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Original article

Dexamethasone, but not meloxicam, suppresses proliferation of bovine $CD25^+CD4^+$ and $CD25^-CD4^+$ T cells

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Abstract

Recently, we found that dexamethasone caused a depletion of $CD25^-CD4^+$ T cells, but it increased the number of $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T cells. We also determined meloxicam-induced increase in the number of $CD25^{high}CD4^+$ T cells. In view of this, and taking into consideration the latest reports indicating that meloxicam shows an anti-proliferative effect on bovine peripheral blood mononuclear cells, it was considered purposeful to determine the effect of both drugs on proliferation of bovine $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and $CD25^-CD4^+$ T cells. Flow cytometry analysis and 5-bromo-2'-deoxyuridine incorporation assay were applied to detect the cell proliferation. It was demonstrated that dexamethasone, but not meloxicam, significantly reduced cell proliferation within all three evaluated $CD4^+$ T cell subpopulations. Thus, the depletion of $CD25^-CD4^+$ T cells by treatment with dexamethasone can partly be the effect of the anti-proliferative action of the drug, however, dexamethasone-induced increase in the number of $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T cells cannot be the result of enhanced proliferation of these cells.

Key words: cattle, $CD4^+$ cells, dexamethasone, meloxicam, proliferation

Introduction

Recently, we investigated the *in vitro* effect of dexamethasone on bovine $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and $CD25^-CD4^+$ T cells (Maślanka and Jaroszewski 2012). It was found that dexamethasone caused considerable loss of $CD25^-CD4^+$ T cells, but it increased the relative and absolute numbers of $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T lymphocytes. It has been demonstrated that changes in cell number were at least partly caused by the pro-apoptotic effect of the drug on $CD25^-CD4^+$ T cells and the anti-apoptotic effect on $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T cells. However, it should be taken into consideration that the above-mentioned changes in the number of bovine $CD4^+$ T lymphocytes could be additionally connected with the effect of the drug on the proliferation of these cells. Therefore, the main aim of this study was to establish the effect of dexamethasone on proliferation of bovine $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and

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$CD25^{-}CD4^{+}$ T cells. In addition, the effect of meloxicam (nonsteroidal anti-inflammatory drug) on these cells was also evaluated in the presented research. In our latest research (Maślanka and Jaroszewski, in press), we found that this drug induced a substantial increase in the number of $CD25^{high}CD4^{+}$ T cells. In view of this, and taking into consideration the latest reports indicating that meloxicam shows an anti-proliferative effect on bovine peripheral blood mononuclear cells (PBMCs) (Maeda et al. 2011), it was considered purposeful to determine the effect of this drug on proliferation of bovine $CD25^{high}CD4^{+}$, $CD25^{low}CD4^{+}$ and $CD25^{-}CD4^{+}$ T cells.

Materials and Methods

PBMCs were derived from clinically healthy heifers (n = 12) (Polish Black and White breed). Isolation of PBMCs was performed as previously described (Maślanka and Jaroszewski 2012). PBMCs were adjusted to the final concentration of 2×10^{6} cells/mL in complete medium (Maślanka and Jaroszewski 2012), seeded in 24-well plates in 1 mL aliquots and incubate 24 hr in the absence (control) or presence of dexamethasone (DEX) or meloxicam (MEL) (both from Sigma-Aldrich, Munich, Germany). PBMCs were treated with drugs in concentrations reflecting their plasma levels achieved *in vivo* at therapeutic doses (DEX 10^{-7} M, MEL 5×10^{-6} M) and at concentrations ten times lower (DEX 10^{-8} M, MEL 5×10^{-7} M). Cell proliferation was evaluated after 24 hrs stimulation with concanavalin A (5 µg/mL Con A, Sigma-Aldrich) in the presence of 5-bromo-2'-deoxyuridine (BrdU) [APC BrdU Flow Kit, Becton Dickinson (BD) Biosciences, San Jose, USA] at a final concentration of 60 µM in cell culture medium during the last 8 hr. Each experiment included 4 wells of PBMCs (obtained from individual heifers) for each condition tested. All experiments were repeated independently three times, using 4 different animals for each experiment (overall n = 12). After the culture, PBMCs were labeled for CD4 and CD25 as previously described (Maślanka and Jaroszewski 2012), and thereafter stained for incorporated BrdU according to the manufacturer's procedure (APC BrdU Flow Kit, BD Biosciences). Flow cytometry analysis was performed using a FACSCanto II cytometer (BD Biosciences). Data were acquired (a total of 200000 events per sample) by FACSDiva version 6.1.3 software (BD Biosciences) and analyzed by FlowJo software (Tree Star Inc., Stanford, CA, USA). Cytometry setup and tracking beads (CST, BD Biosciences) were used to initialize PMT settings. Unstained control cells, as well as single stain control for every fluorochrome, were prepared and used to set up flow cytometric compensation. All data are presented as the mean ± SEM. Student's unpaired t test (GraphPad Prism 3, GraphPad Software, San Diego, USA) was used to compare the results between dexamethasone- or meloxicam-treated (at either concentration) and untreated cells. A P value less than 0.05 was considered to be statistically significant. The data were graphed with Sigmaplot software (version 12, Systat Software, Inc, Chicago, USA).

Results

It was found that dexamethasone in both used concentrations significantly decreased proliferation of cells from both $CD25^{high}CD4^{+}$ ($P < 0.01$), $CD25^{low}CD4^{+}$ ($P < 0.001$) and $CD25^{-}CD4^{+}$ ($P < 0.01$) T cell subpopulations (Fig. 1). It was demonstrated that meloxicam did not statistically significantly affect the proliferation of cells from any of the evaluated $CD4^{+}$ lymphocyte subpopulations (Fig. 1), but a clear trend (P = 0.1014 [Mel 5×10^{-6} M]; P = 0.1052 [Mel 5 x 10-7 M]) was observed towards an increase in the proliferation of $CD25^{high}CD4^{+}$ T cells under the influence of the drug.

Discussion

Analyzing the obtained results, the first issue to be raised is the fact that as regards proliferation in response to Con A stimulation, bovine $CD25^{high}CD4^{+}$ and $CD25^{low}CD4^{+}$ T cells differ from their phenotypic counterparts in humans and mice. In general, human and murine Con A-stimulated $CD25^{+}CD4^{+}$ (regulatory) T cells do not proliferate at all, or proliferate only minimally. Makita et al. (2004) demonstrated in human peripheral blood $CD4^{+}$ T cells that the $CD25^{high}CD4^{+}$ T cell subset was found to be hyporesponsive to Con A stimulation compared to the $CD25^{-}CD4^{+}$ T cells. Proliferation of Con A-stimulated $CD25^{-}CD4^{+}$ T cells was about 5 times greater as compared with $CD25^{high}CD4^{+}$ T cells, which proliferated very poorly. Similarly, $CD25^{+}CD4^{+}$ T subsets were also hyporesponsive to Con A as compared with $CD4^{+}CD25^{-}$ T cells. Moreover, it has been shown (Blache et al. 2009) that murine $CD25^{+}CD8^{-}CD3^{+}$ ($\approx CD25^{+}CD4^{+}$) T cells do not proliferate in response to Con A activation. We demonstrated in our previous research (Maślanka and Jaroszewski 2012) that in respect to the expression of Foxp3 (which is a factor conditioning the regulatory properties of human and murine $CD25^{+}CD4^{+}$ T regulatory cells), bovine $CD25^{high}CD4^{+}$ and $CD25^{low}CD4^{+}$ T cells are not the

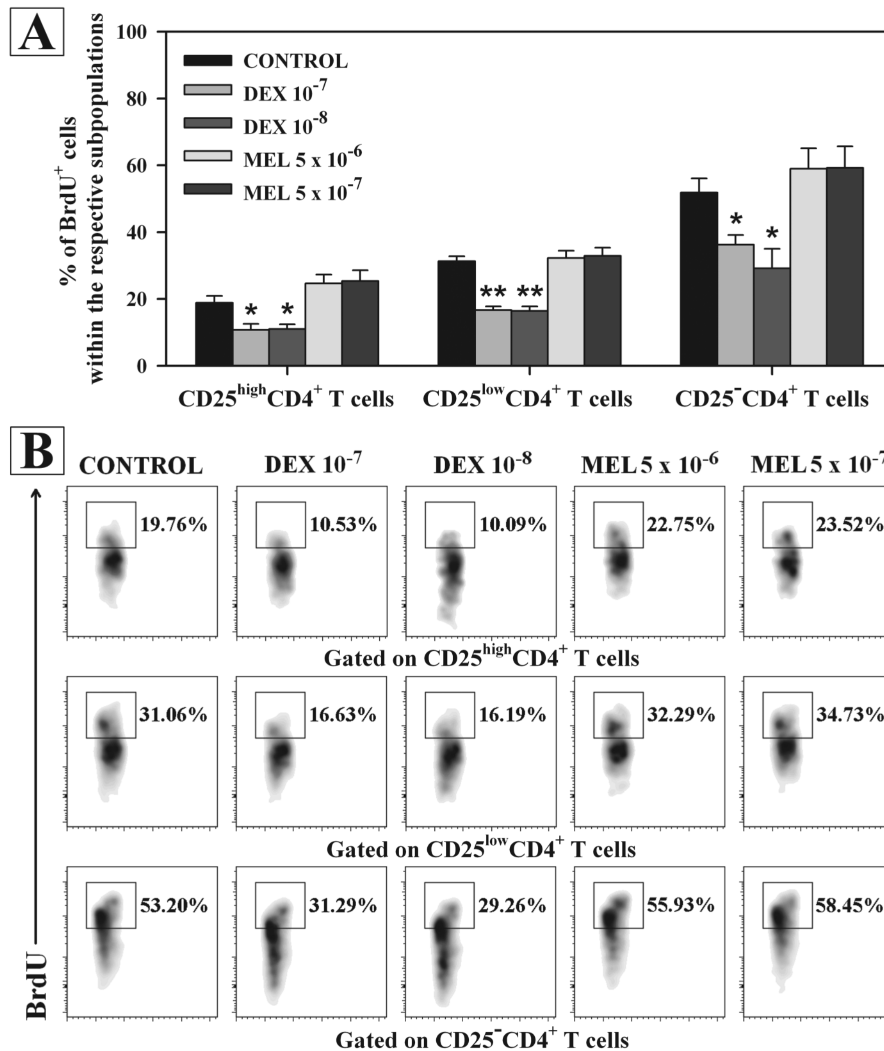

Fig. 1. (A) The effect of dexamethasone (DEX) and meloxicam (MEL) on the proliferation of $CD25^{high}CD4^+$, $CD25^{low}CD4^+$, and $CD25^-CD4^+$ T cells. The data are expressed as a percentage of 5-bromo-2'-deoxyuridine(BrdU)-positive cells within the $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and $CD25^-CD4^+$ T cell subpopulations. The results are the mean (± SEM) of three independent experiments with four animals per experiment (n = 12). *P < 0.01, **P < 0.001 treated cells versus control cells. (B) Typical cytograms demonstrating BrdU incorporation within $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and $CD25^-CD4^+$ T cells.

counterparts of human or murine cells with this phenotype. The ability of $CD25^+CD4^+$ T cells to proliferate in response to polyclonal stimulation as demonstrated in the presented research supports the view that these lymphocytes are not a pool of regulatory cells in cattle.

It was demonstrated that dexamethasone in both used concentrations significantly reduced the proliferation of cells from both $CD25^{high}CD4^+$, $CD25^{low}CD4^+$ and $CD25^-CD4^+$ T cell subpopulations. Thus, the obtained results indicate that the increase in the number of $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T cells, which we demonstrated in our earlier research (Maślanka and Jaroszewski 2012), could not be the result of their increased proliferation under the influence of dexamethasone because this drug not only did not exert a pro-proliferative effect on these cells, but acted anti-proliferatively on them. This, therefore, proves that the anti-apoptotic (Maślanka and Jaroszewski 2012) and not the pro-proliferative action of the drug on bovine $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T lymphocytes lies at the root of dexamethasone-induced increase in the absolute number of these cells. It cannot, however, be excluded that dexamethasone could additionally increase the absolute number of cells from the $CD25^{high}CD4^+$ and $CD25^{low}CD4^+$ T cell subpopulations through induction of the expression of the CD25 molecule on $CD25^-CD4^+$ cells, but the clarification of this issue requires further research. The obtained results also indicate that the loss of bovine $CD25^-CD4^+$ T lymphocytes induced with dexamethasone, which we demonstrated in earlier

research (Maślanka and Jaroszewski 2012), was connected not only with pro-apoptotic, but also with the anti-proliferative effect of the drug on these cells. Research evaluating the influence of dexamethasone on the proliferative capacity of murine $CD25^{-}CD4^{+}$ and $CD25^{+}CD4^{+}$ T lymphocytes demonstrated the anti-proliferative effect of the drug only on cells from the first subpopulation. Chen et al. (2006) found that IL-2 induced proliferation of murine $CD25^{-}CD4^{+}$ T cells was completely abrogated by dexamethasone, whereas IL-2-induced proliferation of $CD25^{+}CD4^{+}$ T cells was preserved in the presence of the same concentration of dexamethasone. Therefore, these authors concluded that treatment with dexamethasone selectively supported the growth of $CD25^{+}CD4^{+}$ regulatory T cells, but not $CD25^{-}CD4^{+}$ T cells. Thus, a fundamental difference exists regarding the *in vitro* effects of dexamethasone on the proliferative capacity of $CD25^{+}CD4^{+}$ T cells also between murine and bovine cells with this phenotype.

No statistically significant effect of meloxicam on the proliferative capacity of cells from any of the studied subpopulations was demonstrated, but a clear trend was observed towards an increase in the proliferation of $CD25^{high}CD4^{+}$ T cells under the influence of the drug. These observations are in accordance with the results of our earlier research (Maślanka and Jaroszewski, in press) in which no effect of meloxicam on the absolute number of $CD25^{low}CD4^{+}$ and $CD25^{-}CD4^{+}$ T cells was found, while demonstrating that a longer (48 and 168 h) culture in the presence of the drug can increase the number of $CD25^{high}CD4^{+}$ T cells.

Finally, it should be noticed that in the context of the influence of the tested drugs on proliferation of bovine $CD4^{+}$ T lymphocytes, meloxicam seems to be safer than dexamethasone to be used in cattle with suppressed immunity or suffering from bacterial or viral infections.

References

Blache C, Adriouch S, Calbo S, Drouot L, Dulauroy S, Arnoult C, Le Corre S, Six A, Seman M, Boyer O (**2009**) Cutting edge: CD4-independent development of functional $FoxP3^{+}$ regulatory T cells. J Immunol 183: 4182--4186.

Chen X, Oppenheim JJ, Winkler-Pickett RT, Ortaldo JR, Howard OM (**2006**) Glucocorticoid amplifies IL-2-dependent expansion of functional $FoxP3^{+}CD4^{+}CD25^{+}$ T regulatory cells *in vivo* and enhances their capacity to suppress EAE. Eur J Immunol 36: 2139-2149.

Maeda Y, Tanaka R, Ohtsuka H, Matsuda K, Tanabe T, Oikawa M (**2011**) Comparison of the immunosuppressive effects of dexamethasone, flunixin meglumine, and meloxicam on the *in vitro* response of calf peripheral blood mononuclear cells. J Vet Med Sci 73: 957-960.

Makita S, Kanai T, Oshima S, Uraushihara K, Totsuka T, Sawada T, Nakamura T, Koganei K, Fukushima T, Watanabe M (**2004**) $CD4^{+}CD25^{bright}$ T cells in human intestinal lamina propria as regulatory cells. J Immunol 173: 3119-3130.

Maślanka T, Jaroszewski JJ (**2012**) *In Vitro* effects of dexamethasone on bovine $CD25^{+}CD4^{+}$ and $CD25^{-}CD4^{+}$ cells. Res Vet Sci 93: 1367-1379.

Maślanka T, Jaroszewski JJ. *In Vitro* effects of meloxicam on the number, Foxp3 expression, production of selected cytokines and apoptosis of bovine $CD25^{+}CD4^{+}$ and $CD25^{-}CD4^{+}$ cells. J Vet Sci, in press.