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Research Article

The Modulatory Effect of 15d-PGJ$_2$ in Dendritic Cells

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Abstract. The PPAR-γ ligands, in special 15-deoxy-$\Delta^{12,14}$-PGJ$_2$ (15d-PGJ$_2$), negatively regulate the cells of innate and adaptative immune system and present excellent results in different models of inflammatory diseases. These findings support the notion that PPAR-γ ligands may be used as therapeutic agents in different diseases. Although PPAR-γ is expressed in different cells and tissues including dendritic cells (DC), few studies have evaluated the effects of these ligands on DCs. Thus, in this study we evaluated the effect of 15d-PGJ$_2$ on DC surface molecule expression, including MHC-II, CD80, and CD86. In addition, we quantified cytokine production in the presence of 15d-PGJ$_2$ or rosiglitazone. Expression of the surface molecules was measured by flow cytometry and cytokines production was measured by ELISA in supernatant of BMDC cultures. The results suggest that 15d-PGJ$_2$ reduced the expression of costimulatory molecules (CD80 and CD86), without altering MCH-class II expression. Furthermore the natural PPAR-γ agonist significantly reduced levels of proinflammatory cytokines (IL-12, IFN-γ, and TNF-α) and appears to also reduce IL-1β levels. Rosiglitazone reduced the expression of these cytokines albeit to a lesser extent. These data suggest the idea that 15d-PGJ$_2$ could be a therapeutic strategy in diseases where DCs play a crucial role, due to its ability to reduce costimulatory molecules expression and modulate the inflammatory environment.

Keywords: PGJ2, inflammation, dendritic cells, PPAR-gamma

1. Introduction

Dendritic cells (DCs) are important professional antigen-presenting cells (APCs) that initiate and modulate immune responses [1, 2]. DCs present antigen to T cells in the context of cell surface major histocompatibility complex (MHC) class II molecules and costimulatory molecules, such as CD40, CD80 (B7-1), and CD86 (B7-2), that are essential for lymphocyte activation [3].

Dendritic cells are present in all tissues in immature state characterized by low surface expression of MHC-II and costimulatory molecules [4]. However, signals associated with inflammation or infectious disease cause maturing of

DCs. This process involves complex phenotypic and functional chances. These mature DCs exhibited high expression of costimulatory molecules, such as CD80, CD86, and CD40, upregulated MHC classes I and II, and produced proinflammatory cytokines, such as IL-12 and TNF-α [5]. Thus, DCs migrate from peripheral organs *via* the lymph to secondary lymphoid organs, where the antigens are presented to naïve T cells, generating effector T cells, that produce more and more proinflammatory cytokines activating other immune cells, causing tissue damage, and besides establishing immunological memory [4].

15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2) is a derivative of prostaglandin D2 and is a natural ligand of peroxisome proliferator-activated receptor-gamma (PPARγ), which is a transcriptional nuclear receptor [6]. Importantly 15d-PGJ_2 differs from other prostaglandins, both chemically and biologically, in several respects [7], especially because it has anti-inflammatory [8–11], antiproliferative [12], and antinociceptive effects [13, 14]. Moreover, PPAR-γ is expressed in macrophages, monocytes, eosinophils, fibroblasts, bone marrow precursors, naive and activated T lymphocytes, and dendritic cells [15–18], which leave PPAR ligands, such as 15d-PGJ_2, a promising therapeutic strategy to treat inflammatory diseases.

Our group has previously demonstrated that 15d-PGJ_2 decreased F-actin polymerization of mouse neutrophils stimulated with MIP-2 [10], downregulated the eosinopoiesis as well as eosinophil recruitment following allergen challenge [19], and at small doses increased the osteoblast activity and the bone-related proteins expression [20]. Besides, it was demonstrated that 15d-PGJ_2 is involved in the regulation of Toll-like receptors and PPAR-γ-mediated signaling in DCs, thus representing a novel negative feedback mechanism involved in the resolution of immunologic responses [21].

Despite many studies demonstrating the anti-inflammatory capacity of 15d-PGJ_2 in various experimental models, there are few studies dedicated to understanding its direct action on immune cells such as DCs. Therefore, in this study we have investigated the influence of 15d-PGJ_2 on cell surface expression of MHC and costimulatory molecules as well as on the ability to inhibit the cytokine release by DCs.

2. Material and Methods

2.1. Animals. C57BL/6 wild-type mice weighing 20–25 g, 6-8 weeks old, were kept in appropriate cages in a temperature-controlled room, with a 12h dark/light cycle, and they had free access to water and food. All animals were manipulated in accordance with the Guiding Principles in The Care and Use of Animals, approved by the Council of the American Physiologic Society. This animal study was deemed to be ethical according to the Brazilian Guidelines (Resolution 11794/2008) and was approved by the Animal Ethics Committee of the São Leopoldo Mandic Faculty (no. 068/2012). The number of animals per group was kept at a minimum and each animal was used once.

2.2. Dendritic cell generation. Dendritic cells were generated *in vitro* from bone marrow cells from 6- to 8-week-old wild-type C57BL/6 mice as described previously [22]. Briefly, femurs were flushed with RPMI 1640 (Gibco-BRL Life Technologies, Grand Island, NY, USA) to release the bone marrow cells that were cultured in 6-well culture plates in RPMI-1640 (Gibco) supplemented with 10% heat-inactivated FCS, 100 μg/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} 2-mercaptoethanol (all from Sigma Chemical Co., St. Louis, MO, USA), and murine GM-CSF (30 ng/ml). On days 3 and 6, the supernatants were gently removed and replaced with the same volume of supplemented medium. On day 9, the nonadherent cells were collected to eliminate the residual macrophage contamination. Flow cytometric evaluation of DCs shows high expression of CD11c (data not shown).

2.3. Treatment of dendritic cells. To evaluate the effect of different concentrations of 15d-PGJ_2 (Sigma–Aldrich, USA) or rosiglitazone (Avandia, Glaxo-Smith Kline, USA) on DCs, these cells ($1x10^6$/mL) were incubated with 15d-PGJ_2 or rosiglitazone and/or LPS, in RPMI 1640 supplemented with 10% FBS. After day 9 from DC generation, DCs were pretreated for 1 hour (37°C in 5% CO_2) with 15d-PGJ_2 (1, 5, or 10 μM) or rosiglitazone (3, 10, or 30 μM) before LPS (50 ng/mL) stimulation for 24 h (overnight at 37°C in 5% CO_2).

2.4. Flow cytometry. To assess the influence of 15d-PGJ_2 treatment on the expression of DC surface molecules, these cells were harvested on plate culture and were characterized by flow cytometry using antibodies against MHC class-II, CD80, and CD86 conjugated to PE or FITC, as well as isotype controls. Afterwards, samples obtained from the abovementioned culture were suspended and incubated for 30 min at 4°C in PBS containing 2% of bovine serum albumin (PBS-BSA) and Fc-block to avoid nonspecific background staining. After the blocking step, DCs were identified by characteristic size (FSC) and granulosity (SSC) combined with two-color analysis. Briefly, DCs were identified as CD11c+ using specific antibody conjugate with PE (BD Biosciences PharMingen, San Diego, CA, USA), and the expression of MHC-II, CD80, and CD86 was identified using antibody conjugate with FITC (BD Biosciences PharMingen, San Diego, CA, USA). The isotype controls used were rat IgG2b PE and Hamster PE/FITC (BD Biosciences PharMingen). After staining, cells were fixed with 1% paraformaldehyde and analyzed by flow cytometry (FACScan and CELLQuest software; BD Biosciences PharMingen).

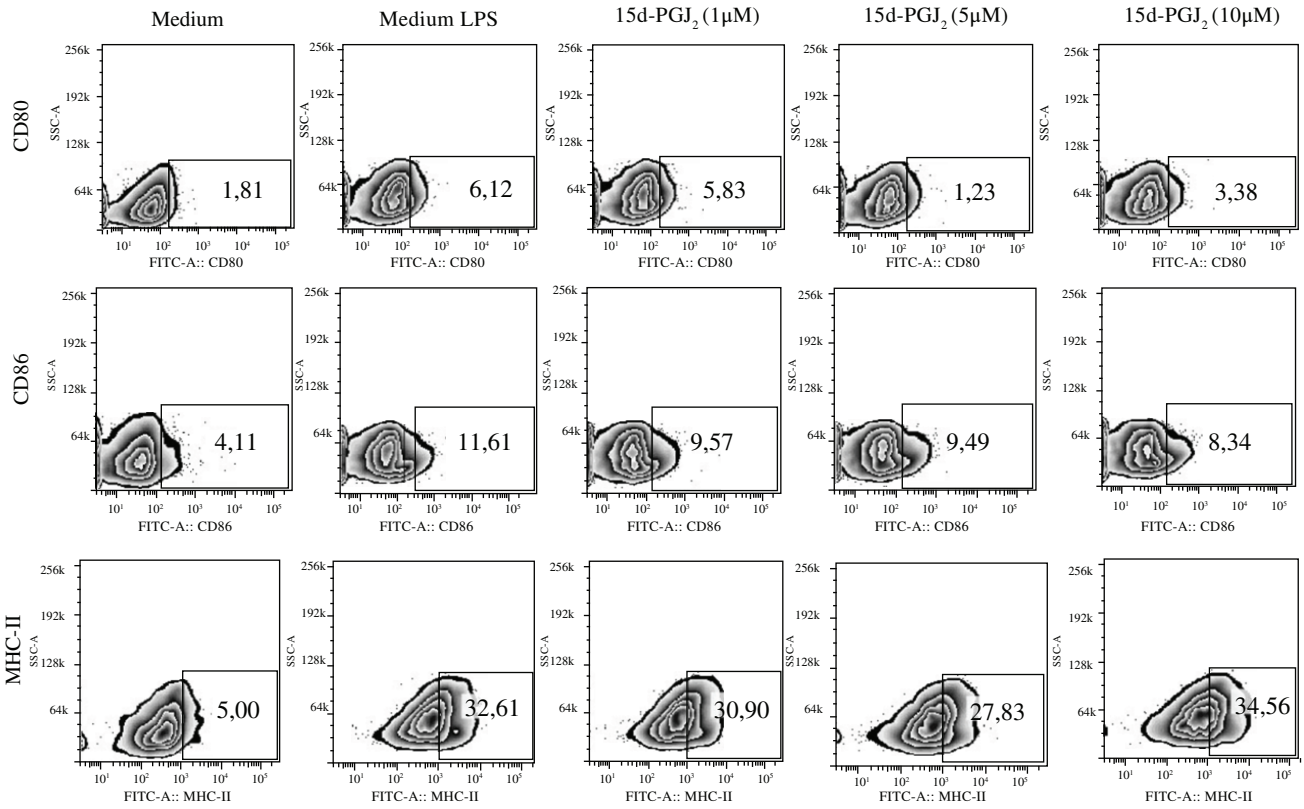

Figure 1: Effect of 15d-PGJ_2 on surface molecules expression. BMDC were subjected to the pretreatment with 15d-PGJ_2(1, 5, and 10μM), for 1 hour, and then stimulated with LPS. After 24 hours of stimulation, the BMDC were harvested and double-stained for CD11c or CD80, CD86, and MHC-II. Monoclonal antibody conjugated PE or FITC was used for staining and was detected through flow cytometry.

2.5. Cytokine measurements (ELISA). The levels of IL-12, IFN-γ, TNF-α, IL-1β, and IL-10 were detected by ELISA using protocols supplied by the manufacturer (R&D Systems, Minneapolis, USA). After all standard procedures, the optical density (OD) was measured at 490 nm. Results are expressed as pg/mL of each cytokine, based on the standard curves. Cytokines levels were measured in supernatant of BMDC cultures.

2.6. Quantitative real time PCR of PPAR-γ. Total RNA was extracted from DCs stimulated, or not, with LPS using RNAspin Mini isolation kit (GE Healthcare, Buckinghamshire, Germany) following the manufacturer's recommendations. Gene expression of PPAR-γ was normalized to the expression of the GAPDH gene.

2.7. Statistical analysis. The means from different treatments were compared using ANOVA. When statistically significant differences were identified, individual comparisons were subsequently made using Bonferroni's t-test for unpaired values. Statistical significance was set at P value $<$ 0.05.

3. Results

FACS analysis was used in an attempt to determine the influence of 15d-PGJ_2 on surface molecules expression of DCs. Cells stimulated with LPS showed elevated expression levels of the CD80 marker (6.12) than nonstimulated DCs (1.18). 15d-PGJ_2 at 1 μM reduced LPS-stimulated levels to 5.83, at 5μM to 1.23, and at 10 μM to 3.38. The same pattern was observed regarding the expression of CD86 molecule. The DC stimulated with LPS showed elevated expression of CD86 marker (11.61) than nonstimulated DC (4.11). 15d-PGJ_2at 1 and 5μM slightly reduced this expression (9.57 and 9.49, resp.) and at 10 μM to 8.34. We also evaluated the expression of the MHC-II by DCs after LPS stimulation, and an elevated expression of MHC-II was observed in the presence of LPS (32.61) compared with nonstimulated DCs (5.00). 15d-PGJ_2 at doses of 1 and 5 μM reduced this expression (30.90 and 27.83, resp.), although this effect was not observed in the presence of 10 μM of 15d-PGJ_2 (34.56). All FACS boxes are summarized in Figure 1.

Next, we analyzed several cytokines in the LPS-stimulated DCs in the absence and presence of 15d-PGJ_2 or rosiglitazone. The levels of IL-12p40 (Figure 2(a)), IFN-γ (Figure

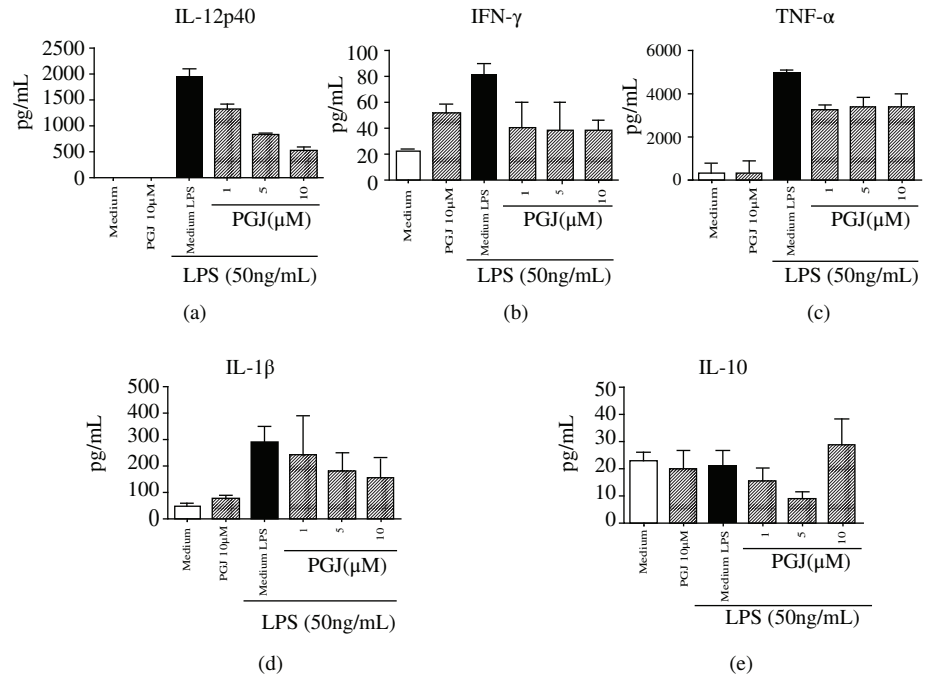

Figure 2: Effect of 15d-PGJ_2 on cytokines release. BMDC were subjected to pretreatment with 15d-PGJ_2 (1, 5, or 10μM) for 1 hour, followed by LPS stimulation. After 24 hours of stimulation, the culture supernatant was harvested and the levels of IL12p40 **(a)**, IFN-γ **(b**, TNF-α **(c)**, IL-1β **(d),** and IL-10 **(e)** were detected through ELISA assay. Data are the mean ± SD and are triplicate representative. #$P < 0.05$ medium group compared with medium + LPS group. *$P < 0.05$ medium + LPS group compared with 15d-PGJ_2 + LPS group.

2(b)), and TNF-α (Figure 2(c)) in the DC stimulated with LPS were statistically higher ($P < 0.05$) than medium alone. All tested doses of 15d-PGJ_2 decreased the release of these cytokines in a dose-dependent fashion. Furthermore, although it was not statistical significant ($P > 0.05$), 15d-PGJ_2 decreased levels of IL-1β (Figure 2(d)) and IL-10 (Figure 2(e)) in DCs stimulated with LPS. In addition, DC stimulated with LPS and treated with rosiglitazone showed statistically decreased levels of IL-12p40 only at higher dose of this PPARγ ligand (Figure 3(a)). The levels of IFN-γ (Figure 3(b)) and IL-1β (Figure 3(d)) were decreased at lower doses of rosiglitazone, while the levels of TNF-α (Figure 3(c)) were decreased with all tested doses. Levels of IL-10 (Figure 3(e)) did not show statistical significance with rosiglitazone.

It is important to point out that DC stimulated with LPS showed elevated levels of PPAR-γ mRNA expression (Figure 4).

4. Discussion

In the present study we have demonstrated that the natural agonist of PPAR-γ, 15d-PGJ_2, exerts an immune-modulatory effect on dendritic cells by promoting a reduction both in the expression of costimulatory surface molecules (MHC-II, CD80, and CD86) and in the secretion of proinflammatory cytokines. The glitazone PPAR-γ agonist, rosiglitazone, showed a lesser modulatory effect.

DCs were discovered in 1973 by Steinman and Cohn [23]. They originate from DC precursors in the bone marrow or from monocytes. Their unique morphology promotes the establishment of sophisticated networks, which allows them to interact with different lymphocyte populations [24]. DCs are regarded as professional APCs and provide an important link between the innate and the adaptive immune responses and play a critical role not only in the host defense against pathogens and cancer but also in the tolerance and prevention against autoimmunity [5, 25]. It has recently been highlighted that DCs can survey the lipid environment through various cell membrane receptors, such as lipid-sensing nuclear hormone receptors, including PPAR-γ and consequently its agonist 15d-PGJ_2 [26].

Previous studies have suggested that PPAR-γ activation negatively affects functional maturation of DCs in response to environmental stimuli [18, 27]. Furthermore, PPAR-γ agonists have been shown to induce the rearrangement of membrane-bound costimulatory molecules [28]. In the present study, a downregulation of B7.1 (CD80) and B7.2 (CD86) as well as MHC-II expression was observed at low doses of 15d-PGJ_2, which corroborates the results from a previous study by Nencioni et al. [29], thus suggesting that PPARγ is involved in the regulatory network by stringently

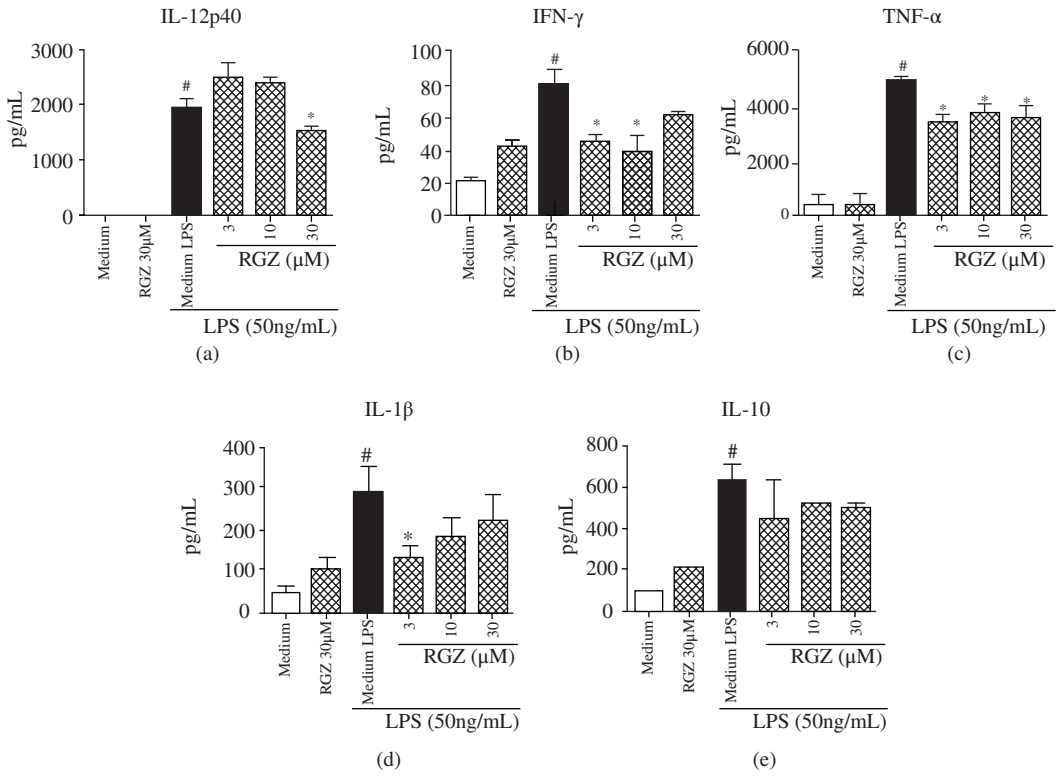

Figure 3: Effect of rosiglitazone on cytokines release. BMDC were subjected to pretreatment with rosiglitazone (3, 10, or 30μM), for 1 hour, followed by LPS stimulation. After 24 hours of stimulation, the culture supernatant was harvested and the levels of IL12p40 **(a)**, IFN-γ **(b)**, TNF-α **(c)**, IL-1β **(d),** and IL-10 **(e)** were detected through ELISA assay. Data are the mean ± SD and are triplicate representative. #$P < 0.05$ medium group compared with medium LPS group. *$P < 0.05$ medium + LPS group compared with rosiglitazone + LPS group.

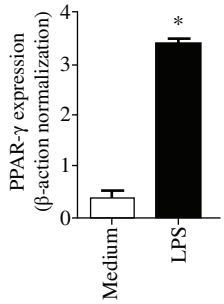

Figure 4: PPAR-γ expression. BMDC were subjected to LPS stimulation and after 24 hours the mRNA was extracted and quantified by RT-PCR. Data are the mean ± SD and are triplicate representative. The symbol * indicates $P < 0.05$.

controlling the immunostimulatory capacity of DCs. Additionally, PPAR-γ activation in DCs resulted in a reduced capacity to induce lymphocyte proliferation and to prime Ag-specific CTL responses [29]. Activation of PPARγ has also been shown to inhibit the nuclear localization of c-Rel and RelB, both of which are members of the NF-κB family of transcription factors and are reported to be essential for normal DC function [21]. Furthermore, PPARγ ligand-activated DCs are not only less stimulatory but also less able to migrate in response to chemokines involved in the homing of DCs to the lymph nodes [30]. Collectively, these findings reinforce the notion that PPARγ plays an important role in regulating DC function.

Previous studies have shown that the activation of PPAR-γ reduces the expression of various cytokines suggesting a therapeutic potential for PPAR-γ agonists [21, 31–34]. The results from the present study corroborate those findings since IL-12, IFN-γ, and TNF-α were significantly down-regulated. IL-1β and IL-10 were also reduced, although this did not reach statistical significance. The activation of PPAR-γ in human monocyte-derived DCs has been reported to decrease the secretion of IL-12, a pivotal cytokine in Th1 polarization [18], which further supports the findings from this study. Dendritic cells are able to produce IL-12, a dominant cytokine involved in the development of IFN-γ-producing T cells [35]. Moreover, interferons are key effector cytokines of the innate and adaptive immune systems. When stimulated with IL-12 produced by DC

[36], the inflammatory cytokine IFN-γ is produced in large quantities by Th1 effector CD4 T cells, by CD8 T cells, and by natural killer (NK) cells. On the other hand, TNF-α and IL-1β are able to act on leukocytes and resident cells inducing the expression of integrins and stimulating the production of platelet-activating factor (PAF), LTB4, and chemokines, which, in turn, can activate neutrophil recruitment [37]. Moreover, TNF-α and IL-1β act on endothelial cells stimulating the expression of selectin and the upregulation of ICAMs [38].

The data presented in this study suggest that 15d-PGJ_2 negatively affects the costimulatory molecules of DCs as well as proinflammatory cytokines in response to environmental stimuli. Significant efforts are currently underway to establish novel PPAR roles and to uncover molecular mechanisms involved in their activation and repression, as well as to develop safer and more effective ways to modulate PPAR as therapeutic targets to treat a myriad of diseases and conditions [39].

5. Conclusion

In conclusion, the results presented herein indicate that the PPAR-γ agonist 15d-PGJ_2 exerts an immunomodulatory effect on DCs via reducing the expression of costimulatory molecules and the secretion of proinflammatory cytokines. These data suggest that 15d-PGJ_2 could be a therapeutic strategy to treat diseases where DCs play a crucial role.

Conflict of Interests

The authors are responsible for the content and writing of the paper and declare that they do not possess any financial interest.

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