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Opposing roles of TGF-β in prostaglandin production by human follicular dendritic cell-like cells

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ABSTRACT

Prostaglandins (PGs) are recognized as important immune regulators. Using human follicular dendritic cell (FDC)-like HK cells, we have investigated the immunoregulatory role of PGs and their production mechanisms. The present study was aimed at determining the role of TGF-β in IL-1β-induced cyclooxygenase-2 (COX-2) expression by immunoblotting. COX-2 is the key enzyme responsible for PG production in HK cells. TGF-β, when added simultaneously with IL-1β, gave rise to an additive effect on COX-2 expression in a dose-dependent manner. However, TGF-β inhibited IL-1β-stimulated COX-2 expression when it was added at least 12 h before IL-1β addition. The inhibitory effect of TGF-β was specific to IL-1β-induced COX-2 expression in HK cells. The stimulating and inhibitory effects of TGF-β were reproduced in IL-1β-stimulated PG production. Based on our previous results of the essential requirement of ERK and p38 MAPKs in TGF-β-induced COX-2 expression, we examined whether the differential activation of these MAPKs would underlie the opposing activities of TGF-β. The phosphorylation of ERK and p38 MAPKs was indeed enhanced or suppressed by the simultaneous treatment or pre-treatment, respectively. These results suggest that TGF-β exerts opposing effects on IL-1β-induced COX-2 expression in HK cells by differentially regulating activation of ERK and p38 MAPKs.

1. Introduction

Follicular dendritic cells (FDCs) are specialized stromal cells located in the primary and secondary follicles of peripheral lymphoid tissues (MacLennan, 1994). FDCs recruit B cells to the follicles, present unprocessed antigens, and provide a microenvironmental niche for the proliferation, differentiation, and selection of B cells (Allen et al., 2007). Some of the cellular interactions between FDCs and B cells are understood at the molecular terms. B cells home to the follicles in response to the B cell chemoattractant CXCL13 which FDCs secrete (Gunn et al., 1998). BAFF is another cytokine produced by FDC and plays important roles in B cell survival and differentiation (Mackay et al., 2003; Zhang et al., 2005). FDCs promote the germinal center (GC) reactions by providing IL-6 to GC B cells that are incapable of producing IL-6 (Burdin et al., 1996; Wu et al., 2009). IL-15 expressed on the surface of FDC supports GC B cell proliferation (Park et al., 2004). Using an in vitro experimental system containing FDC-like cells, we identified FDC as a source of prostaglandins (PGs) (Lee et al., 2005), the physiological significance of which was bolstered by in situ data (Lee et al., 2008). PGs released from FDC-like cells increase CD86 expression on B cells leading to augmented antigen-presenting capability (Kim et al., 2012b, 2011) and survival of GC B cells by preventing cell death (Kim et al., 2013). In an immune response model in vivo, PGI_2 indeed enhances the humoral immune response (Lee et al., 2013).

Paying attention to the emerging role of PGs as an immune regulator (Harris et al., 2002; Kalinski, 2012), we explored the production mechanism of PGs using FDC-like HK cells. Out of several stimuli inducing PG production in HK cells, the regulatory function of TGF-β was investigated extensively in our laboratory. TGF-β stimulated HK cells to increase expression of cyclooxygenase-2 (COX-2), the pivotal enzyme responsible for PG production (Cho et al., 2012). Smad3 and ERK were important intracellular molecules required for TGF-β-triggered COX-2 induction (Cho et al., 2015). In the present study, we demonstrate that TGF-β exhibits

Abbreviations: COX, cyclooxygenase; FDC, follicular dendritic cell; GC, germinal center; IL-1Ra, IL-1 receptor antagonist; MAPK, mitogen-activated protein kinase; mPGES-1, microsomal prostaglandin I synthase-1; PG, prostaglandin.

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opposing roles in IL-1β-induced COX-2 expression. TGF-β augments COX-2 expression when it is added simultaneously with IL-1β. In contrast, pre-treated TGF-β inhibits IL-1β-induced COX-2 expression. As an underlying mechanism, we provide evidence that TGF-β controls the COX-2 expression by differentially regulating phosphorylation of ERK and p38 mitogen-activated protein kinases (MAPKs). The current study reveals another regulatory aspect of TGF-β in the course of immune inflammation.

2. Materials and methods

2.1. Antibodies and other reagents

Following antibodies were used in this study. Antibodies specific to COX-1 and COX-2 were purchased from Santa Cruz Biotechnology, Inc. Specific antibodies against phosphorylated ERK, ERK, phosphorylated p38 MAPK, p38 MAPK were purchased from Cell Signaling Technology. Anti-β-actin antibody and LPS were obtained from Sigma-Aldrich. Secondary antibodies were horseradish peroxidase (HRP)-conjugated anti-mouse IgG (Southern Biotech), HRP-conjugated anti-rabbit IgG, and anti–goat IgG (Koma Biotech). IL-1β and TGF-β were purchased from R&D Systems.

2.2. Cell culture

HK cells were primary cells prepared as described by Kim et al. (Kim et al., 1994) and used until they exhibited degenerate features in culture. The purity of HK cells was assessed by a FACSCalibur (BD Biosciences) as reported recently (Kim et al., 2012a). Human skin fibroblast 1064SK cells were obtained from ATCC. Cell culture media was RPMI-1640 media (GIBCO) containing 10% fetal bovine serum (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin G (Sigma-Aldrich), and 100 μg/ml streptomycin (Life Technologies).

2.3. Immunoblotting

Harvested cells were washed with cold PBS and then lysed in pro-prep cell extract solution (Intron Biotechnology). Centrifugation of lysates at 15,000*g* for 10 min at 4 °C was conducted to remove insoluble materials, followed by protein quantification using a BCA protein assay kit (Thermo scientific). Protein in the lysates were separated on SDS-PAGE and transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 3% nonfat-dried milk for 2 h and then treated with specific antibodies. Visualization of the detected proteins was performed using chemiluminescent solution (Elpis biotech) and X-ray films. Densitometric analysis was carried out on the blots with the LabWorks image acquisition and analysis software (UVP). Results from three independent experiments were statistically analyzed by Prism 5.03 software (GraphPad); $p < 0.05$ were considered significant.

2.4. Enzyme immunoassay to measure PGs

The levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in cell culture supernatants were measured using enzyme immunoassay (EIA) kits according to the manufacturer's protocols (Cayman).

3. Results

3.1. The simultaneous addition of TGF-β and IL-1β to HK cells brings about an additive effect on COX-2 expression

In light of the reported activity of IL-1β to induce COX-2 in human fibroblasts (Farrajota et al., 2005; Yucel-Lindberg et al., 1995), we first examined whether IL-1β would stimulate COX-2 expression in HK cells. HK cells were cultured in the presence of IL-1β for 8 h, and then COX-2 expression levels were measured by the immunoblotting technique. As shown in Fig. 1, IL-1β significantly elevated the protein expression levels of COX-2 from as low as 1 pg/ml of concentration in a dose-dependent manner. We have previously observed that TGF-β increases the expression levels of COX-2 protein and mRNA in HK cells (Cho et al., 2012; Lee et al., 2008). To determine the impact of TGF-β on IL-1β-stimulated COX-2 expression, graded concentrations of TGF-β was added simultaneously with 1 pg/ml of IL-1β (Fig. 1A). TGF-β from the concentration of 0.1 ng/ml increased the COX-2 levels that were induced by IL-1β. The enhancing effect of TGF-β was reproduced when COX-2 expression was induced with higher concentrations of IL-1β (Fig. 1B and C). Since 1 ng/ml of TGF-β consistently augmented IL-1β-stimulated COX-2 expression, this concentration was used to assess the effect of TGF-β on COX-2 levels that were stimulated with different concentrations of IL-1β in a same set of experiments. TGF-β significantly augmented COX-2 expression levels that were induced by 1–10 pg/ml of IL-1β (Fig. 1D). The potentiating effect of TGF-β was specific to COX-2, and this cytokine did not modulate COX-1 expression levels. Of note, we observed the augmenting effect even when TGF-β was added 4 h after IL-1β stimulation (data not shown).

3.2. Pre-treated TGF-β inhibits IL-1β-induced COX-2 expression

Next, the effect of TGF-β on IL-1β-induced COX-2 induction was assessed by treating HK cells with TGF-β 12 h before IL-1β addition. Since COX-2 protein levels were measured after 8-h stimulation with IL-1β, HK cells were treated with TGF-β for 20 h at the point of cell harvest, when COX-2 expression levels were down-regulated to background levels even in the presence of 1 ng/ml of TGF-β alone (Fig. 2). Interestingly, TGF-β pre-treatment resulted in suppression rather than augmentation of IL-1β-triggered COX-2 protein levels. For example, pre-treated TGF-β at 0.1 ng/ml significantly and at 1 ng/ml nearly completely prohibited IL-1β from increasing COX-2 expression (Fig. 2A). The inhibitory activity of TGF-β was viable even HK cells were stimulated with higher concentrations of IL-1β up to 100 ng/ml (Fig. 2B and C). When we examined the effect of TGF-β on COX-2 expression induced by different doses of IL-1β in a same set of experiments, TGF-β consistently inhibited COX-2 expression induced by three different doses of IL-1β (Fig. 2D). TGF-β exerted the inhibitory effect on COX-2 but not COX-1 expression. These results indicate that TGF-β pre-treatment leads to inhibition of IL-1β-induced COX-2 expression. The minimum time required to observe the inhibition was determined by culturing cells with TGF-β for 6 or 12 h and then measuring the levels of IL-1β-induced COX-2 expression. Because HK cells were stimulated for 8 h with IL-1β for COX-2 immunoblotting, we consequently examined whether 14 or 20 h of incubation with TGF-β would affect COX-2 expression levels, which were at around the background levels. Six hours of pre-treatment with TGF-β did not either augment or inhibit IL-1β-induced COX-2 expression. The inhibitory effect was observed when HK cells were pre-treated with TGF-β for 12 or 24 h before the IL-1β addition (Fig. 3A and data not shown). We next examined whether TGF-β would display the inhibitory activity when COX-2 expression was induced by other stimulus than IL-1β. The effect of TGF-β pre-treatment was assessed on LPS-induced COX-2 expression since LPS consistently augmented COX-2 expression in HK cells (Cho et al., 2011; Lee et al., 2008). As shown in Fig. 3B, LPS stimulation of HK cells for 4 h resulted in a significant increase of COX-2 expression. The simultaneous addition of LPS and TGF-β for 4 h led to COX-2 levels that were higher than those achieved by either LPS or TGF-β