STAT6 and JAK1 are essential for IL-4-mediated suppression of prostaglandin production in human follicular dendritic cells: Opposing roles of phosphorylated and unphosphorylated STAT6

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Prostaglandins (PGs) are emerging as important immune mediators. Since our first report on the expression of prostacyclin synthase in the germinal centers, we have investigated production mechanisms and biological functions of PG using human follicular dendritic cell (FDC)-like cells. In the previous report, we observed that TGF-β enhances PG production, and IL-4 prevents this upregulation. To elucidate the inhibitory mechanism of IL-4, its effects on the key enzyme leading to PG production were analyzed in this study. IL-4 but not IL-10 inhibited TGF-β-induced COX-2 expression at both mRNA and protein levels. Next the early signaling molecules of IL-4 were identified by siRNA technology. IL-4 induced tyrosine phosphorylation of STAT1, 3, and 6, but only JAK1-STAT6 pathway was responsible for the prevention of COX-2 augmentation and PG production. Phosphorylated STAT6 accumulated in the nucleus rapidly upon IL-4 addition, and the complete inhibition of COX-2 upregulation required 24 h of pretreatment with IL-4, implying that newly transcribed molecules mediate the inhibitory signals downstream of STAT6. Interestingly, unphosphorylated STAT6 proteins were constitutively expressed in the nucleus, and depletion of STAT6 impaired background level expression of COX-2 and PGs. Our results highlight the crucial roles of TGF-β and IL-4 in the regulation of PG production, which lead us to suggest that T cells play an important role in FDC production of PGs.

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1. Introduction

Follicular dendritic cells (FDCs) are unique stromal cells found normally in the primary and secondary follicles of peripheral lymphoid organs and ectopically in the inflamed tissues of several chronic diseases such as rheumatoid arthritis [1]. In spite of the similar names, FDC is different from dendritic cell (DC) in their cellular origins, anatomic niches, and biological functions. FDCs derive from bone marrow mesenchymal stem cells, localize in the B cell areas of lymphoid organs, and present native antigens to B cells without phagocytosis [2]. In contrast, DCs originate from bone marrow hematopoietic stem cells, establish in the T cell areas of peripheral lymphoid organs, and present processed antigen peptides to T cells following phagocytosis [3]. FDCs are essential components of the humoral immune system, and the development of lymphoid tissues and production of high affinity antibodies are severely compromised in the absence of FDC [4,5]. However, the molecular mechanisms by which FDC plays the essential functions are largely unknown in part due to the practical difficulty in isolating enough FDCs to mimic the germinal center (GC) reactions in vitro. We have established a method of preparing FDC-like primary cells, HK, from human tonsils [6] and have reported several interesting observations previously. FDC and HK cells express prostacyclin synthase [7] and have a distinct prostacyclin production mechanism [8]. Prostacyclin enhances the APC capability of B cells by increasing CD86 expression levels [9]. In addition, we demonstrated that prostaglandins (PGs) produced by HK cells inhibit proliferation and apoptosis of T cells [10] and PG production from HK cells is controlled by the inhibitory effect of IL-4 on COX-2 expression [11]. These findings suggest that FDCs also interact with another cellular component of the GC, T cells, via PGs and cytokines.

Paying attention to the emerging concept of PGs as critical immune modulators [12–14], our laboratory conducted several experiments to understand the cellular and molecular mechanisms of
PG production regulation in the GC. We have previously demonstrated that both LPS and TGF-β stimulate PG production in HK cells, which is suppressed by IL-4 [10]. As an underlying mechanism, IL-4, via the JAK1-STAT6 pathway, inhibits COX-2 expression and PG secretion that is stimulated with LPS [11]. Although LPS and TGF-β share the feature of stimulating COX expression in HK cells, they trigger intracellular signaling by binding distinct receptors and via unconnected pathways. For example, LPS signaling involves innate immune receptors and MyD88-dependent pathway [15], whereas TGF-β signaling is associated with its cognate receptors and Smad molecules [16]. Therefore, we reasoned that IL-4 might regulate LPS- and TGF-β-induced COX-2 expression by different mechanisms. The current results reveal that IL-4 inhibits TGF-β-induced COX-2 expression and resultant PG production via the essential mediation of JAK1 and STAT6 molecules and that other STATs are not required for the inhibition. The critical role of STAT6 in this process is clearly demonstrated by the nuclear translocation and accumulation of phosphorylated STAT6 upon IL-4 addition. In addition, we observe that unphosphorylated STAT6 proteins are constitutively expressed in the nucleus, and depletion of STAT6 impairs background level COX-2 expression and PG production. The distinct roles of JAK1 and STAT6 in LPS- and TGF-β-induced COX-2 expression are discussed with the significance of our new findings.

2. Materials and methods

2.1. Culture of HK cells

HK cells are primary cells prepared on a regular basis from human tonsils which are obtained from children undergoing tonsillectomy at Asan Medical Center (Seoul, Korea). This study was approved by the Institutional Review Board of Asan Medical Center and written informed consent was obtained. HK cells at passages 5–8 were used to ensure purity. The purity and phenotype of typical HK cells are presented elsewhere [17]. They are prepared as described by Kim et al. [6] and maintained in RPMI-1640 (Irvine Scientific, Santa Ana, CA) containing 10% fetal calf serum (Hyclone, Logan, UT), 2 mM L-glutamine and maintained in RPMI-1640 (Irvine Scientific, Santa Ana, CA) with 10% FBS and 0.1% triton X-100. For each plate, HK cells were cultured with TGF-β for 48 h to harvest the supernatants. The amounts of PGE2 and 6-keto PGF1α, stable metabolites of COX-1, COX-2 (Cayman Chemical, Ann Arbor, MI), were measured using enzyme immunoassay (EIA) kits as described previously [10].

2.2. Immunoblotting

The whole cell lysates of HK cells were subject to immunoblotting as previously described [18]. The cytosol or nuclear fractions of HK cells were obtained as follows. Cell membranes were lysed by buffer A (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 0.5 mM PMSF, 0.5 mM leupeptin, 0.1 mM sodium vanadate, 50 mM sodium fluoride, 0.6% NP40, pH 7.9) at 4°C for 30 s, followed by centrifugation at 14,000 rpm for 5 min. The supernatants (cytosol fraction) were collected. The pellets were lysed by Proprep lysis buffer at 4°C for 30 min and then centrifuged at 14,000 rpm for 5 min to collect the supernatants (nuclear fraction). The protein concentrations of the each fraction were assayed with a BCA assay. Used antibodies were against COX-1, COX-2 (Cayman Chemical, Ann Arbor, MI), active and total forms of JAK1, JAK2, JAK3, TYK2, STAT1, STAT2, STAT3, STAT5, STAT6 (Cell Signaling Technology, Danvers, MA), β-actin (Sigma-Aldrich), HRP-conjugated anti-mouse IgG (Jackson Immunoresearch, West Grove, PA), and HRP-conjugated anti-rabbit IgG (KOMA Biotech, Seoul, Korea). The membranes were incubated with SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) and exposed to X-ray films.

2.3. Quantitative real-time PCR

After HK cells were cultured for 4 h, total RNA was purified using easy-BLUE™ RNA extraction kit (iNtron Biotechnology, Seongnam, Korea). cDNA synthesis from total RNA was performed using Oligo-dT (T) primers and MuLV reverse transcriptase (Roche, Indianapolis, IN). The following primers were used for PCR amplification: COX-2 forward (5′-CCCGCAGTACAAAGGATC-3′) and reverse (5′-ATTCA-TAGGCCCTTACGATA-3′), GAPDH forward (5′-CCTCCAAAT-CAAGTGGG-3′) and reverse (5′-GCCACAGRRCCGGAGG-3′). Quantitation of cDNA was accomplished by real-time, quantitative PCR (ABI7700; Applied Biosystems, Carlsbad, CA) using Taq™ SYBR®-Green Supermix With ROX (Bio-Rad, Hercules, CA). Cycling parameters were 53°C (COX-2) or 55°C (GAPDH) for 1 min and 72°C for 1 min. Calculations of expression were normalized using the Ct method; Ct is the cycle number of the detection threshold.

2.4. Confocal microscopy

HK cells were cultured to 80% confluence on 18 mm round cover slip in 12-well plate. Each well was incubated with IL-4 for indicated times, fixed in 4% parafomaldehyde for 30 min, and then blocked and permeabilized with PBS solution containing 10% FBS and 0.1% triton X-100. The slides were incubated with control, anti-STAT6, or anti-tyrosine phosphorylated STAT6 rabbit polyclonal antibodies (Cell Signaling Technology, Danvers, MA) and then stained with FITC-conjugated anti-rabbit IgG (Invitrogen, Carlsbad, CA) and propidium iodide for nuclear staining. The relative distribution of fluorochromes was visualized and scanned using a Fluoview FV1000 confocal laser microscope (Olympus, Tokyo, Japan).

2.5. siRNA transfection

The siRNA duplexes used (Ambion Inc, Austin, TX) were constructed with the following target sequences. Control (Neg-siRNA#2, sequence not disclosed by Ambion); JAK1, sense (5′-CCACCAACUGAUGACAAATT-3′), antisense (5′-UUUCAUCUAGCCGUAGG-3′); JAK2, sense (5′-CCACCGGAAUUUUGGGUAT-3′), antisense (5′-UACCCCAAAUUUCGGCGGT-3′); JAK3, sense (5′-GUAUCGGUGGUACCUAAT-3′), antisense (5′-AUAGGUGUAUGUGUUGGATG-3′); TYK2, sense (5′-CAUCCCAUUGGACAUAATT-3′), antisense (5′-UUUAUGUGCGAAUGUGAUG-3′); STAT1, sense (5′-CCACGGAACAGCGCUAT-3′), antisense (5′-AUAGGGUAUGUUCGUGGATG-3′); STAT3, sense (5′-GAUCCAAACGCGAAATT-3′), antisense (5′-GGTATCAUUCGGUUGGATG-3′). HK cells were cultured to 50–60% confluence in 100 mm plates. For each plate, 40 nm of each siRNA and 24 μl Lipofectamine™ (Invitrogen) were separately diluted in 400 μl serum-free medium without antibiotics, mixed together, and incubated at RT for 45 min. The plates were then washed with serum-free medium, added with 5 ml serum-free medium, and then with the diluted solutions. The plates were incubated at 37°C for 8 h, followed by the addition of a growth medium containing 10% serum. After 48 h of additional incubation, cells were used for experiments. The degree of gene-silencing was assayed by immunoblotting.

2.6. Enzyme immunoassay to measure prostaglandins

HK cells were cultured with TGF-β for 48 h to harvest the supernatants. The amounts of PG_E2 and 6-keto PGF1α, stable metabolite of PG_E2, were measured using enzyme immunoassay (EIA) kits as described previously [10].
2.7. Statistical analysis

Statistical analysis and graphic presentation were carried out with GraphPad Prism 4.0. Results are presented as means of triplicates plus SEM. The statistical significance of differences was determined by Student’s t-test; P<0.05 was considered significant.

3. Results

3.1. IL-4 represses TGF-β-induced COX-2 expression in HK cells

In order to understand the mechanism of controlling PG production in HK cells, we examined the effect of IL-4 on the key enzyme involved in PG generation, COX-2. Treatment of HK cells with TGF-β enhanced the expression of COX-2 but not COX-1 protein, which was prevented by IL-4 pretreatment (Fig. 1A). The inhibitory effect was specific to IL-4 because such an effect was not observed with IL-10 pretreatment. Dose-response experiments revealed that IL-4 exhibited the inhibitory effect from 10 U or 0.125 ng/ml of concentration in a dose-dependent manner, whereas IL-10 did not modulate COX-2 levels up to 50 ng/ml (Fig. 1B). By adding IL-4 at different time points before TGF-β stimulation, the duration of IL-4 stimulation that was required to bring out the inhibitory effect was determined. The impact of IL-4 was demonstrated from the simultaneous addition with TGF-β. Twenty-four hours of pre-incubation with IL-4 completely inhibited COX-2 up-regulation, and TGF-β stimulation did not increase COX-2 expression beyond the background levels. The prevention of COX-2 up-regulation indeed resulted from IL-4 treatment but not from a certain resistance to TGF-β in HK cells due to the prolonged culture period, because the control cultures that were maintained parallel for the same period without IL-4 responded normally to TGF-β by increasing COX-2 (Fig. 1C). The effect of IL-4 on COX-2 was examined at mRNA levels by real-time RT-PCR analysis. HK cells responded to TGF-β stimulation by increasing COX-2 mRNA about 10-fold over the media control, which was clearly inhibited by IL-4 but not IL-10 (Fig. 1D). These results indicate that IL-4 represses TGF-β-induced COX-2 expression in HK cells from the transcription stage.

3.2. JAK1 and STAT6 mediate the inhibitory effect of IL-4

Since cytokines generally utilize the JAK-STAT pathway to transduce the signals coming from membrane-bound cytokine receptors, we explored which STAT proteins would mediate the inhibitory effect of IL-4 in HK cells. We have recently demonstrated the essential requirement for STAT6 in the inhibitory effect of IL-4 [11]. However, the previous study does not exclude the possibility that IL-4 mobilizes other STAT molecules in addition to STAT6 to display its inhibitory effect. First, we examined whether HK cells express all the STAT molecules. All the six STATs except STAT4 were expressed in considerable amounts in HK cells. Peripheral blood mononuclear cells were used to ensure the integrity of STAT4 reagents. Compared with blood mononuclear cells, the expression levels of STAT4 in HK cells were very low. None of them was found in tyrosine-phosphorylated forms before IL-4 stimulation, but IL-4 induced slight phosphorylation of STAT1 and STAT3 and strong phosphorylation of STAT6 (Fig. 2A). The weak phosphorylation of STAT1 and STAT3 was specific to IL-4 stimulation since they were potently phosphorylated by IFN-α and IFN-γ. In contrast, IL-12 failed to induce phosphorylation of any STAT molecules. Consistent with the common inhibitory effect on PG production from HK cells [10], IL-13 induced phosphorylation of STAT1, STAT3, and STAT6 in similar patterns to IL-4. Because we used culture supernatants that contained recombinant IL-4, we adopted an IL-4-blocking antibody in the experiment. The cytokine specificity of IL-4 was proven by the results that IL-4-neutralizing antibody completely abolished the activity of IL-4 but not IL-13 (Fig. 2B).

STATs are tyrosine-phosphorylated by JAKs. To determine the JAK that phosphorylates STATs upon IL-4 stimulation, the four JAK family members were considered. In order to understand the mechanism of controlling PG production in HK cells, we examined the effect of IL-4 on the key enzyme involved in PG generation, COX-2. Treatment of HK cells with TGF-β enhanced the expression of COX-2 but not COX-1 protein, which was prevented by IL-4 pretreatment (Fig. 1A). The inhibitory effect was specific to IL-4 because such an effect was not observed with IL-10 pretreatment. Dose-response experiments revealed that IL-4 exhibited the inhibitory effect from 10 U or 0.125 ng/ml of concentration in a dose-dependent manner, whereas IL-10 did not modulate COX-2 levels up to 50 ng/ml (Fig. 1B). By adding IL-4 at different time points before TGF-β stimulation, the duration of IL-4 stimulation that was required to bring out the inhibitory effect was determined. The impact of IL-4 was demonstrated from the simultaneous addition with TGF-β. Twenty-four hours of pre-incubation with IL-4 completely inhibited COX-2 up-regulation, and TGF-β stimulation did not increase COX-2 expression beyond the background levels. The prevention of COX-2 up-regulation indeed resulted from IL-4 treatment but not from a certain resistance to TGF-β in HK cells due to the prolonged culture period, because the control cultures that were maintained parallel for the same period without IL-4 responded normally to TGF-β by increasing COX-2 (Fig. 1C). The effect of IL-4 on COX-2 was examined at mRNA levels by real-time RT-PCR analysis. HK cells responded to TGF-β stimulation by increasing COX-2 mRNA about 10-fold over the media control, which was clearly inhibited by IL-4 but not IL-10 (Fig. 1D). These results indicate that IL-4 represses TGF-β-induced COX-2 expression in HK cells from the transcription stage.

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proteins were efficiently silenced by siRNA technology (Fig. 3A). In a stark contrast to JAK2, JAK3, and TYK2, the silencing of JAK1 almost completely prevented IL-4-induced phosphorylation of STAT1, STAT3, and STAT6 (Fig. 3B), suggesting the essential role for JAK1 in IL-4 signaling in HK cells. Next we examined whether those three STATs are all involved in the inhibitory effect of IL-4. As shown in Fig. 4, TGF-β up-regulated COX-2 expression in HK cells that were transfected with control siRNA, which was inhibited by IL-4 almost to background levels. However, the inhibitory effect of IL-4 was not observed when JAK1 or STAT6 were silenced, indicating the essential role for these proteins. In contrast, the inhibitory effect of IL-4 was viable in HK cells that were transfected with STAT1 or STAT3 siRNAs. This result implies that STAT1 and STAT3 may participate in other activities of IL-4 than COX-2 inhibition in HK cells. It is interesting to note that TGF-β-stimulated COX-2 up-regulation was inhibited consistently when STAT6 was silenced in HK cells. The results that a prolonged pretreatment of HK cells with IL-4 up to 24 h resulted in a more evident inhibitory effect (Fig. 1C) suggest that IL-4 inhibits TGF-β signals by inducing transcription of certain proteins in the nucleus. As an initial test of this hypothesis, we performed immunoblotting and confocal microscopic analyses to examine whether phosphorylated STAT6 (P-STAT6) indeed migrates to the nucleus. STAT6 proteins were distributed in both cytoplasm and nucleus of HK cells as unphosphorylated forms prior to IL-4 stimulation (Fig. 5A and B). STAT6 proteins became phosphorylated and were observed in the nucleus as early as 5 min after IL-4 addition.
of P-STAT6 were maintained until 30 min and then gradually declined. The staining by anti-STAT6 and anti-P-STAT6 was specific because staining by control antibody was negative (data not shown). These results imply that STAT6 is an important transcription factor mediating the inhibitory IL-4 signal in HK cells.

3.3. IL-4 suppresses PG production via JAK1-STAT6 pathway

Based upon the results that JAK1-STAT6 axis conveys IL-4 signals in HK cells, we asked whether JAK1 and STAT6 proteins indeed would mediate IL-4 signals leading to the ultimate suppression of PG production. These molecules were knocked down by siRNA technology, and the effects on PG production were evaluated by EIA kits. HK cells that were transfected with control siRNA responded to TGF-β by giving rise to 4.3- and 1.5-fold increases of PGE2 and 6-keto-PGF1α, respectively. This PG induction was almost completely prevented by IL-4 pretreatment (Fig. 6), in line with the potent inhibition of COX-2 expression by IL-4 (Fig. 1). TGF-β stimulation of HK cells that were transfected with JAK1 siRNA also resulted in 3.5- and 1.5-fold increases of PGE2 and 6-keto-PGF1α, respectively. However, IL-4 failed in inhibiting the PG induction in these cells. After transfection of HK cells with STAT6 siRNA, we observed that the production of PGE2 and 6-keto-PGF1α was severely diminished. The concentrations of PGE2 and 6-keto-PGF1α were only 30% and 17%, respectively, of those in control cells. These results are compatible with the impaired expression of COX-2 in HK cells transfected with STAT6 siRNA (Fig. 4). Nevertheless, the addition of TGF-β gave rise to 10.3-fold and 6.2-fold increases of PGE2 and 6-keto-PGF1α, respectively, which was unaffected by IL-4 pretreatment. Taken together, our data indicate that JAK1 and STAT6 are the early signaling molecules of IL-4 leading to the suppression of PG production in HK cells.

4. Discussion

TGF-β was used in this study to induce PG production. TGF-β is a well-known cytokine that displays pleiotropic effects on the immune cells by regulating their generation, survival, proliferation, differentiation, and apoptosis [16,19,20]. The cellular sources of TGF-β include GC T cells [21] as well as Foxp3+ Treg cells, CXCR5+ T follicular helper (Tfh) cells, interleukin-10 (IL-10)-producing T regulatory-1 (Tr1) cells, DCs, stromal cells, B cells, and endothelial cells [22]. The immunoregulatory impact of TGF-β on GC B cells has been demonstrated previously. For example, it is the best characterized switching factor to IgA isotypes [23,24]. Strong increase of smad1 that is involved in TGF-β signaling is observed in GC B cells [25]. TGF-β also induces apoptosis in human centroblasts [26]. Although Lee et al. suggested a protective role for TGF-β in the apoptosis of FDC [27], the physiological importance of TGF-β in FDC functions is unclear. Using an in vitro experimental model containing primary FDC-like cells, we suggested that TGF-β induces PG production from FDC and then secreted PG inhibits proliferation and apoptosis of T cells [10]. PG also enhances the APC capability of B cells by increasing CD86 expression levels [9]. The immunostimulatory effect of TGF-β on FDC appears to be counterbalanced by IL-4 in the manner as presented in the current investigation. However, TGF-β may have a dominant effect over IL-4 on FDC, considering the findings that FDC requires pretreatment with IL-4 to display its inhibitory function and that the simultaneous stimulation with TGF-β and IL-4 results in only a partial inhibitory activity. The dominant effect of TGF-β over IL-4 was also reported in other immune cells [28].

We have herein shown that STAT1, 3, and 6 are tyrosine-phosphorylated upon IL-4 stimulation and the phosphorylation of these three molecules is abrogated by JAK1 knockdown. But the
phosphorylation degrees of STAT1 and STAT3 were much weaker than that of STAT6, and knockdown of these two proteins did not affect the inhibitory activity of IL-4 on COX-2 upregulation. These results indicate that IL-4 signaling to PG regulation involves the JAK1-STAT6 pathway but does not utilize STAT1 and STAT3 proteins. Then what molecule mediates IL-4 signals between STAT6 and COX-2? We do not know the identity yet, but it may be a molecule newly transcribed through the action of phosphorylated STAT-6 (P-STAT6). Supportive of this speculation, pretreatment is required for IL-4 to exhibit the complete inhibition of COX-2 upregulation. In addition, P-STAT6 accumulated in the nucleus right after IL-4 stimulation. Regarding the biological function of STAT6, it is worthwhile to note the observations that unphosphorylated STAT6 (U-STAT6) molecules were dispersed in the nucleus as well as in the cytoplasm of HK cells before IL-4 stimulation and that STAT6 knockdown drastically disturbed TGF-β-stimulated COX-2 upregulation and background PG production. These observations imply that U-STAT6 plays a role in the nucleus distinct from that of P-STAT6 because P-STAT6 accumulates in the nucleus only after IL-4 addition. U-STAT6 may contribute to the constitutive expression of COX-2 proteins at the background levels, explaining why STAT6 knockdown hampers COX-2 upregulation since TGF-β-induced COX-2 upregulation is not disturbed by silencing of STAT1, STAT3, or JAK1 and STAT6 depletion does not affect COX-2 upregulation that is induced by LPS [11]. In contrast, JAK1 depletion disturbs LPS-stimulated COX-2 induction [11], suggesting that JAK1 and STAT6 appear to be involved in LPS- or TGF-β-stimulated PG production in distinct manners. TGF-β does not induce phosphorylation of STAT6 in HK cells (our unpublished observations). Taken together, P-STAT6 and

Fig. 5. IL-4 induces nuclear translocation of phosphorylated STAT6 in HK cells. (A) HK cells (3×10⁵ cells) were cultured in the presence or absence of IL-4 (100 U/ml) for indicated time periods. Collection of cytosol and nuclear fractions of cultured cells and subsequent immunoblotting were carried out as described in Materials and methods. Representative of three reproducible experiments. (B) HK cells (1×10⁴ cells) were cultured in the presence of IL-4 (100 U/ml) for indicated time periods and then analyzed by a confocal microscope after staining with FITC-conjugated anti-STAT6 or anti-tyrosine phosphorylated STAT6 antibodies and propidium iodide. Scale bars, 50 μm. Representative results of two reproducible experiments.
U-STAT6 appear to have opposing roles in the expression of COX-2; P-STAT6 inhibits COX-2 expression whereas TGF-β-induced COX-2 upregulation depends on the presence of U-STAT6. In line with our speculation, Cui et al. reported that U-STAT6 associates with p300 and binds to the COX-2 GAS element, resulting in constitutive COX-2 transcription in human non-small cell lung cancer cells [29]. The contribution of unphosphorylated STAT3 to oncogenesis has been observed [30]. Our study implies that U-STATs may play significant roles also in normal cells.

In summary, our study provides evidence that JAK1 and STAT6 may be universal mediators of IL-4 in the suppression of PG production in human FDCs. Both molecules are required for the inhibitory activity of IL-4 irrespective of PG production stimuli; TGF-β or LPS. Our findings reveal that IL-4 represses TGF-β or LPS-induced COX-2 expression via JAK1-STAT6 pathway, subsequently leading to the suppression of PG production. The cellular and molecular mechanisms revealed here suggest that T cells play an important role in the regulation of PG production from FDC by timely providing counteracting cytokines, TGF-β and IL-4, in line with the recent reports on the crucial impact of follicular Treg on GC reactions by several investigators [31–33].

Acknowledgments

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Fig. 6. IL-4 suppresses TGF-β-stimulated prostaglandins production in HK cells via JAK1-STAT6 pathway. Control, JAK1, or STAT6 siRNA-transfected HK cells (1×10⁶ cells) were cultured in the presence or absence of IL-4 (100 U/ml) for 24 h, followed by a further incubation with or without TGF-β (1 ng/ml) for 48 h. The amounts of PGE₂ and 6-keto-PGF₁α in the culture supernatants were measured by EIA. Representative results of at least three reproducible experiments. Asterisks indicate significant differences (**p<0.01, ***p<0.001, NS, non-significant).

References


