Associations Between Function and Composition of Blood Mononuclear Leukocyte Populations from Holstein Bulls Treated with Dexamethasone

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ABSTRACT
To characterize further the effects of corticosteroid-induced stress on the immune system of dairy cattle, functional and phenotypic characteristics of populations of blood mononuclear leukocytes from control and treated (0.04 mg dexamethasone/kg per d for 3 consecutive d) Holstein bulls were evaluated concurrently. In vivo administration of dexamethasone caused a ≥97% reduction in in vitro secretion of interferon-γ by pokeweed mitogen-stimulated mononuclear leukocytes by d 2 after the first treatment. In vitro secretion of immunoglobulin M was reduced by >50% on d 2 and 3 after the first treatment, but returned to normal concentrations sooner than did interferon-γ secretion. Concurrent with changes in the secretion of these proteins were changes in the mean fluorescence intensities of major histocompatibility class I and II antigens and the WC1 antigen and in the proportion of B cells, CD3 T cells, \( \gamma \delta \) T cells, and cells in the leukocyte population expressing major histocompatibility class II antigens. Examination of the relationships between protein secretion in vitro and the composition of the blood mononuclear leukocyte population indicated that secretion was associated positively with the proportion of CD3 T cells (primarily the \( \gamma \delta \) T-cell subset) and the expression of major histocompatibility class I and II molecules and associated negatively with the proportion of cells expressing major histocompatibility class II antigens. Overall, these results suggest that corticosteroid-mediated stress in dairy cattle impairs secretion of proteins that are critical to normal cellular and humoral immune responses, an effect that is strongly linked with changes in the composition of the circulating mononuclear leukocyte population. (Key words: immunosuppression, flow cytometry, dairy cattle, corticosteroid, interferon-γ)

Abbreviation key: DEX = dexamethasone, IFN-γ = interferon-γ, MFI = mean fluorescence intensities, MHC = major histocompatibility, PBML = peripheral blood mononuclear leukocytes, PBST = PBS containing Tween 80, PBST-g = PBST and gelatin, PWM = pokeweed mitogen.

INTRODUCTION
Corticosteroids regulate virtually all components of the immune system and inflammatory responses. Elevations in endogenous corticosteroids as a consequence of physical or emotional stress may serve to prevent excessive or unrestrained amplification of immune and inflammatory responses (22). Corticosteroids, including the synthetic compound dexamethasone (DEX), are frequently used in veterinary medicine as anti-inflammatory drugs for the treatment of inflammation, autoimmunity, and shock. Their use results in a state of generalized immunosuppression (10, 25) and exacerbation of infectious disease processes (9, 29). This characteristic has also made corticosteroids useful in studies evaluating immunosuppression induced by stress and the efficacy of immunomodulators in dairy cattle (3, 24, 25).

The release of corticosteroids from the adrenal cortex in response to stress can have profound effects on the circulation and functional capacities of immune cells (11, 12, 18, 24). The peripartum period of the dairy cow has been associated with a dramatic elevation in the concentrations of plasma corticosteroids, a pronounced reduction in the functional capacities of blood neutrophils and lymphocytes, neutrophilia, and an increased susceptibility to mastitis (19, 20, 25). Recent research using dairy cattle suggests that part of the anti-inflammatory action of corticosteroids is to prevent the migration of neutrophils from the blood by down-regulating the expression of leukocyte adhe-
sion molecules (L-selectin and β2-integrin) that play an essential role in the movement of circulating neutrophils into peripheral tissues (8). Because L-selectin is also the homing receptor for blood lymphocytes in dairy cattle (5), the effects of in vivo administration of DEX on L-selectin expression and the composition of the circulating mononuclear leukocyte populations in dairy bulls were subsequently examined by Burton and Kehrli (7). Results indicated that DEX promoted migration of the γδ T-cell subset, which is considered an important first-line of defense (4, 17), out of the circulation by a mechanism that is not dependent on L-selectin; results also indicated that DEX did not alter the migration of αβ T cells. Burton and Kehrli (6) and Burton et al. (8) postulated that, if a redistribution of the monocyte populations in dairy cattle (5), the effects of vivo ad-

Conceivably, changes induced by DEX in the composition of the population of mononuclear leukocytes circulating in blood would influence the functional capacity of the population. The objectives of this study were, first, to evaluate in young dairy bulls the effects of the administration of DEX on the in vitro capacity of the peripheral blood mononuclear leukocyte (PBML) population to secrete interferon-γ (IFN-γ) and polyclonal Ig and, second, to determine whether associations existed between functional capacity and the composition of the PBML population.

MATERIALS AND METHODS

DEX Injections and Blood Collection Schedule

Young Holstein bulls were used that had reached sexual maturity before the studies were initiated. Bulls were housed at a facility in Stewartville, Minnesota for the duration of the study. Four bulls were treated with DEX (Azium; Schering Corp., Kenilworth, NJ), and four other bulls served as untreated controls. The DEX was injected i.m. once daily, for 3 consecutive d, at 0.04 mg/kg (mean dose of 16.8 mg/d per bull). This protocol has been described (7).

Blood from control and treated bulls was collected into acid-citrate-dextrose by jugular venipuncture on d -5, -4, and -3 before the first DEX injection (d 0) and on d 2, 3, 4, 5, 9, 10, and 11 after the 1st d of injection. Blood samples were collected on d 2 and were taken immediately before the third injection of DEX. Samples were transported to the laboratory of the USDA, Agricultural Research Service, National Animal Disease Center (Ames, IA) for functional and phenotypic analyses. Blood mononuclear cells were enriched from whole blooduffy coats as previously described (7).

In Vitro Production of IFN-γ and Measurement

Secretion of IFN-γ was evaluated in 200-μl cultures employing PBML that had been enriched with Percoll. Individual wells of 96-well microtiter plates were inoculated with 100 μl of PBML suspension that had been adjusted to 4 million cells/ml. The PBML were suspended in RPMI 1640 medium with 25 mM HEPES buffer (Gibco, Grand Island, NY), 200 mM L-glutamine (Sigma Chemical Co., St Louis, MO), and antibiotics and an antimycotic (100 IU/ml of penicillin G sodium, 100 μg/ml of streptomycin sulfate, and 0.25 μg/ml of amphotericin B; Gibco). Cultures of PBML were stimulated with pokeweed mitogen (PWM; 0, 5, and 10 μg/ml in culture; Sigma Chemical Co.). Mitogens were solubilized in RPMI-1640 medium with fetal bovine serum (5%, vol/vol, in culture; Hyclone, Logan, UT) and added as a 100-μl component of each culture. Cultures of PBML from each bull were prepared in triplicate and evaluated on the same plate. Cultures were incubated at 39°C for 48 h in a humidified atmosphere of 5% CO₂. Supernatants were then harvested (100 μl per well) after centrifugation (400 × g for 5 min at 18°C) of the plate and stored at -80°C.

Interferon-γ in the supernatants from cell cultures was measured using an IFN-γ capture ELISA (1). Assays were performed in Immunolon II microtiter plates (Dynatech Laboratories, Inc., Chantilly, VA). Reagents consisted of a capture antibody [mouse anti-recombinant bovine IFN-γ, IgG fraction; Veterinary Infectious Disease Organization, Saskatoon, SK, Canada], detection antibody (rabbit anti-bovine IFN-γ, IgG fraction; Veterinary Infectious Disease Organization), recombinant bovine IFN-γ (Veterinary Infectious Disease Organization), biotinylated goat anti-rabbit IgG (Zymed Laboratories, Inc., South San Francisco, CA), horseradish peroxidase-conjugated streptavidin-biotinylated complex (Amersham Corporation, Arlington Heights, IL), and substrate (N-ethylbenzothi-azolinone-6-sulfonic acid azine in citrate buffer and H₂O₂ at 0.1% vol/vol).

Internal standards consisting of serially diluted recombinant bovine IFN-γ were prepared in PBS with Tween 80 (PBST; 0.1%, vol/vol) and gelatin (PBS-g; 0.1%, vol/vol). Positive and negative control samples and test samples from mitogen-stimulated whole
TABLE 1. Antibodies used in the immunostaining of peripheral blood mononuclear leukocytes (PBML) from control bulls and bulls treated with dexamethasone.

| Antibody specificity | Designation | Isotype | Source | Working solution
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>CD3 (Pan T cell)</td>
<td>MM1A</td>
<td>IgG1</td>
<td>VMRD</td>
<td>14 μg/ml</td>
</tr>
<tr>
<td>CD4 (Helper/inducer)</td>
<td>IL-A11</td>
<td>IgG1</td>
<td>ILRAD</td>
<td>1/50</td>
</tr>
<tr>
<td>CD8 (Cytotoxic/suppressor)</td>
<td>CACT80C</td>
<td>IgG1</td>
<td>VMRD</td>
<td>7 μg/ml</td>
</tr>
<tr>
<td>WC1 (Subset of γδ T cell)</td>
<td>IL-A29</td>
<td>IgG1</td>
<td>ILRAD</td>
<td>1/400</td>
</tr>
<tr>
<td>B Cell</td>
<td>BAQ155A</td>
<td>IgG1</td>
<td>VMRD</td>
<td>3.5 μg/ml</td>
</tr>
<tr>
<td>MHC Class I</td>
<td>HS8E</td>
<td>IgG3</td>
<td>VMRD</td>
<td>7 μg/ml</td>
</tr>
<tr>
<td>MHC Class II</td>
<td>TH14B</td>
<td>IgG3</td>
<td>VMRD</td>
<td>7 μg/ml</td>
</tr>
<tr>
<td>Mouse IgG</td>
<td>...</td>
<td>IgG/F(ab’)2</td>
<td>BM</td>
<td>1/100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(phycoerythrin-conjugated)</td>
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</table>

1VMRD = VMRD, Inc. (Pullman, WA), ILRAD = International Laboratory Research for Animal Diseases (Nairobi, Kenya), and BM = Boehringer Mannheim (Indianapolis, IN).
2Antibody diluted in PBS containing BSA (0.01% vol/vol).
3Major histocompatibility complex.

blood and PBML cultures were serially diluted in PBST-g. Capture antibody was diluted 1:4000 (vol/vol) in carbonate buffer, and detection antibody was diluted 1:1000 (vol/vol) in PBST-g. Biotinylated goat anti-rabbit IgG was diluted 1:10,000 (vol/vol), and horseradish peroxidase-conjugated streptavidin-biotinylated complex was diluted 1:2000 (vol/vol) in PBST. Intervening washes were done using PBST. Absorbances of standards, controls, and test samples were read at 405 nm using an automated ELISA plate washer and reader (Dynatech MR7000; Dynatech Laboratories Inc., Guernsey Channel Islands, UK). Interferon-γ in test samples was determined from a standard absorbance curve regressed on IFN-γ concentration for the dilution of test samples that gave absorbance readings falling inside the linear portion of the curve. The concentration (nanograms per milliliter) of IFN-γ in supernatants was determined by multiplying the value from the standard curve by the dilution factor.

In Vitro Production of IgM and Measurement

Secretion of IgM by PBML was assayed in flat-bottom, 24-well polystyrene tissue culture plates inoculated with 1.5 × 10⁶ PBML in 1.5-ml cultures. The PBML were cultured in RPMI-1640 medium with 25 mM HEPES buffer (Gibco) and supplemented with fetal bovine serum (5% vol/vol in culture; Hyclone), 200 mM L-glutamine (Sigma Chemical Co.), and antibiotics and an antimycotic. The PBML were stimulated with PWM (0 and 0.08 μg/ml). After incubation for 14 d at 39°C in a humidified atmosphere containing 5% CO₂, the plates were centrifuged (400 × g, for 5 min at 18°C). Culture supernatants (100 μl per well) were harvested from each culture and stored at −80°C until analysis for total IgM.

The IgM in supernatants from unstimulated and PWM-stimulated PBML cultures was assayed by ELISA as previously described (23). The concentration of IgM (micrograms per milliliter) was calculated by comparison of the absorbance of unknowns with the absorbance of the standards (serially diluted bovine IgM; Sigma Chemical Co.) fit within a linear curve.

Immunostaining and Flow Cytometric Analysis of PBML

Percoll-enriched PBML were washed twice, resuspended in PBS, and inoculated at 5 × 10⁵ cells per well (100 μl per well) into 96-well, U-bottomed microtiter plates for immunostaining. Indirect fluorescent antibody immunostaining was used to identify T-cell subsets, B cells, and major histocompatibility (MHC) class I and class II positive cells in the PBML suspensions. Primary antibodies recognized the CD3 antigen present on all T cells, the CD4 antigen present on helper and inducer T cells, the CD8 antigen present on cytotoxic and suppressor T cells, and the WC1 antigen present on 80 to 90% of γδ T cells in peripheral blood. Sources, specificities, isoforms, and working dilutions of monoclonal antibodies and secondary antibody are given in Table 1. The immunostaining procedure has been described in detail elsewhere (7).

Antibody-labeled cell suspensions were analyzed by a FACScan flow cytometer (Becton Dickinson, San
Jose, CA) as previously described (7). Acquired data were analyzed using Cell Quest software (Becton Dickinson). Variables recorded for each marker were the percentages of cells that stained positive and the mean fluorescence intensities (MFI) of those cells.

Statistical Analysis

Data were assessed for normality of distributions using the univariate procedure of SAS (26) and were log_{10}-transformed prior to statistical analyses. The GLM procedure of SAS (26) was then used for the least squares ANOVA of the log_{10}-transformed data files. The statistical model included fixed effects of treatment, day, and interaction of day and treatment, as well as a random effect of bull nested within treatment. The expected mean square value for the interaction of day and treatment was used as the denominator for the type III sums of squares F test of the treatment effect, and all other hypothesis tests were performed using the residual error mean square. Statistical significance was determined at \( P \leq 0.05 \). For simplicity of reporting, DEX effects were described according to least squares means for the interaction of day and treatment. The least squares means for interaction of day and treatment were converted back to original units of measurement for the purposes of data presentation. Indicated significant differences between treatment means within a day were taken from the matrices of the Student's two-sample t test, which accompanied the least squares means from ANOVA of log_{10}-transformed data, when the interaction of day and treatment was significant.

Pearson's product-moment correlations were computed between the PBML phenotypes and the IFN-\( \gamma \) and IgM variables using log_{10}-transformed data and the correlation procedure of SAS. Correlations were judged to be significant at \( P \leq 0.05 \).

RESULTS

Effects of DEX on Secretion of IFN-\( \gamma \) and IgM by PBML

Effects of in vivo administration of DEX on the in vitro capacity of the unstimulated and PWM-stimulated PBML cultures to secrete IFN-\( \gamma \) and polyclonal IgM were evaluated concurrently. Results from these assays were correlated with results from flow cytometric analyses of the cellular composition of the PBML suspensions that were used to inoculate the cultures.

Secretion of IFN-\( \gamma \) by unstimulated PBML cultures was undetectable (data not shown). Secretion by PBML cultures stimulated with PWM (10 \( \mu \)g/ml) was almost abrogated on 2 d or more after the first administration of DEX (Figure 1). The amount of IFN-\( \gamma \) that was present in these cultures declined from \( \sim 6 \) ng/ml during the pretreatment period to \(<0.1 \) ng/ml on d 2 and 3 after the first injection of DEX. Secretion of IFN-\( \gamma \) returned to pretreatment concentrations by \( >5 \) d after the first injection of DEX. Effects of DEX on IFN-\( \gamma \) secretion by parallel cultures stimulated with PWM at 5 and 20 \( \mu \)g/ml were similar in magnitude and duration (data not shown).

The low concentration of IgM secreted by unstimulated cultures of PBML was not affected (\( P >0.05 \)) by DEX (data not shown). Effects of DEX on the capacity of PWM-stimulated PBML to secrete polyclonal IgM are shown in Figure 2. The mean concentration of IgM produced by PBML during the pretreatment period was 6.3 \( \mu \)g/ml, which declined to 3.6 and 2.8 \( \mu \)g/ml on d 2 and 3 after the first treatment. These latter concentrations were less (\( P <0.05 \)) than those produced in parallel cultures using cells from controls bled on the same days. The magnitude of DEX-induced inhibition of IgM secretion (\( \sim 50\% \)) was considerably less than that observed for IFN-\( \gamma \) secretion (\( \sim 98\% \)). Secretion of IgM returned to normal by d 4 after the first treatment, but secretion of IFN-\( \gamma \) was inhibited longer.
CORTICOSTEROIDS AND BOVINE LEUKOCYTES

Phenotype of Cells in PBML Suspension

Results from flow cytometric analysis of PBML from control and treated bulls are summarized in Figures 3 and 4. Relative to control values, the MFI of MHC class I and II antigens was reduced (P<0.001) by 27.7% (MHC class I) and 40.13% (MHC class II) for up to 4 d after the first treatment (Figure 3). The MFI of MHC molecules was maximally inhibited on d 2. In contrast, DEX did not affect the percentage of cells expressing MHC class I antigen (overall range was 82.5 to 99.9%) and increased (P<0.001) the percentage of cells expressing MHC class II antigen on d 2 and 3 by more than twofold (Figure 4) relative to pretreatment values. When posttreatment values were compared with pretreatment values within the treated group, percentages of B cells in PBML suspensions decreased (P<0.01) by 50.2, 49.5, 21.5, and 21.6% on d 2, 3, 4, and 9, respectively (Figure 4).

Percentages of CD3+ T cells in PBML populations from the treated group were consistently lower than those from controls (data not shown). The mean percentage of CD3+ cells was lower (P<0.001) by 35.6% on d 2 and 3 after the first treatment (Figure 4) than pretreatment. This reduction was reflected in a 58.7% decrease (P<0.001) in the percentage of γδ T cells in PBML suspensions on these days. Reductions (P<0.01) of 34.9, 23.6, and 26.9% in percentages of CD4+ and CD8+ T cells occurred in treated bulls. The MFI of CD3 (Figure 3), CD4, and CD8 antigens (not shown) were unaffected by DEX, but the MFI of the WC1 antigen increased (P<0.001) an average of 116.7% over d 2, 3, and 4 (Figure 3) relative to pretreatment values.

Significant (P<0.05) Pearson's product-moment correlations between PBML phenotype and protein secretion are shown in Table 2. The percentage of CD3+ T cells in the PBML population was correlated positively to in vitro secretion of IFN-γ and IgM. This correlation was reflected by strong positive relationships between percentages of CD4+ T cells and γδ T cells in PBML suspensions and secretion of both proteins. The percentage of CD8+ T cells was correlated positively only to IFN-γ secretion, and the percentage of B cells was correlated only to IgM secretion. The MFI was correlated positively (CD3 and CD4 antigens) and negatively (WC1 antigen) to IFN-γ secretion. The MFI of CD3, CD4, and WC1 antigens was unrelated to IgM secretion. Associations between IFN-γ secretion that were induced by PWM at 5 and 20 μg/ml and expression of leukocyte antigens were almost identical to those shown for IFN-γ that were induced by PWM at 10 μg/ml (data not shown).

Correlations between protein secretion and the percentages and MFI of MHC class I and class II were
Figure 4. Mean (±SEM) percentages of major histocompatibility (MHC) class II cells that were positive for antigen (●), B cells (♦), CD3 T cells (○), and WC1+ cells (◊) in mononuclear leukocyte populations recovered from the peripheral blood of bulls treated with dexamethasone (n = 4) during the experimental period. The cells were used to inoculate cultures evaluating the capacity of peripheral blood mononuclear leukocytes to secrete interferon-γ and polyclonal IgM. Pretreatment versus treatment values (*P < 0.01; **P < 0.001).

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TABLE 2. Pearson's product-moment correlations between peripheral blood mononuclear leukocyte (PBML) phenotype and the capacity of pokeweed mitogen (PWM)-stimulated mononuclear leukocytes to secrete interferon-γ (IFN-γ) and polyclonal IgM in vitro.

<table>
<thead>
<tr>
<th>Leukocyte antigen</th>
<th>Antigen expression1</th>
<th>Cell product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IFN-γ2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgM3</td>
</tr>
<tr>
<td>CD3</td>
<td>%</td>
<td>0.5518***</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>0.2351*</td>
</tr>
<tr>
<td>CD4</td>
<td>%</td>
<td>0.3201**</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>0.2970**</td>
</tr>
<tr>
<td>CD8</td>
<td>%</td>
<td>0.3984***</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>NS</td>
</tr>
<tr>
<td>WC1</td>
<td>%</td>
<td>0.4716***</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>-0.3024**</td>
</tr>
<tr>
<td>B Cell</td>
<td>%</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>NS</td>
</tr>
<tr>
<td>MHC Class II antigen</td>
<td>%</td>
<td>-0.5263***</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>0.5572***</td>
</tr>
<tr>
<td>MHC Class I antigen</td>
<td>%</td>
<td>-0.4176***</td>
</tr>
<tr>
<td></td>
<td>MFI</td>
<td>0.5572***</td>
</tr>
</tbody>
</table>

1Percentage of mononuclear leukocytes expressing specific antigen and mean fluorescence intensity, an indicator of antigen density per cell.

2Secretion of IFN-γ induced with PWM at 10 μg/ml.

3Secretion of IgM induced with PWM at 0.08 μg/ml.

4Correlation was not significant and, therefore, is not shown.

*P < 0.05.

**P < 0.01.

***P < 0.001.

more pronounced than those of other antigens (Table 2). Strong negative correlations existed between the percentage of cells expressing MHC class II antigen and secretion of both proteins. In contrast, a strong positive correlation existed between the MFI of the MHC class II molecule and secretion of both proteins. The percentage of cells expressing MHC class I antigen was correlated negatively with IFN-γ secretion, and the MFI of the MHC class I antigen was correlated positively with the secretion of IFN-γ and IgM.

**DISCUSSION**

In the present study, the in vivo administration of DEX to young Holstein bulls resulted in the simultaneous inhibition of the IFN-γ and IgM production by PBML. Changes in protein secretion were associated with dramatic changes in the composition of the PBML population. These results suggest that corticosteroids affect changes in the PBML population that would modulate the capacity to mount normal cellular and humoral immune responses. Effects of DEX on the bovine PBML population can be explained by recent studies (2, 28) indicating that corticosteroids can inhibit translocation of the transcription factor, nuclear factor IκB, to the nucleus, where it normally associates with the response elements essential for the induction of genes for a variety of immunoreceptors, cell adhesion molecules, cytokines (i.e., IFN-γ), hematopoietic growth factors, and acute phase proteins. Previously reported changes in the expression of L-selectin and CD18 on neutrophils of dairy cows (8) and compositional changes in circulating T-cell populations in cattle (7) may also be consequences of the effects of DEX on multiple genes encoding key immunoregulatory proteins.

Positive correlations between in vitro secretion of IFN-γ and the percentage of WC1+ T cells, consisting mainly of γδ T cells, in the PBML population are interesting because published data (15) indicate that γδ T cells secrete IFN-γ and that peak production of IFN-γ by this T-cell subset during infection coincides with peak activation and recruitment of macrophages. Results from the present study suggest that the γδ T cell is closely linked to the production of IFN-γ in cattle. Because several lines of evidence suggest that γδ T cells may act as a first line of defense and have a protective role (4), the effects of corticosteroids on this T-cell subset likely compromise host defenses in cattle. Effects of corticosteroids in young cattle, in which γδ T cells comprise a high proportion of the
The effects of corticosteroids on the distribution and function of B cells in dairy cattle are not clearly defined (7, 10, 24). Pharmacological dosages of corticosteroids or elevations in plasma cortisol concentrations that are induced by stress can reduce antibody production in previously sensitized cattle (24). Burton and Kehrli (7) showed that administration of DEX caused a transient increase in the percentage of B cells in the circulation of dairy bulls, but results of other studies (14, 31) suggest that the circulating population of B cells in dairy cattle is resistant to the effects of corticosteroids. In the present study, DEX caused a reduction in B-cell percentages in the PBML population on d 2 and 3 after the first treatment. Concurrent with the decrease in the proportion of B cells was a dramatic reduction in secretion of Ig by the B cells within this population.

Because PWM is a T-dependent, B-cell mitogen for bovine lymphocytes (16, 21), the positive correlations between IgM secretion by PWM-stimulated PBML and percentages of T cells (CD3+, CD4+, and γδ T cells) in the initial PBML population were not surprising. These results suggest that the reduced secretion of IgM was not due only to the reduction in the number of B cells that had been induced by DEX, but also was likely due to the effect of DEX on T-cell populations supporting the Ig-secreting capacity of the B cell. The positive correlation between the percentage of γδ T cells in the PBML population and the secretion of IgM and IFN-γ suggests that the γδ T cell subset contributes to normal cellular and humoral immune responses in dairy cattle.

A major consequence of the administration of DEX on the PBML population was the dramatic reduction in percentages of CD3+ T cells, which was attributable mainly to a reduction in γδ T cells and, to a lesser extent, to reductions in percentages of αβ T cells, consisting of the CD4+ and CD8+ T cell subsets. Burton and Kehrli (7) proposed that the loss of circulating γδ T cells after administration of DEX is not a consequence of apoptosis, but more likely is due to the migration of γδ T cells out of the circulating pool. Conceivably, movement of the γδ T cells out of the peripheral circulation to other sites (i.e., gut or skin), where they can function as a first line of defense against pathogens (4), may benefit the stressed animal. This subset of T cells has been shown to be the predominant cell type infiltrating cutaneous reactions 6 to 24 h after injection of tuberculin into sensitized cattle (13) and only a minor component of cellular response at 72 h (14). For this reason, a more complete evaluation of the effects of DEX on peripheral blood and γδ T cells at the reaction site during early stages of the cutaneous delayed-type hypersensitivity reaction might provide new information regarding the circulation and function of γδ T cells in stressed cattle.

Administration of DEX increased the percentages of cells expressing MHC class II antigens and simultaneously reduced MHC class I and II molecule MFI in the PBML population, confirming results of studies of dairy cattle (8) and other species (28, 30). Because of the essential role of MHC molecules in antigen presentation and recognition (28, 30), the changes in MHC molecule expression (MFI and percentage positive cells) that were associated with administration of DEX would be expected to affect adversely the adaptive arm of the immune response. Strong correlations between MHC antigen expression and specific functional capacities of the PBML, demonstrated for the first time in cattle, support this supposition.

CONCLUSIONS

Administration of DEX to young bulls was used in the present study as a model of corticosteroid-induced immunosuppression. Using this approach, DEX caused phenotypic changes in the PBML population that were strongly associated with reduced capacity of this population to secrete IFN-γ and IgM. These results support the recommendation (14) that diagnostic tests measuring cell-mediated immunity by IFN-γ production in response to antigenic stimulation be interpreted with extreme caution in stressed cattle and cattle given corticosteroids because the chance for a false-negative result would be greater. Similarly, the potential of a natural or therapeutically induced elevation in corticosteroids to reduce the responsiveness of cattle to vaccines that rely on antibody production for a protective effect should be considered. Results from the present study, linking functional and compositional characteristics of the PBML population, suggest that the best overall predictor of the capacity of a young bull to mount a normal immune response, as measured by in vitro IFN-γ and IgM secretion, may be expression (MFI and percentage of cells) of MHC II molecules and the percentage of γδ T cells in the PBML population. These phenotypic variables might serve as sensitive and easily measured indicators of stress in dairy cattle if associations between the composition and functional capacity of the PBML population are confirmed by subsequent studies.
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