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IL-27 affects helper T cell responses via regulation of PGE_2 production by macrophages

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ABSTRACT

IL-27 is a heterodimeric cytokine that regulates both innate and adaptive immunity. The immunosuppressive effect of IL-27 largely depends on induction of IL-10-producing Tr1 cells. To date, however, effects of IL-27 on regulation of immune responses via mediators other than cytokines remain poorly understood. To address this issue, we examined immunoregulatory effects of conditional medium of bone marrow-derived macrophages (BMDMs) from WSX-1 (IL-27Rα)-deficient mice and found enhanced IFN-γ and IL-17A secretion by $CD4^+$ T cells as compared with that of control BMDMs. We then found that PGE_2 production and COX-2 expression by BMDMs from WSX-1-deficient mice was increased compared to control macrophages in response to LPS. The enhanced production of IFN-γ and IL-17A was abolished by EP2 and EP4 antagonists, demonstrating PGE_2 was responsible for enhanced cytokine production. Murine WSX-1-expressing Raw264.7 cells (mWSX-1-Raw264.7) showed phosphorylation of both STAT1 and STAT3 in response to IL-27 and produced less amounts of PGE_2 and COX-2 compared to parental RAW264.7 cells. STAT1 knockdown in parental RAW264.7 cells and STAT1-deficiency in BMDMs showed higher COX-2 expression than their respective control cells. Collectively, our result indicated that IL-27/WSX-1 regulated PGE_2 secretion via STAT1–COX-2 pathway in macrophages and affected helper T cell response in a PGE_2-mediated fashion.

1. Introduction

IL-27 is a novel heterodimeric cytokine consisting of EBI-3 and p28 which are structurally related to the IL-12/IL-23 subunits p40 and p35/p19, respectively [1]. IL-27 is produced by activated antigen presenting cells (APCs) including dendritic cells (DCs) and macrophages [2–4]. IL-27 signals through its heterodimeric receptor consisting of WSX-1 and gp130 [2–4]. Initial studies demonstrated that IL-27 plays a role in Th1 induction and enhances proliferation of naïve $CD4^+$ T cells [1–4]. Recent studies, however, have demonstrated the anti-inflammatory function of IL-27 by generating Tr1 cells [5]. In addition to its regulation of T cell function, IL-27 also regulates APCs in an autocrine manner [4].

Prostaglandins (PGs) are a group of biologically active lipid mediators that are derived from arachidonic acid, and mediate a variety of functions. While cyclooxygenase (COX)-1 constitutively exists and mediates PG production in various tissues/organs, COX-2 is induced in immune cells, such as macrophages, by stimulation including growth factors, and mediates PG production in inflammatory venues [6]. Among various PGs produced, PGE_2 has a dominant role in inflammation/immune response [7,8]. Interestingly, recent reports have demonstrated that PGE_2 promotes Th1 differentiation and Th17 expansion *in vitro* via its receptors, EP2 and EP4 [9,10]. Regulation of PGs, especially PGE_2, may thus be a potent target of anti-inflammation strategies.

To date, functions of IL-27 on lipid mediators are poorly understood. In this study, we firstly demonstrated that lack of IL-27 signaling resulted in enhanced production of PGE_2 by bone marrow-derived macrophages (BMDMs), which led to increased production

Abbreviations: DC, dendritic cell; PG, prostaglandin; COX, cyclooxygenase; BMDM, bone marrow-derived macrophage; BM, bone marrow; CM, conditioned medium; mPGES-1, microsomal prostaglandin E_2 synthase-1; cPGES-1, cytosolic prostaglandin E_2 synthase; H-PGDS, hematopoietic prostaglandin D synthase; APCs, antigen presenting cells.

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of IL-17A and IFN-γ by $CD4^+$ T cells. We also demonstrated that IL-27 negatively regulated COX-2 expression in a STAT1-dependent way. These findings clearly showed that IL-27 regulates PGE_2 production by macrophages and "indirectly" regulates Th1/17 differentiation. IL-27 modulation of PGE_2 production is a novel mechanism by which IL-27 shows its anti-inflammatory/immunosuppressive function.

2. Materials and methods

2.1. Mice

Female *WSX-1*$^{-/-}$ mice were generated as described previously [11]. Female *STAT1*$^{-/-}$ mice were provided by Dr. Yoshimura (Keio University, Japan). C57BL/6J mice as a control were purchased from Japan CLEA, Inc. Mice were maintained under specific pathogen-free conditions and used between 8 and 14 weeks of age. All experiments were approved by the Animal Care and Use Committee at Nippon Boehringer Ingelheim Co., Ltd. or Saga University.

2.2. Reagents

Recombinant murine (rm) M-CSF was purchased from eBioscience. rmIL-27 was purchased from R&D systems. LPS (Re595) was purchased from Sigma–Aldrich. Indomethacin, NS-398, GW627368X, AH6809, anti-COX-1, anti-mPGES-1 were purchased from Cayman Chemicals. SC-58125, anti-β-actin, anti-pSTAT1 were purchased from Santa Cruz Biotechnology. Anti-COX-2, anti-pSTAT3, anti-STAT1 and anti-STAT3 were purchased from Cell Signaling Technology. Anti-CD3ε (145-2C11) and anti-CD28 (37.51) were purchased from BD Biosciences.

2.3. Cells preparation

BMDMs were prepared from bone marrow (BM) suspension of femurs and tibias of mice. Briefly, BM cells were cultured in RPMI1640 supplemented with 10% FBS and 10 ng/ml murine M-CSF for 6 days and used as macrophages. Naïve $CD4^+$ T cells were isolated from splenocytes of C57BL/6J mice by MACS beads, $CD4^+$-$CD62L^+$ T Cell Isolation Kit II, mouse (Miltenyi Biotech). The murine macrophage cell line RAW264.7 cells were obtained from the RIKEN Cell Bank (Japan).

2.4. LPS stimulation of macrophages

BMDMs (2×10^5 cells or 4×10^4 cells/well) were seeded at 24- or 96-well plate, respectively. Cells were pretreated with or without indomethacin or COX-2 inhibitors (NS-398, SC-58125) for 10 min, and then stimulated with LPS for additional 20 h. Culture supernatants and cells were collected and used for qPCR, Western blotting, cytokine and PGE_2 assay.

2.5. $CD4^+$ T cells culture with conditional medium of BMDMs

Naïve $CD4^+$ T cells (5×10^4 cells/well) were cultured with conditioned medium (CM) of BMDM in the presence of anti-CD3ε (precoated, 1 μg/ml) and anti-CD28 (0.5 μg/ml). Cells were pretreated with or without EP2 antagonist (AH6809) or EP4 antagonist (GW627368X) for 10 min. After 4 days culture, culture supernatants were collected and used for cytokine ELISA.

2.6. Establishment of mWSX-1-expressing RAW264.7 cells

RAW264.7 cells were transfected with murine WSX-1-FLAG plasmid by Lipofectamine 2000 (Life technologies). After 2 days, transfected cells were selected by resistance to neomycin (G418, Sigma–Aldrich) for more than 2 weeks.

2.7. siRNA transfection to RAW264.7 cells

RAW264.7 cells (2×10^4 well/well) were transfected with 30 nM siRNA (Qiagen) for mouse STAT1 (pools of SI02668862, SI02688763, SI02710729, SI02735054) and STAT3 (pools of SI01435294, SI01435301, SI01435287, SI01435308) by Lipofectamine RNAiMAX (Life technologies). AllStars Negative Control siRNA was used as a negative control.

2.8. PGE_2 and cytokine assays

PGE_2 was measured by homogeneous time resolved fluorescence (HTRF) assay (Cisbio Bioassay). Cytokines were determined by ELISA. ELISA kits for IL-17A, IFN-γ, and IL-4 were from eBioscience and for TNF-α, IL-6 and IL-12p40 were from R&D systems.

2.9. Quantification of eicosanoids

BMDMs (2×10^5 cells/well) were stimulated with LPS for 20 h. Supernatants were collected and stored at −80 °C. The amounts of eicosanoids were measured as described previously [12]. In brief, the samples were diluted with 2 ml of methanol and 7 ml of water containing 0.1% formic acid containing a mixture of deuterium-labeled eicosanoids as internal standards, and then loaded on Oasis HLB cartridges (Waters). The column was washed with 1 ml of water, 1 ml of 15% methanol, 1 ml of petroleum ether, and then eluted with 0.2 ml of methanol containing 0.1% formic acid. Eicosanoids levels were quantified by reversed phase HPLC–electrospray ionization-tandem mass spectrometry method.

2.10. Western blotting analysis

Cells (2×10^5 cells) were lysed and samples were boiled and electrophoresed with 10–20% polyacrylamide SDS–PAGE, transferred to PVDF membrane (Bio-Rad), and blotted with primary antibodies followed by HRP-conjugated secondary antibodies. Immunoblots were visualized with Western Lightning Plus ECL (Perkin Elmer) and densitometric analysis was performed using an image analyzer LAS-3000 (Fujifilm).

2.11. RNA isolation and PCR

Total RNA was extracted with RNeasy mini kits (Qiagen) and was reverse transcribed using PrimeScript RT reagent (Takara Bio) and analyzed for gene expression by RT-PCR or quantitative real-time PCR using an ABI PRISM 7000 (Applied Biosystems). The level of gene expression was normalized to GAPDH expression.

2.12. Plasmid construct

For the STAT1-FLAG and WSX-1-FLAG, murine STAT1 and murine WSX-1 cDNAs were amplified and ligated into pCMV14-3xFLAG (Sigma).

2.13. Statistical analysis

Data were presented as the mean ± SEM. Student's *t* test was used to determine the significance of differences.

3. Results

3.1. WSX-1-deficient BMDM conditioned medium promoted IFN-γ and IL-17A secretion by $CD4^+$ T cells

LPS stimulation of BMDMs induced expression of IL-27 subunits, EBI-3 (peak at around 12 h after stimulation) and p28 (peak