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15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits IL-13 production in T cells via an NF-κB-dependent mechanism

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ABSTRACT

Interleukin (IL)-13 is a cytokine produced by activated $CD4^+$ T cells that plays a critical role in promoting allergic responses and tumor cell growth. The 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is a natural ligand for the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR-γ), a known regulator of anti-inflammatory activities. We determined the effects of 15d-PGJ_2 on IL-13 expression in the Jurkat E6.1 T-cell line and in peripheral blood mononuclear cells. Semi-quantitative reverse transcription-polymerase chain reaction and enzyme-linked immunosorbent assay revealed that treatment of activated T cells with 15d-PGJ_2 significantly decreased IL-13 mRNA transcription and secretion, respectively. This inhibition by 15d-PGJ_2 was independent of PPAR-γ since treatment with GW9662, an irreversible antagonist of the nuclear receptor, produced no effect. Our data also revealed the involvement of nuclear factor-κB in mediating 15d-PGJ_2-dependent down regulation of *IL-13* expression. Collectively, these results demonstrate the potential of 15d-PGJ_2 in attenuating expression and production of IL-13 in activated T cells.

1. Introduction

The 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), a downstream metabolite of prostaglandin D_2 (PGD_2), is an emerging key immunoinflammatory mediator [1,2]. The 15d-PGJ_2 is a natural ligand for the peroxisome proliferator-activated receptor gamma (PPAR-γ). However, 15d-PGJ_2 also exhibits PPAR-γ-independent effects, such as regulation of the nuclear factor (NF)-κB pathway by direct inhibition and covalent modification of the IKKβ subunit of IKK [3,4].

Interleukin (IL)-13 is an immunoregulatory cytokine secreted predominantly by activated T-helper type 2 (Th-2) cells [5]. IL-13 was originally described as a cytokine involved in regulating inflammation and immune responses in monocytes and B cells [6]. More recently, however, this cytokine has been associated with many biological processes, including regulation of gastrointestinal parasite expulsion [7], airway hyper-responsiveness, allergic inflammation [8–10], IgE production in B cells [11], ulcerative colitis [12], fibrosis [13], and tumor cell growth [14].

IL-13 shares some functions with IL-4 but its regulation is poorly understood in T cells. Both cytokines play pivotal roles in regulating Th-2 cytokine-mediated immune responses and acting as a counter-regulatory system for Th-1 immune responses [15]. IL-4 and IL-13 share a 25–30% amino acid homology and their similar biological roles have been attributed to common receptor components [15].

In this study, we investigated the ability of 15d-PGJ_2 to regulate IL-13 gene expression and production. We demonstrate that 15d-PGJ_2 strongly attenuated *IL-13* expression from activated T cells through transcriptional repression involving the NF-κB transcription factor. Our data suggests a potential role for 15d-PGJ_2 in regulating *IL-13* expression in activated T cells.

2. Materials and methods

2.1. Reagents

Phorbol 12-myristate 13-acetate (PMA), phytohemagglutinin-L (PHA-L), ionomycin, IL-2, and GW9662 were purchased from Sigma (Oakville, ON, Canada). 15d-PGJ_2 was purchased from EMD Chemicals (Mississauga, ON, Canada). α-CD3 antibody was purchased from eBioscience (San Diego, CA, USA) and α-CD28 antibody was obtained from BD Biosciences (Mississauga, ON, Canada).

2.2. Cell culture

The human acute $CD4^+$ T-cell line Jurkat E6.1 (ATCC, Manassas, VA, USA) was grown in 5% CO_2 in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 0.5 mM sodium pyruvate, 100 U/ml penicillin–streptomycin, and non-essential

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amino acids (all from Wisent, St.-Bruno, QC, Canada). Peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors by lymphocyte separation medium (Wisent) according to the manufacturer's instructions and stimulated for 3 days with PHA-L (1 μg/ml) and IL-2 (30 U/ml) in RPMI 1640 containing10% FBS.

2.3. Transient transfections and luciferase assays

The plasmid pIL-13-luc (courtesy of Dr. Jana Stankova, Université de Sherbrooke) expresses the luciferase reporter gene under control of the *IL-13* promoter (−940 to +48). Jurkat E6.1 cells were transfected using the DEAE-Dextran method [19] with 15 μg of pIL-13-luc vector. Cells were stimulated with PMA (20 ng/ml) and PHA (1 μg/ml) for 30 min, and treated with 15d-PGJ_2 (10 μM) for 24 h at 37 °C. Cell lysis buffer (50 μl) was added to each well before addition of 100 μl luciferase assay buffer [16]. Cell lysates were evaluated for luciferase activity using the LUMIstar Galaxy software (BMG Labtechnologies, Ortenburg, Germany).

2.4. Cell stimulations for mRNA analysis

Jurkat E6.1 cells (5×10^6 cells) or PBMCs (3×10^6 cells) were left untreated or stimulated in RPMI containing 10% FBS with PMA/ionomycin (20 ng/ml per 1 μM) or α-CD3 (5 μg/ml) and α-CD28 (2.5 μg/ml) for 0, 4, 6, 8, or 24 h. Alternatively, cells were cultured in the presence of 15d-PGJ_2 (10 μM) for 30 min before (pre-treatment) or after (post-treatment) T-cell activation with PMA/ionomycin or α-CD3/α-CD28.

2.5. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was prepared from 2×10^6 stimulated cells using the QIAzol Lysis Reagent (QIAGEN, Valencia, CA, USA). Two micrograms were reverse-transcribed into cDNA in the presence of 200 U Moloney murine leukemia virus RT (Promega, Madison, WI, USA), 0.5 μg oligonucleotide $d(T)_{15}$, and 500 μM dNTP at 42 °C for 1 h. One microliter of cDNA was amplified with 0.25 μM each of forward (5′-GACCTTGTGCGGGCAGAAT-3′) and reverse (5′-TGCAGTGCCATCGAGAACAC-3′) primer, GAPDH primers [17]), 0.4 mM dNTP, 2 mM $MgCl_2$, and 1.25 units of Taq DNA polymerase (Roche, Indianapolis, IN, USA). PCR cycling conditions consisted of initial denaturation at 95 °C for 5 min and 30 cycles of 95 °C for 15 s, 60 °C for 1 min, 72 °C for 45 s, and an additional 7-min extension using the Power SYBR® Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) and the 7500 Real Time PCR System

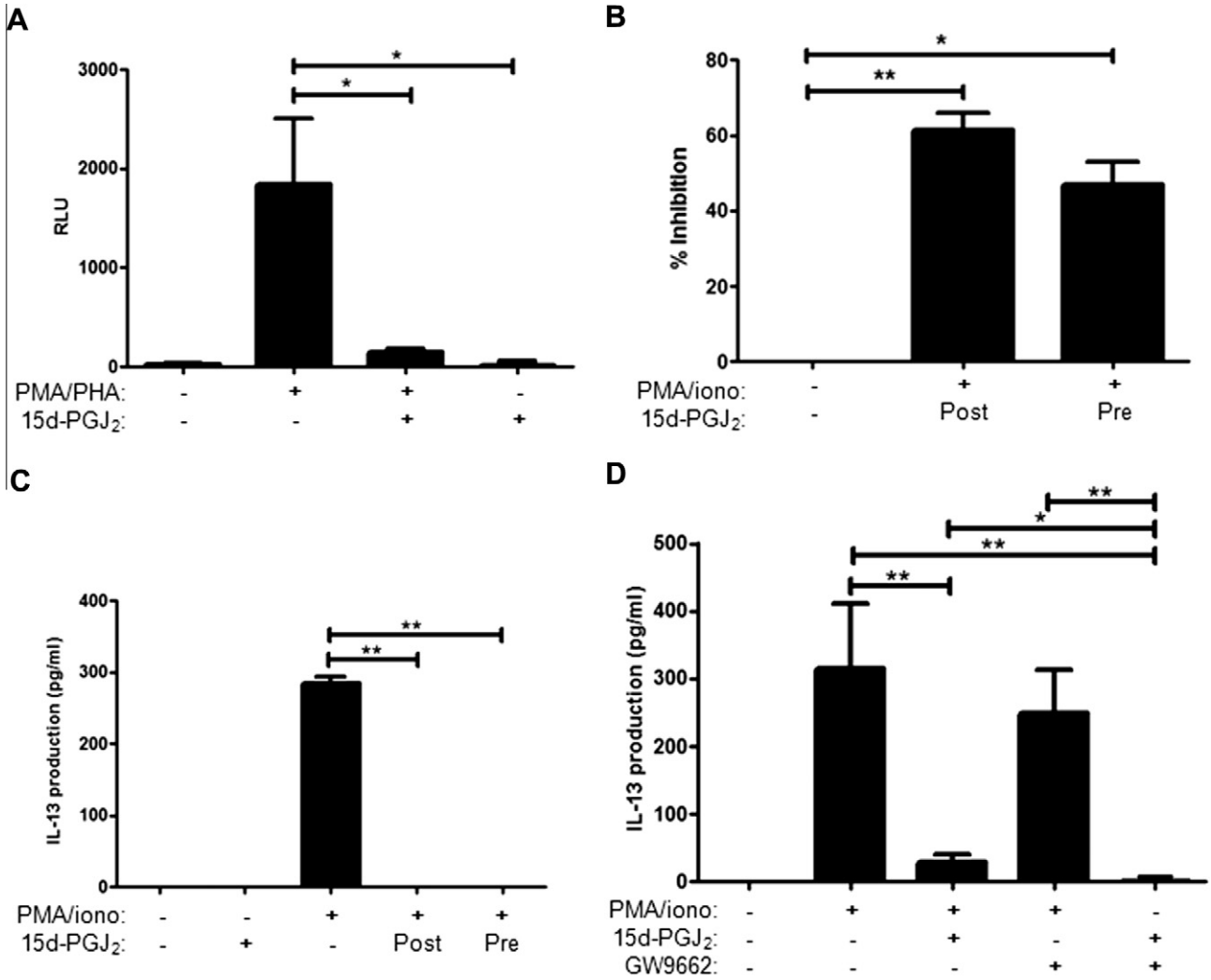

Fig. 1. 15d-PGJ_2 inhibits IL-13 expression in Jurkat E6.1 T cells via a PPAR-γ-independent mechanism. (A) Jurkat E6.1 cells were transiently transfected with pIL-13-luc, stimulated with PMA/PHA, and treated with 15d-PGJ_2. Cells were then lysed and luciferase activity was monitored. Data are graphed as the mean (± standard deviation) of four replicates and are expressed in relative luciferase units (RLUs). These results are representative of four independent experiments. PMA/ionomycin-activated Jurkat E6.1 cells were pre- or post-treated with 15d-PGJ_2 before measurement of (B) IL-13 mRNA by real-time RT-PCR ($n = 4$) and (C) secreted IL-13 protein by ELISA ($n = 3$). (D) Cells were pre-treated with the PPAR-γ inhibitor GW9662 before stimulation with PMA/ionomycin or 15d-PGJ_2. Secreted IL-13 was measured by ($n = 4$). $^*p < 0.05$ and $^{**}p < 0.01$.