used the results (i.e. counts per minute) without further manipulation. Analyses comparing the differences between responses to PHA, ConA and PWM in the DM-treated group used the mean response to each mitogen (in cpm) between days 7 and 20 post-treatment for individual animals, expressed as a percentage of the mean response for the control (untreated) group to the same mitogen over the same period.

RESULTS

Clinical observations

Calves receiving DM treatment developed slight hair loss, exopthalmos and apathy after about 1 week of treatment. There were no more infections in the immunosuppressed group compared with the control group but this was expected as the calves were gnotobiotes and were maintained in isolators as described in the Materials and Methods Section.

Haematological changes following DM administration

The number of circulating white blood cells (WBC) rose within 24 h of the first injection of DM and was highest after 48 h (Fig. 1). The increased WBC count seen on Days 1–4 following DM treatment (Fig.1(a)) was accounted for by a large increase in the numbers of circulating neutrophils (Fig. 1(b)). However, while total leukocyte counts had returned to normal by Day 5 of DM treatment, the numbers of neutrophils, after an initial decline from peak numbers, remained elevated for a further 11 days (Fig. 1(b)). In contrast, the numbers of mononuclear cells in the circulation fell sharply within 24 h of the start of DM treatment and remained at these reduced levels (approximately 25% of the pre-DM level) until the end of the period of treatment (Fig. 1(c)).

The total and differential leukocyte counts of the control calves (Group A) remained relatively constant throughout the experimental period (Figs. 1(a)–1(c)) except for one calf which had an unexplained high neutrophil count on Day 14 (Fig. 1(b)).

Proliferative responses to mitogens following in vivo DM administration

Lymphocytes from DM-treated calves showed significantly ($P<0.01$) decreased responsiveness to the mitogens PHA, ConA and PWM when compared with non-treated calves. However, the level of suppression was similar for all three mitogens. The mean level of suppression between Days 7 and 20 after the start of DM treatment was 83.7% for PHA, 68.7% for ConA and 75.3% for PWM. The results presented in Fig. 2 are from two representative calves from each group and demonstrate the kinetics of suppression to the three mitogens following DM treatment. Despite continued daily injections of DM, all calves showed transient recoveries in the immune responsiveness of their circulating lymphocytes on one or more occasions during the course of treatment (Fig. 2).

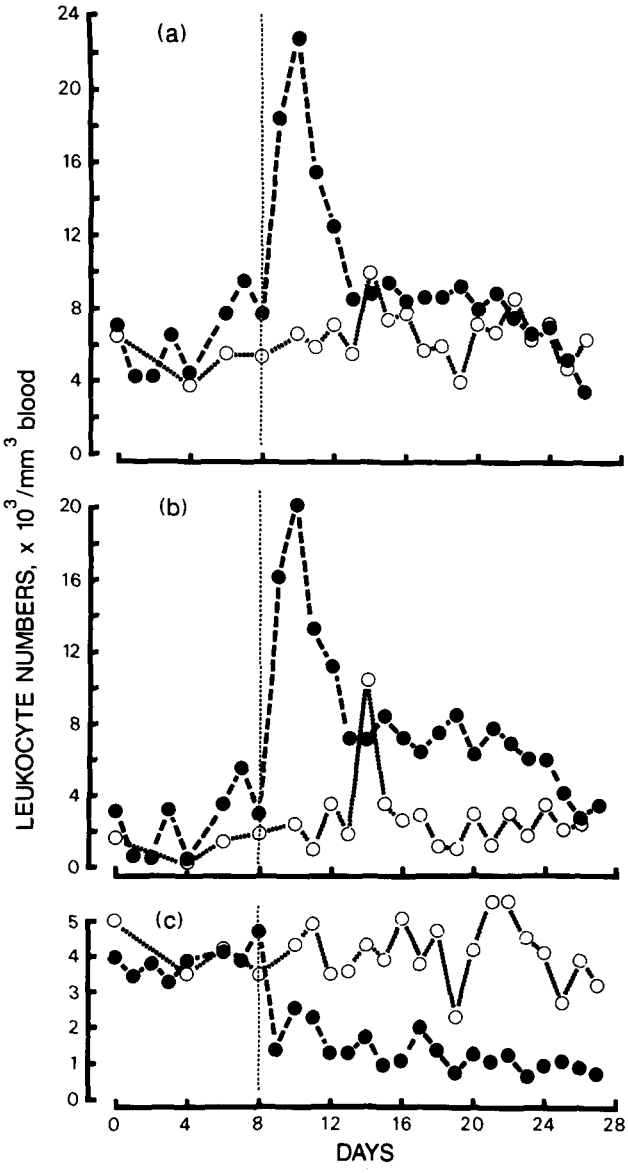

Fig. 1. Effect of dexamethasone on mean numbers of circulating leukocytes in gnotobiotic calves: (a) total leukocytes; (b) neutrophils; (c) mononuclear cells. Dexamethasone (0.5 mg day^{-1} kg^{-1} body weight) was administered from day 8 (¦) to one group of five calves (Group B, ●); five control, age-matched calves (Group A, ○) received no treatment.

In vitro effects of DM on mitogen responses

In vitro addition of DM to cultures of lymphocytes from control calves

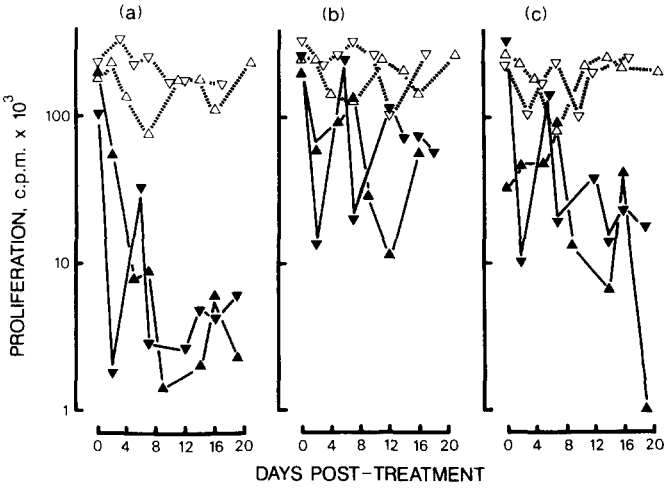

Fig. 2. Effect of in vivo dexamethasone administration on in vitro responses by peripheral blood lymphocytes to: (a) phytohaemagglutinin; (b) concanavalin A; (c) pokeweed mitogen. Two representative animals are shown from the dexamethasone-treated group (closed symbols) and the control, untreated group (open symbols).

markedly suppressed PHA responses (results not shown). The proliferative response to PHA was suppressed at low concentrations of DM (5.1×10^{-9} M) in 11 out of 12 calves. However, cells from six of these 11 calves with suppressed proliferative responses showed a recovery of their PHA responsiveness when the concentration of DM was increased to 5.1×10^{-6} M or greater. Dexamethasone had no effect on the in vitro PHA response by lymphocytes from one calf. In marked contrast, the response to ConA was virtually unaffected even at very high concentrations (5.1×10^{-4} M) of DM in eight out of ten calves tested (results not shown). In one of the two calves where DM suppressed the ConA response, the suppression was only modest (approximately 50% of the control proliferation) and recovery of responsiveness was seen at higher DM concentrations as with PHA responses in some calves. Proliferative responses to PWM were variable; responses by lymphocytes from some animals were enhanced, some were suppressed while others were unaffected (results not shown). The responses of spleen and mesenteric lymph node lymphocyte to PHA, ConA and PWM were affected by DM in the same manner as blood lymphocytes (Figs. 3(a)–3(c)).

Addition of exogenous IL-2 restores PHA responsiveness in the presence of DM

One possible explanation for the in vitro suppression of PHA responsiveness was that DM was inhibiting IL-2 production. In order to test this possi-

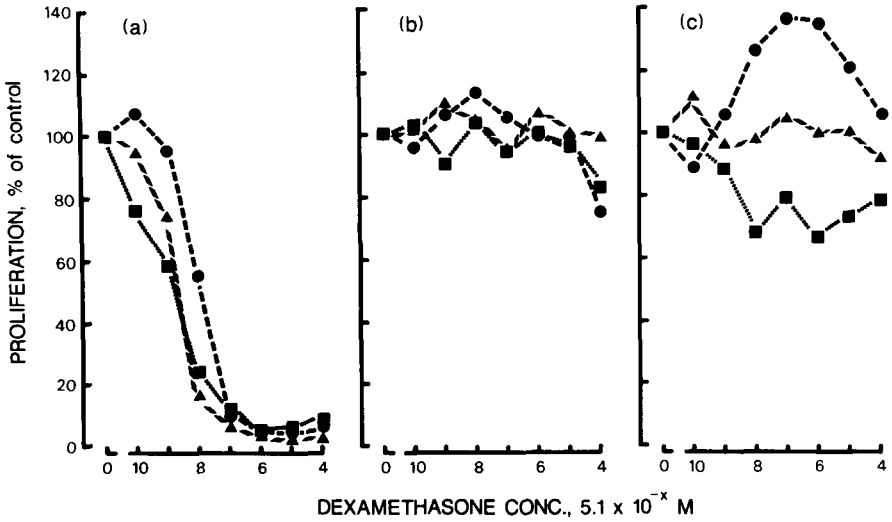

Fig. 3. Effect of different concentrations of dexamethasone in vitro on proliferative responses by blood (■), spleen (●) and mesenteric lymph node (▲) lymphocytes to: (a) phytohaemagglutinin; (b) concanavalin A; (c) pokeweed mitogen. The results presented are from a single calf.

bility, IL-2 was added to PHA-stimulated lymphocyte cultures in the presence or absence of DM.

These tests were carried out using lymphocytes from three calves (Fig. 4). Two different patterns of suppression of the PHA response were observed. Lymphocytes from Calf 1 behaved in a similar way to that seen in previous calves with suppression increasing with increasing concentration of DM. However, lymphocytes from the other two calves exhibited maximal suppression at a DM concentration of 5.1×10^{-8} M and as the DM concentration increased above this, a lower degree of suppression was obtained. Despite these differences, addition of recombinant human IL-2 to the cultures at either 1 unit ml^{-1} (Calves 2 and 3) or 4 units ml^{-1} (Calf 1) was able to, at least partially, restore responsiveness.

Changes in circulating lymphocyte sub-populations following in vivo DM treatment

There was a depletion in the absolute numbers of B cells (sIg^+), T cells ($CD5^+$, although this also includes a minor sub-population of B cells) and

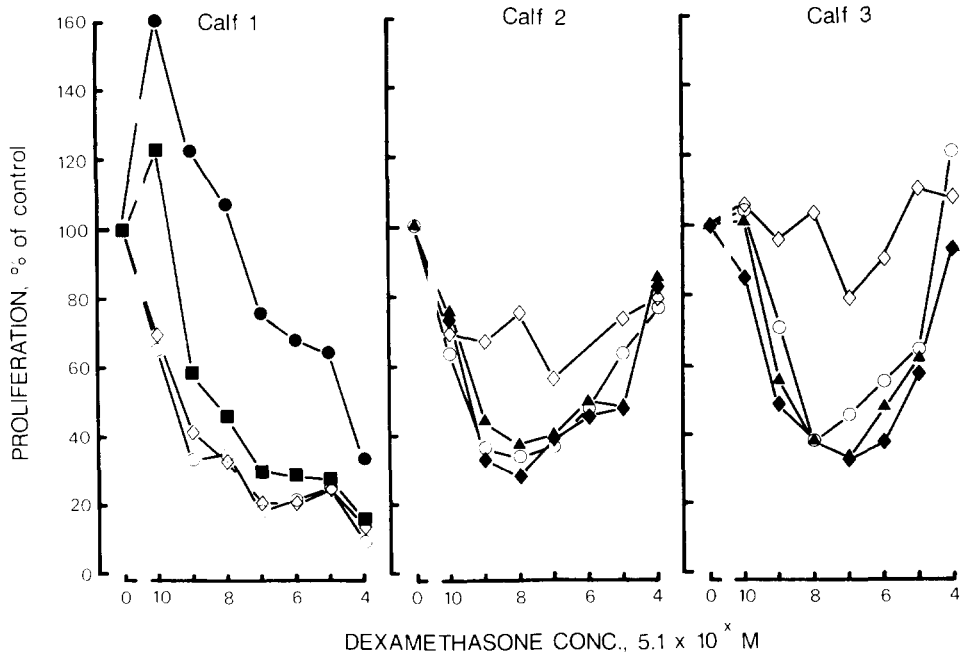

Fig. 4. Effect of different concentrations of recombinant human IL-2 on in vitro dexamethasone suppression of phytohaemagglutinin-induced proliferative responses by lymphocytes from three calves: ○, control; no IL-2; ♦, 0.01 units IL-2 ml^{-1}; ▲, 0.1 units IL-2 ml^{-1}; ◇, 1.0 units IL-2 ml^{-1}; ■, 2.0 units IL-2 ml^{-1}; ●, 4.0 units IL-2 ml^{-1}.

macrophages from the circulation following DM treatment (Table 2). However, the reduction in B cell numbers was more marked than the reduction of either T cells or macrophages. Similar results were obtained with two other mAbs to B cell antigens, either surface IgM or the 145 kDa B cell antigen (data not shown). Table 2 also demonstrates that the percentage of B cells present in the lymphocyte population declined while the percentage of total T cells present remained fairly constant.

The effect of DM administration on T cells was further studied by analysing the different T cell sub-populations present (Table 3). Treatment with DM reduced both the number and percentage of circulating $BoWC1^+$ cells, whereas, although the numbers of $BoCD2^+$ T cells were reduced, their proportion of the total population did not decline. When the $BoCD2^+$ T cell population was subdivided into $BoCD4^+$, $BoCD8^-$ and $BoCD4^-$, $BoCD8^+$ sub-populations, no differences were seen between these two cell types in their sensitivity to DM.

Cell surface markers following in vitro culture with DM

Suppression of the in vitro proliferative response of peripheral blood lymphocytes to PHA by DM was seen within 72 h of culture. Expression of sur-

TABLE 2

Numbers ($\times 10^5$) and percentages (in parentheses) of circulating B cells, T cells and monocytes in the peripheral blood of two calves following in vivo dexamethasone treatment

Cell[1] (marker)	Calf	Days post-dexamethasone treatment			
		0	6	12	19
B (sIg)[2]	C43	6.9 (17.3)	1.1 (6)	0.3 (2.6)	–[5]
	C44	6.8 (16)	2.1 (10.5)	0.6 (6.6)	0.5 (4.2)
T (BoCD5)[3]	C43	30.0 (62.5)	12.9 (80.8)	8.4 (60)	–
	C44	28.5 (63.4)	13.7 (54.6)	10.2 (84.8)	3.7 (41)
Ma/Mo[4]	C43	9.2 (19.1)	3.2 (3.1)	2.9 (20.4)	–
	C44	4.4 (9.8)	6.7 (26.7)	1.3 (11.1)	2.9 (31.9)

[1]Cell phenotype (antigen stained for).
[2]B cells detected by staining for surface membrane immunoglobulin (sIg).
[3]T cells detected by staining for BoCD5 (this population will include the $CD4^+$, $CD8^+$ and $BoWC1^+$ T cell sub-populations and a small percentage of $BoCD5^+$ B cells).
[4]Macrophages/monocytes detected by staining with ILA-24.
[5]High percentage of immature neutrophils present in cell preparation for this day, data not used.

TABLE 3

Numbers ($\times 10^5$) and percentages (in parentheses) of circulating T cells and T cell sub-populations in the peripheral blood of two calves following in vivo dexamethasone treatment

Cell marker[1]	Calf	Days post-dexamethasone treatment			
		0	6	12	19
BoWC1	C43	10.2 (21.3)	0.2 (1.4)	0.2 (1.6)	–[2]
	C44	13.3 (29.5)	1.9 (7.4)	0.3 (2.5)	0.4 (4.1)
BoCD2	C43	25.8 (53.7)	12.6 (78.9)	11.4 (81.2)	–
	C44	21.3 (47.3)	11.5 (46.1)	9.9 (82.8)	3.6 (39.5)
BoCD4	C43	16.1 (27.4)	8.8 (55.1)	8.3 (59.2)	–
	C44	15.6 (34.7)	8.2 (32.6)	8.1 (67.6)	3.1 (34.6)
BoCD8	C43	12.0 (24.9)	3.5 (22)	2.0 (13.9)	–
	C44	8.7 (19.4)	2.4 (9.7)	1.3 (10.8)	0.5 (5.5)

[1]Cell phenotype (antigen stained for).
[2]High percentage of immature neutrophils present in cell preparation for this day, data not used.

face antigens was analysed to determine whether this suppression was associated with a phenotypic change in the cells present in culture.

There was no marked alteration in the percentage of cells expressing the majority of cell surface antigens following culture with DM (Table 4). How-

TABLE 4

Effect of in vitro culture of peripheral blood lymphocytes with dexamethasone on expression of cell surface antigens (percentage of cells staining)

Cell marker	Control[1]			PHA			ConA		
	0 DM	10^{-9} M DM	10^{-6} M DM	0 DM	10^{-9} M DM	10^{-6} M DM	0 DM	10^{-9} M DM	10^{-6} M DM
BoWC1	35	20	21	35	38	ND[2]	39	42	29
BoCD2	43	55	59	65	58	66	54	67	54
BoCD5	88	83	91	94	96	95	92	91	92
BoCD4	14	23	40	29	31	37	47	36	35
BoCD8	23	35	26	22	19	50	21	25	26
BoCD6	ND	58	55	ND	59	63	55	57	68
IgM	4	ND	6	4	3	6	4	2	3
B-145 kDa	5	ND	7	3	1	5	2	1	4
Ma/Mo	8	15	11	3	2	8	3	3	7
MHC II	47	12	10	89	86	70	80	69	63

[1]Cells were cultured for 72 h either in medium alone or in the presence of mitogen (PHA or ConA).
[2]Not done.

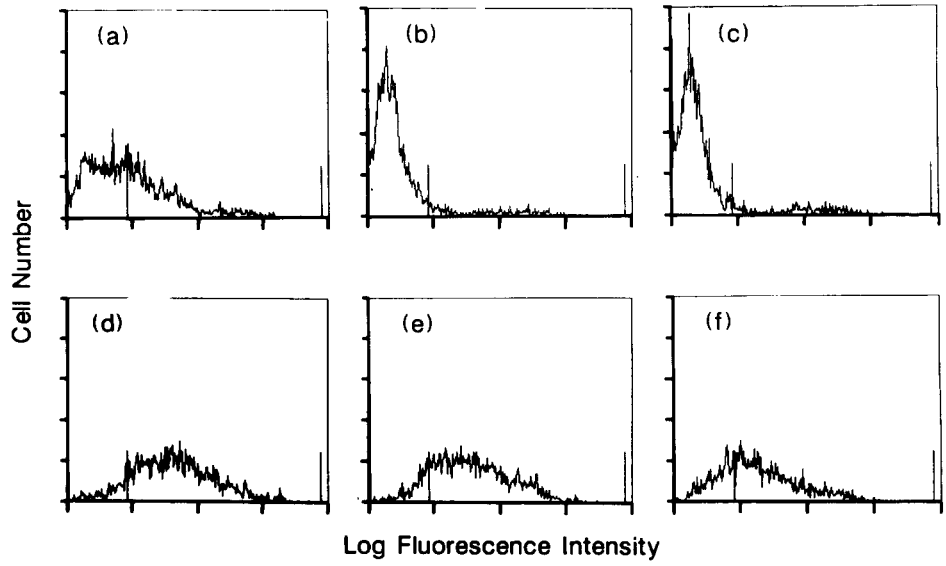

Fig. 5. Effect of dexamethasone on expression of MHC II expression by bovine mononuclear cells after 72 h in culture without PHA ((a)-(c)) or with PHA ((d)-(f)). Cells were cultured without DM ((a) and (d)), with 10^{-9} M DM ((b) and (e)), or with 10^{-6} M DM ((c) and (f)).

ever, BoCD8 and MHC II expression were affected. In the presence of PHA, DM (10^{-6} M) increased the percentage of cells expressing BoCD8. In contrast, MHC II expression was reduced by DM. The latter finding was most marked with cells cultured in the absence of mitogen, while the increased MHC II expression induced by culture with either PHA or ConA showed a more modest decrease. In addition to the change in the percentage of cells staining for MHC II antigens there was also a decrease in the overall intensity of staining for this molecule in the absence of PHA (Fig. 5).

DISCUSSION

The results presented here show that the DM regimen used for the immunosuppression of young calves induces marked changes in haematological values. The magnitude of these changes compared with the minor changes seen by others (Pruett et al., 1987) is due probably to the high dose of DM and age of animals used in our studies.

Our finding that responses to mitogens are suppressed following in vivo DM treatment are similar to those of Roth et al. (1984) who demonstrated suppression of both PHA and PWM in DM-treated steers. However, Pruett and colleagues found in stressed heifers that responses to ConA were more sensitive to suppression than those to PHA (Pruett et al., 1986) and PWM responses were enhanced in DM-treated steers (Pruett et al., 1987). The differences between the results of the present study and those of Pruett et al. (1987) are unclear, but, as above, are probably a result of differences in the doses of GC used.

The in vitro studies carried out in parallel with our in vivo studies confirmed the sensitivity of the PHA response to the presence of dexamethasone. In contrast to the in vivo studies however, ConA responses were unaffected by in vitro culture with DM, even at very high concentrations. This finding would indicate that GC suppressed responses to PHA and ConA by different mechanisms or that PHA and ConA stimulate differently. Given in vivo, GC result in a redistribution of lymphocytes from the blood to other lymphoid organs (Cupps and Fauci, 1982) and this effect may have caused the depressed ConA and PWM responses seen following in vivo DM treatment. Redistribution of lymphocytes in vivo may have affected the PHA responses but the results obtained in vitro with DM suggested that DM also exerted a specific physiological effect on responses to PHA.

Glucocorticosteroids have been shown to inhibit the production of IL-2 (Bettens et al., 1984), tumour necrosis factor (Waage and Bakke, 1988), IL-1 (Lee et al., 1988) in man and IL-3 (Culpepper and Lee, 1985) in mice. On the other hand, IL-4 production by a mouse Th clone was not affected by DM (Bertoglio and Leroux, 1988). Gillis et al. (1979) found that addition of uncharacterised exogenous T cell growth factor to GC-suppressed cultures of

mouse lymphocytes completely restored responsiveness. Similarly, in this study, PHA responsiveness was restored by the addition of exogenous recombinant human IL-2. This finding implies that the suppression of PHA responses by DM is due, at least partially, to blocked induction of IL-2 expression. Additionally, the inhibition of IL-2 receptor expression (Reed et al., 1986) may account for the incomplete restoration of the proliferative response by exogenous IL-2.

The effects of GC treatment on the expression of cell membrane components are variable, some being increased while others are decreased (Duval et al., 1983). Another explanation for the difference in results seen with PHA and ConA is that there is a selective GC-mediated suppression of a molecule involved with the PHA responsiveness of lymphocytes. MHC II expression by the mitogen-presenting cell has been demonstrated by Torbett et al. (1986) to be essential for PHA (but not ConA) responses in the mouse. Of all the surface antigens examined in this study, only MHC II expression was reduced. However, the modest level of suppression seen with DM in mitogen-stimulated cultures was not considered sufficient to explain the observed lack of responsiveness. The CD2 molecule on the T cell has been reported to bind PHA and to be involved in PHA-induced stimulation (O'Flynn et al., 1985). The equivalent molecule in our study, BoCD2, was not affected by DM in vitro.

As stated above, Pruett et al. (1987) found that while PHA and ConA responses were suppressed, PWM responses were enhanced and suggested that this differential responsiveness of lymphocytes to mitogens was the result of a change in the sub-populations of T lymphocytes. No data were presented by these authors to support this suggestion. Although we did not observe this differential responsiveness to the mitogens, we did observe changes in the populations of lymphocytes present in the circulation. Firstly, our results confirmed the reports of others (Wilkie et al., 1979; Pruett et al., 1987) that there was no selective loss of total T cells. However, we were able to demonstrate a selective loss from the circulation of the BoWC1$^+$, γ/δ T lymphocyte sub-population. Previous findings have shown that in vivo depletion with mAbs in cattle resulted in a reduced response to all three mitogens in vitro if the BoCD4$^+$ lymphocytes were depleted, but an enhanced response to PWM alone if the BoWC1$^+$ lymphocytes were depleted (Howard et al., 1989). There is no obvious simple relationship between these observations and DM may have additional physiological effects on lymphocytes. The functions of the BoCD4$^+$ and BoCD8$^+$ cells in cattle are equivalent to those of the same phenotype in other animal species. The function of the BoWC1$^+$ sub-population, that is highly sensitive to DM in vivo, is not fully understood. This phenotype characteristically produces the γ/δ form of TCR in ruminants (MacKay, 1988; Clevers et al., 1990) and may have a modulating effect on the immune response (Howard et al., 1989). The relative lack of effect on cell surface mark-

ers seen following in vitro culture with DM probably suggests that the changes seen in vivo are the result of a selective redistribution of cells from the circulation into peripheral lymphoid organs.

In conclusion, we have shown that DM selectively depletes some lymphocyte populations from the circulation and suppresses peripheral blood lymphocyte responses to mitogens. We have also demonstrated that the immunosuppressive effects of DM differ depending on the mitogen being used and whether exposure to DM was carried out in vivo or in vitro. Clearly, different interpretations would be reached as to the cortisone sensitivity of the calves if only in vivo or in vitro studies had been conducted or if only one mitogen had been used in the proliferation tests. We would therefore suggest that the classification of a species as being corticosteroid-resistant or corticosteroid-sensitive requires careful definition, not only of the age of the animal and dose of GC used, as others have demonstrated, but also of the tests used to assess immunosuppression and whether the tests are conducted following in vivo or in vitro exposure to GC. These results would also suggest that the mechanisms of stimulation by the mitogens PHA and ConA act through different pathways, perhaps at the second messenger level, and this system may provide a means whereby these pathways could be analysed.

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