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*Short communication*

# Prostaglandin E<sub>2</sub> inhibits IL-10 production by bovine CD4<sup>+</sup> T cells

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## Abstract

Although prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is a pro-inflammatory mediator, it also produces some effect which is anti-inflammatory in character. It is suggested that one of the mechanisms responsible for the latter effect is the increased synthesis of IL-10. The aim of this study has been to determine the influence of PGE<sub>2</sub> on IL-10 production by bovine CD4<sup>+</sup> and CD8<sup>+</sup> T cells and NK cells. With this aim, peripheral blood mononuclear cells collected from 12-month-old heifers (n = 10) were treated without or with PGE<sub>2</sub> (10<sup>-6</sup> M). Flow cytometric analysis showed that PGE<sub>2</sub> caused a reduction in the percentage of IL-10 producing CD4<sup>+</sup> T cells (P < 0.001), while leaving the secretion of this cytokine by CD8<sup>+</sup> T cells and NK cells unaffected. This seems to indicate that PGE<sub>2</sub> in cattle does not produce an anti-inflammatory effect by increasing the synthesis of IL-10; contrary to this, it may aggravate an inflammatory response by inhibiting the secretion of this cytokine by CD4<sup>+</sup> T cells.

**Key words:** prostaglandin E<sub>2</sub>, interleukin 10, T cells, NK cells, flow cytometry, bovine

## Introduction

Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is a ubiquitous eicosanoid which exerts a variety of physiological effects, for example it regulates multiple aspects of inflammation and multiple functions of different immune cells (Kalinski, 2012). On the one hand, PGE<sub>2</sub> demonstrates a pro-inflammatory effect by inducing local vasodilation as well as local attraction and activation of various immunocompetent cells (Phipps et al. 1991, Wang et al. 2007). On the other hand, there are several research results indicating that PGE<sub>2</sub> has certain anti-inflammatory and immunosuppressant properties, which may stem from induced production of IL-10 and indirect inhibition of the secretion of

pro-inflammatory cytokines (Phipps et al. 1991, Wang et al. 2007). Therefore, PGE<sub>2</sub> has the paradoxical status of a pro-inflammatory factor with some immunosuppressive activity (Kalinski 2012). It has been shown that PGE<sub>2</sub> increased production of IL-10 by dendritic cells (Harizi et al. 2002), splenocytes (Stolina et al. 2000) and whole blood cells (van der Pouw Kraan et al. 1995). Furthermore, it has been demonstrated that PGE<sub>2</sub> augmented IL-10 signaling and function (Cheon et al. 2006). The available references lack any data regarding the question whether PGE<sub>2</sub> affects secretion of IL-10 by CD4<sup>+</sup> and CD8<sup>+</sup> T cells and NK cells in human and animal subjects. Thus, it appeared worthwhile to undertake research on the effect of PGE<sub>2</sub> on production of IL-10 by these cells in cattle.

## Materials and Methods

Blood was collected from ten heifers (Hereford), aged 12 months, originating from a dairy farm located in Bałdy (Poland). The animals were housed and treated in accordance with the rules approved by the Local Ethics Commission (Ethic Commission Opinion No 82/2010). Peripheral blood mononuclear cells (PBMCs) were isolated by Histopaque 1.077 (Sigma-Aldrich, Munich, Germany) density gradient centrifugation. PBMCs were adjusted to a final concentration of  $3 \times 10^6$  cells/mL in complete medium and seeded in 24-well plates in 1 mL aliquots. Cells were pre-incubated for 1 h without (control) or with  $\text{PGE}_2$   $10^{-6}$  M (Sigma-Aldrich), followed by 9 h stimulation with Concanavalin A ( $5 \mu\text{g/mL}$ , Sigma-Aldrich) in the presence of brefeldin A ( $10 \mu\text{g/mL}$ , Sigma-Aldrich) during the last 5 h. The concentration of  $\text{PGE}_2$  was chosen on the basis of earlier reports on the effects of this eicosanoid on T and NK cells. Each experiment included 10 wells of PBMCs, which were obtained from ten individual heifers. Cells were removed from the wells by pipetting and rinsing with FACS buffer [FB, 1x Dulbecco's PBS with 2% (v/v) heat-inactivated FBS (Sigma-Aldrich)] and transferred into individual tubes and centrifuged. After additional washing in 2 mL FB, the cells were re-suspended in FB and stained with FITC-conjugated mouse anti-bovine CD4 (1:20, CC8, IgG2a) or with FITC-conjugated mouse anti-bovine CD8 (1:20, CC63, IgG2a) or with AF 488-conjugated mouse anti-bovine CD335 (1:20, AKS1, IgG1) (all from Serotec, Oxford, UK). After 45 min incubation, the cells were washed in 2 mL FB and fixed with 200  $\mu\text{L}$  2% paraformaldehyde in Dulbecco's PBS for 15 min on ice. Intracellular staining for IL-10 was performed as previously described (Maślanka and Jaroszewski 2013). Flow cytometry analysis was performed using a FACSCanto II cytometer (BD Biosciences). The data were analyzed and graphed using SigmaPlot software (version 12, Systat Software, Inc, Chicago, USA).

## Results and Discussion

Studies on the influence of  $\text{PGE}_2$  on various types of mouse and human cells [dendritic cells (Harizi et al. 2002), splenocytes (Stolina et al. 2000), whole blood cells (van der Pouw Kraan et al. 1995), THP-1 cell line (Cheon et al. 2006)] have proven that this eicosanoid is a strong inducer of the secretion of IL-10. The current results imply that this way of acting of  $\text{PGE}_2$  does not appear in bovine  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells and NK cells. It has been found that exposure

of PBMCs to  $\text{PGE}_2$  did not influence the secretion of IL-10 by  $\text{CD8}^+$  T and NK cells, while causing a significant reduction in the percentage of IL-10 producing  $\text{CD4}^+$  T cells ( $p < 0.001$ , Fig. 1). Our experiments, however, do not give us grounds to claim that this effect was a consequence of the direct influence of  $\text{PGE}_2$  on  $\text{CD4}^+$  T cells or else was of secondary character, i.e. it stemmed from the effect of the eicosanoid on other immunocompetent cells. Nonetheless, whether primary or secondary in nature, the observed effect suggests that one of the elements responsible for the pro-inflammatory properties of  $\text{PGE}_2$  in cattle can be a reduction in the production of IL-10, i.e. an anti-inflammatory and immunosuppressive cytokine, by  $\text{CD4}^+$  T cells. Theoretically,  $\text{PGE}_2$  synthesis inhibitors, i.e. nonsteroidal anti-inflammatory drugs

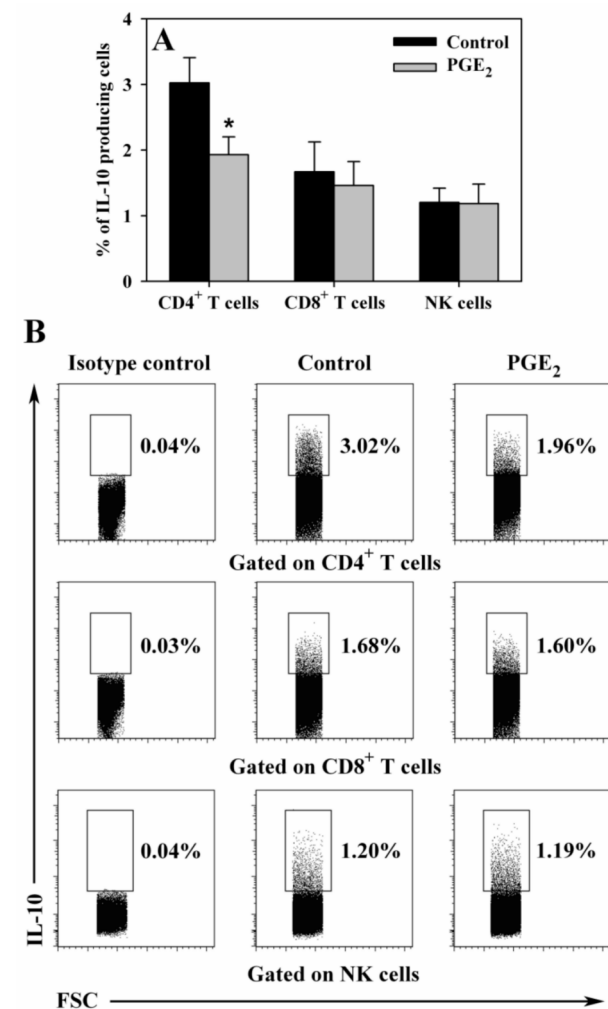


Fig. 1. Production of IL-10 by  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells as well as NK cells. Results are expressed as a percentage (A) of IL-10 producing cells among  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells and NK cells. Data are presented as a mean ( $\pm$  SD) of ten animals. \* $p < 0.001$ , treated cells versus control cells. Representative cytograms (B) illustrating IL-10 production by  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells as well as NK cells.

(NSAIDs), should produce opposite effects to those induced by PGE<sub>2</sub>. However, our earlier studies showed that meloxicam (NSAID) did not affect IL-10 production by bovine CD4<sup>+</sup> T cells (Maślanka and Jaroszewski 2013). These results suggest that the influence of NSAIDs on cytokine production does not have to be congruent with that which could be expected from their major mechanism of action, i.e. inhibition of the synthesis of PGE<sub>2</sub>.

In conclusion, regarding the effect of the studied eicosanoid on production of IL-10 by CD4<sup>+</sup> and CD8<sup>+</sup> T cells and NK cells, the results obtained herein do not confirm that PGE<sub>2</sub> in cattle has a paradoxical status, being a pro-inflammatory factor with some immunosuppressive activity, as it does in humans and mice (Kalinski 2012).

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