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Short communication

Prostaglandin E_2 inhibits IL-10 production by bovine $CD4^+$ T cells

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Abstract

Although prostaglandin E_2 (PGE_2) 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_2 on IL-10 production by bovine $CD4^+$ and $CD8^+$ 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_2 (10^{-6} M). Flow cytometric analysis showed that PGE_2 caused a reduction in the percentage of IL-10 producing $CD4^+$ T cells ($P < 0.001$), while leaving the secretion of this cytokine by $CD8^+$ T cells and NK cells unaffected. This seems to indicate that PGE_2 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 $CD4^+$ T cells.

Key words: prostaglandin E_2, interleukin 10, T cells, NK cells, flow cytometry, bovine

Introduction

Prostaglandin E_2 (PGE_2) 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_2 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_2 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_2 has the paradoxical status of a pro-inflammatory factor with some immunosuppressive activity (Kalinski 2012). It has been shown that PGE_2 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_2 augmented IL-10 signaling and function (Cheon et al. 2006). The available references lack any data regarding the question whether PGE_2 affects secretion of IL-10 by $CD4^+$ and $CD8^+$ T cells and NK cells in human and animal subjects. Thus, it appeared worthwhile to undertake research on the effect of PGE_2 on production of IL-10 by these cells in cattle.

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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 x 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 PGE_2 10^{-6} M (Sigma-Aldrich), followed by 9 h stimulation with Concanavalin A (5 µg/mL, Sigma-Aldrich) in the presence of brefeldin A (10 µg/mL, Sigma-Aldrich) during the last 5 h. The concentration of 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 individuals 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 µL 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 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 PGE_2 does not appear in bovine $CD4^+$ and $CD8^+$ T cells and NK cells. It has been found that exposure of PBMCs to PGE_2 did not influence the secretion of IL-10 by $CD8^+$ T and NK cells, while causing a significant reduction in the percentage of IL-10 producing $CD4^+$ 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 PGE_2 on $CD4^+$ 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 PGE_2 in cattle can be a reduction in the production of IL-10, i.e. an anti-inflammatory and immunosuppressive cytokine, by $CD4^+$ T cells. Theoretically, PGE_2 synthesis inhibitors, i.e. nonsteroidal anti-inflammatory drugs

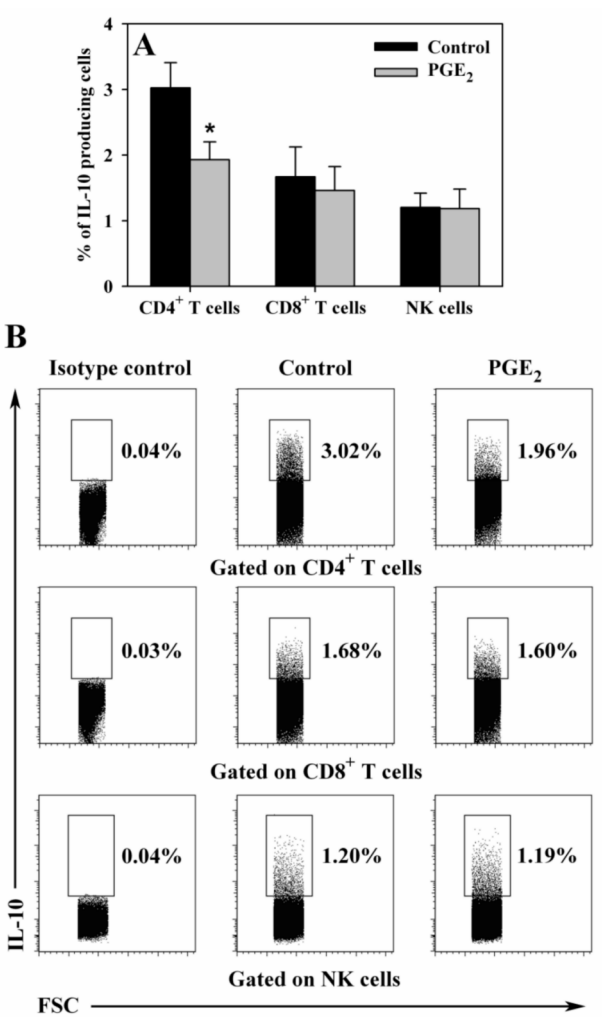

Fig. 1. Production of IL-10 by $CD4^+$ and $CD8^+$ T cells as well as NK cells. Results are expressed as a percentage (A) of IL-10 producing cells among $CD4^+$ and $CD8^+$ T cells and NK cells. Data are presented as a mean (± SD) of ten animals. *$p < 0.001$, treated cells versus control cells. Representative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

(NSAIDs), should produce opposite effects to those induced by PGE_2. However, our earlier studies showed that meloxicam (NSAID) did not affect IL-10 production by bovine $CD4^+$ 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_2.

In conclusion, regarding the effect of the studied eicosanoid on production of IL-10 by $CD4^+$ and $CD8^+$ T cells and NK cells, the results obtained herein do not confirm that PGE_2 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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