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Prostaglandin E_2 down-regulates the expression of CD25 on bovine T cells, and this effect is mediated through the EP4 receptor

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ABSTRACT

A crucial event in the initiation of an immune response is the activation of T cells, which requires IL-2 binding to its high-affinity IL-2 receptor for optimal signaling. The IL-2 receptor α-chain (CD25) is needed for the high affinity binding of IL-2 to effector cells and is potently induced after T cell activation. The aim of this research has been to determine whether prostaglandin E_2 (PGE_2) affects the CD25 expression on bovine T cells, and if it does, then which of the PGE_2 receptor (EP) subtype(s) mediate(s) this effect. Herein, we report that exposure of peripheral blood mononuclear cells (PBMC) to PGE_2 considerably reduces the percentage and absolute counts of $CD25^+CD4^+$, $CD25^+CD8^+$ and $CD25^+WC1^+$ T cells, significantly increases the value of these parameters with respect of $CD25^-CD4^+$, $CD25^-CD8^+$ and $CD25^-WC1^+$ T cells, and does not affect counts of the total populations of $CD4^+$, $CD8^+$ and $WC1^+$ T cells. These results indicate that PGE_2 down-regulates the CD25 expression on bovine T cells. Moreover, we show that the selective blockade of EP4 receptor, but not EP1 and EP3 receptors, prevents this effect. Interestingly, the exposure of PBMC to a selective EP2 receptor agonist leads to a substantial increase in the percentage and absolute number of $CD25^+CD4^+$, $CD25^+CD8^+$ and $CD25^+WC1^+$ T cells. In conclusions, the PGE_2-induced down-regulation of CD25 expression on bovine $CD4^+$, $CD8^+$ and $WC1^+$ T cells should be considered as immunosuppressive and anti-inflammatory action, because these lymphocytes primarily represent effector cells and adequate CD25 expression is essential for their correct functioning. The PGE_2-mediated down-regulation of the CD25 expression on bovine T cells is mediated *via* the EP4 receptor, although selective activation of the EP2 receptor up-regulates the CD25 expression on these cells. Thus, with respect to the effect of PGE_2 on the CD25 expression on bovine T cells, EP4 receptor serves as an inhibitory receptor, whereas EP2 receptor functions as a stimulatory receptor. The fact that non-selective

Abbreviations: CM, complete medium; CST, cytometry setup and tracking beads; EP receptors, E-type prostanoid receptors; FB, facs buffer; FITC, fluorescein isothiocyanate; IL-2R, IL-2 receptor; IL-2Rα/CD25, IL-2 receptor α chain; PE, phycoerythrin; PGE_2, prostaglandin E_2; RT, room temperature; Teffs, effector T cells; Tregs, regulatory T cells.

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stimulation of EP receptors, *i.e.* triggered by PGE_2, leads to weaker CD25 expression proves that inhibitory actions prevail over stimulatory ones. These results indicate the possibility of pharmacological manipulation of the CD25 expression on T cells *via* selective antagonists and agonists of EP2 and EP4 receptors.

1. Introduction

IL-2 is a pleiotropic cytokine which plays pivotal roles in orchestrating immune responses. IL-2 can act both in an autocrine and paracrine fashion by binding to the IL-2 receptor (IL-2R) (Létourneau et al., 2009). Three distinct receptor chains have been identified as components of IL-2R: α (IL-2α, CD25), β (IL-2β, CD122) and γ (IL-2γ, CD132) chains. The receptor is known to exist in three isoforms: the monomeric low-affinity receptor ($CD25^+CD122^-CD132^-$), the dimeric intermediate-affinity receptor ($CD25^-CD122^+CD132^+$), and the trimeric high affinity receptor ($CD25^+CD122^+CD132^+$); however, only the two latter ones are functional receptor forms (Liao et al., 2013). The dimeric intermediate-affinity receptor is expressed by naive and memory $CD4^+$ and $CD8^+$ T cells and NK cells (Létourneau et al., 2009). On the contrary, the trimeric high affinity receptor is expressed on recently activated T cells; CD25 is rapidly induced after T cell activation, increasing responsiveness to IL-2 (Liao et al., 2013). However, this molecule is not exclusively the marker of T lymphocyte activation because human and murine Foxp3-expressing $CD4^+$ regulatory T cells with constitutive CD25 expression are well known (Curotto de Lafaille and Lafaille, 2009).

A critical point in the development of an immune response is the activation of T lymphocytes that requires IL-2 binding to its high-affinity IL-2R for optimal signaling. Of crucial importance for the transmission of IL-2 signals into T cells is the expression of CD25, which, along with CD122 and CD132, confers high affinity binding to IL-2. Therefore, down-regulation of CD25 on activated Teffs represents strong immune suppression. There are a few reports in literature (Rincón et al., 1988; Anastassiou et al., 1992; Baratelli et al., 2005) indicating that prostaglandin E_2 (PGE_2) down-regulates the CD25 expression on human T cells. Although PGE_2 is typically perceived as a pro-inflammatory mediator, this eicosanoid actually shows a "Janus face" in terms of its effect on an inflammatory reaction because it exerts both pro- and anti-inflammatory effects (Konya et al., 2013). The effects of PGE_2 are mediated *via* four distinct G protein-coupled E-type prostanoid (EP) receptors, referred to as EP1, EP2, EP3 and EP4 receptors (Kalinski, 2012). All these receptor subtypes can be expressed by mouse T cells (Tilley et al., 2001). However, the results obtained by Boniface et al. (2009) indicate that in naive and activated human T cells, EP2 and EP4 receptors appear to be the most abundant, while the expression of EP1 and EP3 receptors by these cells is low. The influence of PGE_2 on the CD25 expression on T cells or other bovine immunocompetent cells remains unknown. However, our latest studies have revealed that exposure of bovine PBMC to meloxicam (non-steroidal anti-inflammatory drug), which is an inhibitor of PGE_2 synthesis, leads to a reduced expression of CD25 on $CD4^+$ (Maślanka and Jaroszewski, 2013a), $CD8^+$ (Maślanka et al., 2013) and γδ ($WC1^+$) T cells (Maślanka and Jaroszewski, 2013b). Because administration of a PGE_2 synthesis inhibitor causes reduction of the CD25 expression on bovine T cells, in cattle – contrary to humans – PGE_2 is implied to up-regulate CD25 expression. Consequently, the principal objective of this study has been to determine the effect of PGE_2 on the CD25 expression on main populations (*i.e.* $CD4^+$, $CD8^+$ and $WC1^+$) of bovine T cells. The experiments proved that this eicosanoid reduces the CD25 expression on the analyzed cells, hence the subsequent research aim was to identify the EP receptor subtype(s) which mediate(s) this effect.

2. Materials and methods

2.1. Animals

Blood was obtained from the jugular vein of clinically healthy Hereford heifers from the farm at the Research Station of the University of Warmia and Mazury, located in Bałdy (Poland).

2.2. Isolation of PBMC and culture conditions

Blood was collected into 10 mL heparinized sterile vacutainer tubes [Becton Dickinson (BD) Biosciences, San Jose, CA, USA]. PBMC were isolated by Histopaque 1.077 (Sigma-Aldrich, Munich, Germany) density gradient centrifugation at $400 \times g$ for 30 min at room temperature (RT). PBMC were recovered from the interface, washed ($300 \times g$ for 10 min at 4 °C; the same parameters were used for all cell-washing procedures) three times and re-suspended in complete medium [CM; RPMI 1640, 10% FBS, 10 mM HEPES buffer, 10 mM nonessential amino acids, 10 mM sodium pyruvate and 10 U/mL penicillin/streptomycin (all from Sigma-Aldrich)]. PBMC were adjusted to a final concentration of 4×10^6 cells/mL in CM and seeded in 24-well plates in 1 mL aliquots.

In order to determine whether PGE_2 affects the CD25 expression on T cells, PBMC were incubated for 24 h in the absence (control) or presence of PGE_2 (10^{-6} M; Sigma-Aldrich) with accompanying ConA stimulation (5 μg/mL; Sigma-Aldrich). Each experiment included five wells of PBMC (obtained from individual heifers) for each condition tested. Two experiments were performed, in each one using five different animals (overall $n = 10$).