

DOI 10.2478/pjvs-2013-0031

Original article

Dexamethasone, but not meloxicam, suppresses proliferation of bovine CD25⁺CD4⁺ and CD25⁻CD4⁺ T cells

T. Maślanka, J.J. Jaroszewski

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Warmia and Mazury, Oczapowskiego 13, 10-718 Olsztyn, Poland

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 part-

ly 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

Correspondence to: T. Maślanka, e-mail: tomasz.maslanka@uwm.edu.pl, tel.: +48 89 523 3758

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 x 10⁶ 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 x 10⁻⁶ M) and at concentrations ten times lower (DEX 10⁻⁸ M, MEL 5 x 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 x 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⁺ (≈ 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

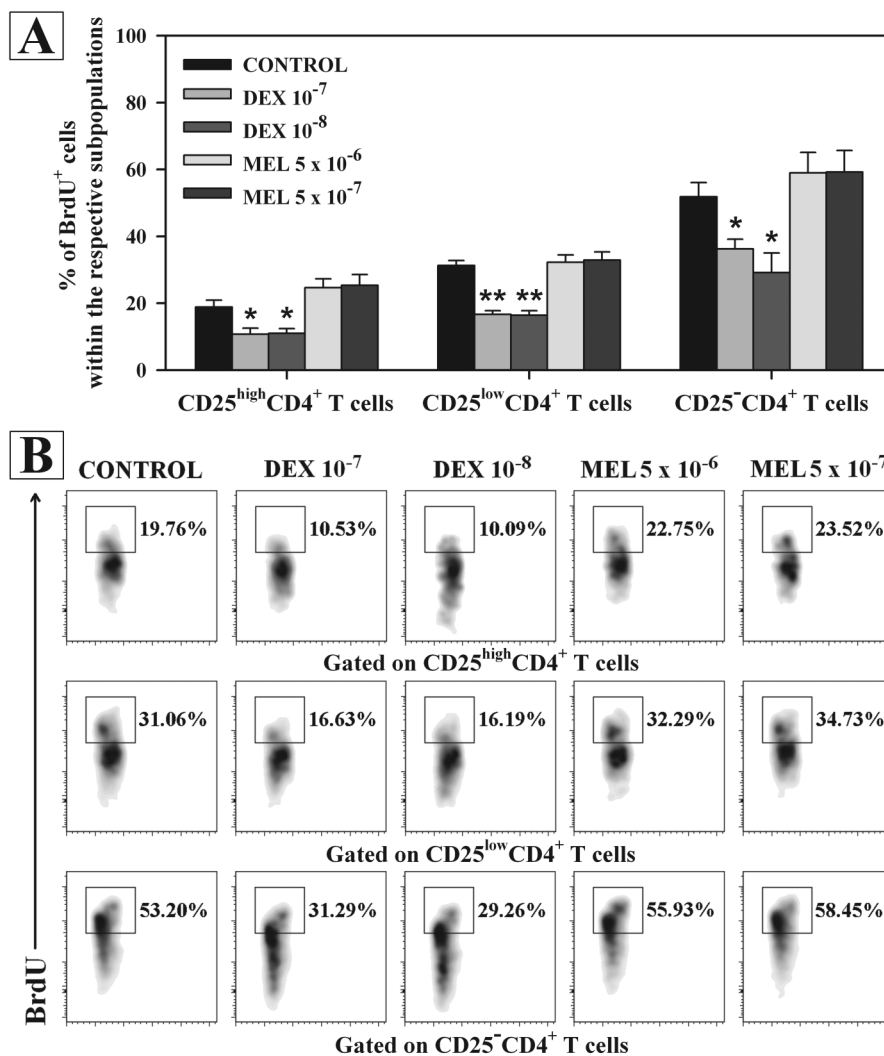


Fig. 1. (A) The effect of dexamethasone (DEX) and meloxicam (MEL) on the proliferation of CD25^{high}CD4⁺, CD25^{low}CD4⁺, and CD25⁻CD4⁺ T cells. The data are expressed as a percentage of 5-bromo-2'-deoxyuridine (BrdU)-positive cells within the CD25^{high}CD4⁺, CD25^{low}CD4⁺ and CD25⁻CD4⁺ T cell subpopulations. The results are the mean (\pm 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^{low}CD4⁺ and CD25^{high}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^{low}CD4⁺ T cells was completely abrogated by dexamethasone, whereas IL-2-induced proliferation of CD25^{high}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^{high}CD4⁺ regulatory T cells, but not CD25^{low}CD4⁺ T cells. Thus, a fundamental difference exists regarding the *in vitro* effects of dexamethasone on the proliferative capacity of CD25^{high}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^{high}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^{low}CD4⁺ and CD25^{high}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^{low}CD4⁺ and CD25^{high}CD4⁺ cells. *J Vet Sci*, in press.