Effects of 15-Deoxy-Δ^{12,14}-Prostaglandin J2 (15d-PGJ2) and Rosiglitazone on Human Vδ2+ T Cells

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Abstract

Background: Thiazolidinediones (TZD) class of drugs, and 15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) are immune regulators predicted to modulate human autoimmune disease. Their effects on γδ T cells, which are involved in animal model and human and animal autoimmune diseases, are unknown.

Methodology/Principal Findings: We characterized the activity of rosiglitazone (from the TZD class of drugs) and 15d-PGJ2 in human Vδ2 T cells. We found that 15d-PGJ2 and rosiglitazone had different effects on Vδ2 T cell functions. Both 15d-PGJ2 and rosiglitazone suppressed Vδ2 T cell proliferation in response to IPP and IL2. However, only 15d-PGJ2 suppressed functional responses including cytokine production, degranulation and cytotoxicity against tumor cells. The mechanism for 15d-PGJ2 effects on Vδ2 T cells acts through inhibiting Erk activation. In contrast, rosiglitazone did not affect Erk activation but the IL2 signaling pathway, which accounts for rosiglitazone suppression of IL2-dependent, Vδ2 T cell proliferation without affecting TCR-dependent functions. Rosiglitazone and 15d-PGJ2 are designed to be peroxisome proliferator-activated receptor gamma (PPARγ) ligands and PPARγ was expressed in Vδ2 T cell. Surprisingly, when PPARγ levels were lowered by specific siRNA, 15d-PGJ2 and rosiglitazone were still active, suggesting their target of action induces cellular proteins other than PPARγ.

Conclusions/Significance: The current findings expand our understanding of how the immune system is regulated by rosiglitazone and 15d-PGJ2 and will be important to evaluate these compounds as therapeutic agents in human autoimmune disease.

Introduction

The incidence of autoimmune disease has been growing in recent years and the contribution to disease of various immune cell subsets are being defined. Research in autoimmunity focuses primarily on cells of the adaptive immune system and their roles in disease. Several studies implicated γδ T cells in animal models and human autoimmune diseases including multiple sclerosis (MS) [1,2], experimental allergic encephalomyelitis (EAE) [3], polymyositis [4,5], Bechet’s disease [6,7], rheumatoid arthritis (RA) [8], atopic dermatitis (AD) [9] and systemic lupus erythematosus (SLE) [10]. Although the exact role for γδ T cells remains unknown, they possess potent cytotoxic activity, are major sources of cytokines including IFN-γ and TNF-α, and produce chemokines involved in recruiting monocyte/macrophages [11,12,13]. Recently, γδ T cells in mouse were reported to be an important source of IL17 [14,15,16,17,18]. These functions of activated γδ T cells could contribute significantly towards inflammatory processes and promote autoimmunity.

In humans, γδ T cells represent 1 to 10% of circulating T cells in blood, with the majority (≥80%) expressing a Vγ2Vδ2 (also termed Vγ9Vδ2) TCR (hereafter referred as Vδ2 T cells) [19] that mediates broad reactivity against microbial agents and tumors. Cells in this subset recognize low molecular weight, non-peptidic compounds termed “phosphoantigens,” including isopentyl pyrophosphate (IPP) [20,21], an intermediate in sterol and isoprenoid biosynthesis. Following stimulation by phosphoantigens, Vδ2 T cells proliferate, release cytokines (particularly IFN-γ and TNF-α) [22,23] or chemokines [24,25], and acquire cytotoxic activity against tumor cells [26,27] or infected cells [28]. In view of the similarity between inflammatory processes in pathogen responses and autoimmune diseases, it is not surprising that Vδ2 T cells might participate in both. Thus, potential treatments for autoimmune diseases may involve modulating human γδ T cell function.

Peroxisome proliferator-activated receptor gamma (PPARγ) is a ligand-dependent transcription factor that was recognized originally as a key regulator of adipocyte function [29,30]. Recent studies reported that PPARγ are expressed in many immune cells [31]; PPARγ ligands down-regulated dendritic cell [32], NK cell [33], B cell [34] and helper T cell [35], and enhanced regulatory T cell responses [36]. Some of the effects were proved to be PPARγ-independent [33,34,36]. Consequently, there have been many studies using PPARγ ligands in animal models of autoimmunity including experimental allergic encephalomyelitis, asthma, arthritis, and colitis [37,38,39,40,41,42,43,44,45,46]. The success of this approach has led to the potential use of PPARγ ligands as therapeutic agents in human autoimmune disease [47], even with the knowledge that these compounds may target molecule other than PPARγ.
15d-PGJ2 and the TZD class of drugs are two types of PPARγ ligands that are studied most often. In the present study, we used synthetic PPARγ ligands from the thiazolidinediones (TZD) class of drugs that are used widely for treating type 2 diabetes because they enhance insulin sensitivity [48] and the endogenous PPARγ ligand cyclopentenone prostaglandin15-deoxy-D12,14-prostaglandin J2 (15d-PGJ2) [49]. We tested their effects on human V82 T cell function as a model for their impact on γδ T cells in autoimmune diseases. We also tested several different TZD class drugs, including rosiglitazone, troglitazone and ciglitazone all with similar results. We uncovered a mechanism for V82 T cell inhibition that surprisingly, was partly independent of PPARγ.

Results

Both 15d-PGJ2 and Rosiglitazone Suppressed V62 T Cell Proliferation

We tested the effects of PPARγ ligands on V82 T cell proliferative response to phosphoantigen. Freshly isolated PBMC were treated with 15d-PGJ2 or rosiglitazone for 1 hour before adding IPP plus IL2. Cells were cultured for 10 days with IL2 added every 3 days. V82 T cell frequency was measured every 3 days. As shown in figure 1, both 15d-PGJ2 (Figure 1A) and rosiglitazone (Figure 1B) suppressed IPP-driven V82 T cell expansion in a dose-dependent manner. To reach similar effects, a 10-fold higher concentration of rosiglitazone was needed compared to 15d-PGJ2.

15d-PGJ2, but not Rosiglitazone, Suppressed Cytokine Production, Degranulation and Cytotoxicity

It was reported previously that expanded V82 T cells (V82 T cell line) but not fresh cells can kill tumor cell targets [27]. Here, we tested whether PPARγ ligands affect phosphoantigen-driven cytokine production and degranulation in V82 T cell lines. Freshly isolated PBMC contained 1–10% of V82 T cells; after 10 to 14 days of culture with IPP plus IL2, the percentage of V82 T cells was more than 90% (Figure 2A). V82 T cell lines were rested after washing twice and culturing in fresh medium for 24 hours without stimulation. Then, cells were treated with 15d-PGJ2 or rosiglitazone for 1 hour, then washed and stimulated with IPP. There was a dose-dependent suppression by 15d-PGJ2 of V82 T cell IFN-γ (Figure 2B) or TNF-α (Figure 2C) production and degranulation (CD107α expression) (Figure 2D). However, rosiglitazone had no effect on cytokine expression or degranulation, even at very high concentrations (Figure 2B, C and D).

![Figure 1. Both 15d-PGJ2 and rosiglitazone suppressed IPP-stimulated V62 T cell proliferation.](image-url)

We also tested the effect of PPARγ ligands on V82 T cell line cytotoxicity. Tumor cell lines were Daudi (a Burkitt’s lymphoma) and TU167 (a squamous carcinoma). Lysis of both Daudi (Figure 2E) and TU167 (Figure 2F) cells were reduced significantly and in a dose dependent manner when V82 T cells were treated with 15d-PGJ2 before adding to targets. Rosiglitazone did not alter V82 cytotoxicity against Daudi (Figure 2G) or TU167 (Figure 2H) at any of the concentrations tested.

15d-PGJ2 Suppressed V82 T Cell Functions by Inhibiting Erk Activation

The V82 T cell responses to phosphoantigen depends on TCR signaling. We tested 15d-PGJ2 for effects on the V82 TCR signaling pathway as a possible explanation for inhibition of cytokine expression or cytotoxicity. The V82 T cell lines were washed and incubated in fresh medium for 24 hours without stimulation. Then, cells were treated with 15d-PGJ2 or rosiglitazone for 1 hour followed by the addition of IPP. After 30 minutes, cells were collected for western blotting analyses. We measured phosphorylation of several signaling molecules implicated in TCR signal transduction: NFκB, Erk, p38 and PI3-K-associated Akt. Our results demonstrated that NFκB, p38 and Akt were constitutively activated (phosphorylated) in expanded V82 T cell lines, although p38 and Akt were phosphorylated at a lower level compared to NFκB (Figure 3A, lane 1). Phosphorylated Erk was not detected (Figure 3A, lane 1). IPP-stimulation activated Erk and Akt but not NFκB (Figure 3A, lane 2). 15d-PGJ2 but not rosiglitazone suppressed IPP-activated Erk phosphorylation. Neither 15d-PGJ2 nor rosiglitazone affected NFκB, p38, or Akt activation (Figure 3A, lane 3 and 4).

We also tested whether Erk activation is important for V82 T cell function. A highly selective inhibitor of MEK1/2, U0126, was used to inhibit Erk activation. The U0126 inhibited IPP-stimulated Erk activation in V82 T cells in a dose-dependent manner (Figure 3B). When Erk activation was inhibited, the V82 T cells, including cytokine production in response to IPP (Figure 3C, D) and cytotoxicity against tumor cells (Figure 3E, F) were suppressed. These results indicated that Erk activation is a key factor in V82 TCR signaling pathway for functional responses. U0126 also suppressed V82 T cell proliferation responses (Figure 3G). Based on these data, we believe that 15d-PGJ2 inhibits V82 T cell functionality by inhibiting Erk activation.

15d-PGJ2 and Rosiglitazone Suppressed IL2-Induced Phosphorylation of STAT5 in V82 T Cells

IPP-driven V82 T cell proliferation depends on IL2. Rosiglitazone inhibited V82 T cell proliferation without affecting the TCR signal. Thus, we postulated that rosiglitazone might inhibit
Figure 2. 15d-PGJ2, but not rosiglitazone, suppressed cytokine production, degranulation and cytotoxicity functions of Vδ2 T cell.

(A) Freshly isolated PBMC contained 1–10% of Vδ2 T cells (left panel); after 10 to 14 days of culture with IPP plus IL-2, the percentage of Vδ2 T cells was more than 90% (right panel). (B, C and D) Vδ2 T cells were treated with 15d-PGJ2 or rosiglitazone at various concentrations for 1 hour and then washed and stimulated with IPP (50 μM). After stimulating for 4 hours, the levels of IFN-γ (B) or TNF-α (C) in cell-free supernatant were detected by antigen capture ELISA. The experiments were done in triplicate and statistical tests compared drug and vehicle (DMSO). CD107a expression (D) was analyzed by flow cytometry. (E and F) Vδ2 T cells were pretreated with 15d-PGJ2 at various concentrations for 1 hour. The cytotoxicity of Vδ2 T cell against Daudi (E) or TU167 (F) was evaluated at different E:T ratios in triplicate. The statistical significance of specific lysis compared with a drug vehicle (DMSO) control at E:T = 5:1 was analyzed. (G and H) Vδ2 T cells were pretreated with rosiglitazone at various concentrations for 1 hour. The cytotoxicity of Vδ2 T cell against Daudi (G) or TU167 (H) was evaluated at different E:T ratios in triplicate. (I) PBMC was stimulated with PMA (10 ng/ml) and ionomycin (1 μM) for 4 h. IL-17 production in different cell type was detected by flow cytometry. *, P<0.05; **, P<0.01; ***, P<0.001. Data are representative of three independent experiments using different donors.

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the IL2 signaling pathway. Purified Vδ2 T cells from fresh PBMC were pretreated with 15d-PGJ2 or rosiglitazone for 1 hour, then incubated with IL2 for 15 minutes. The phosphorylated STAT5 was stained with a specific antibody and detected by flow cytometry. Both 15d-PGJ2 (Figure 4A) and rosiglitazone (Figure 4B) suppressed IL2-induced phosphorylation of STAT5 in Vδ2 T cells.

Primary and Expanded Vδ2 T Cells Express PPARγ

15d-PGJ2 and rosiglitazone are also PPARγ ligands. We assessed PPARγ expression in primary Vδ2 T cell and IPP-expanded Vδ2 T cell lines. We examined the expression of PPARγ by intracellular staining and flow cytometry. PPARγ was present among Vδ2 cells (Figure 5A). We confirmed the result by western blotting using purified primary or expanded Vδ2 T cells (Figure 5B).
PPARγ-Independent Effects of 15d-PGJ2 and Rosiglitazone on V\textsubscript{d}2 T Cell

PPARγ ligands have the curious property of acting in both PPARγ-dependent and independent ways. To test whether 15d-PGJ2 or rosiglitazone regulated V\textsubscript{d}2 T cell through PPARγ-dependent or independent mechanism, we used the PPARγ inhibitor GW9662 that covalently modifies the PPARγ ligand-binding domain and acts as an irreversible antagonist at concentrations of 100 nM or less [34,56]. We used the optimized concentration of 100 nM GW9662 for V\textsubscript{d}2 T cell. As shown in Figure 6A, GW9662 alone did not inhibit IPP-driven V\textsubscript{d}2 proliferation (Figure 6A) or cytokine production (Figure 6B, C). Furthermore, GW9662 did not relieve the inhibitory effect of 15d-PGJ2 or rosiglitazone on V\textsubscript{d}2 proliferation (Figure 6A). Also, GW9662 did not prevent the inhibitory effect of 15d-PGJ2 on cytokine production (Figure 6B, C). We next used siRNA to knock down PPARγ and repeated the inhibition studies. A specific siRNA knocked down PPARγ protein levels (Figure 6D) but did not prevent the effects of 15d-PGJ2 or rosiglitazone on V\textsubscript{d}2 T cells (Figure 6E, F and G). These data argue that 15d-PGJ2 and rosiglitazone regulate V\textsubscript{d}2 T cells through PPARγ-independent mechanisms and the molecular target for these drugs has not yet been defined in human γδ T cells. As a positive control, we show here that rosiglitazone increased the expression of CD36 in human monocytes, while GW9662 suppressed the effect of rosiglitazone (Figure 6H), which is consistent with a previous report [57].

Discussion

In the present study, we report that 15d-PGJ2 and the TZD drug rosiglitazone had different effects on V\textsubscript{d}2 T cell functions. We also elucidated the underlying mechanisms by evaluating signal transduction pathways. This work will be important for understanding the effects of 15d-PGJ2 and the TZD drugs on immune responses and evaluating their application as therapeutic agents in human autoimmune disease.

15d-PGJ2 and rosiglitazone both suppressed V\textsubscript{d}2 T cell proliferation in response to IPP and IL2. Although TZD drugs have a higher binding affinity for PPARγ [31,48], rosiglitazone was less potent for inhibiting V\textsubscript{d}2 T cell proliferation. Only 15d-PGJ2 suppressed V\textsubscript{d}2 T cell functional responses including cytokine production, degranulation and cytotoxicity against tumor cells. Consequently, the effects of 15d-PGJ2 and rosiglitazone on V\textsubscript{d}2 T cell responses to antigen appear to be independent of PPARγ. This hypothesis is supported by recent reports showing 15d-PGJ2 or TZD class drugs act independently of PPARγ. For example, 15d-PGJ2 or TZD drugs modulated regulatory T cell [36], NK

![Figure 6](https://example.com/image6.png)

**Figure 6.** The effects of 15d-PGJ2 and rosiglitazone on V\textsubscript{d}2V\textsubscript{d}2 T cells are PPARγ-independent. (A) Fresh isolated PBMC was pretreated with GW9662 (100nM) for 1 hour, then treated with 15d-PGJ2 (10 μM) or rosiglitazone (50 μM) and cultured with IPP (15 μM) plus IL2 (100 U/ml). Cells were cultured for 10 days by adding IL2 every 3 days. V\textsubscript{d}2 T cell frequencies were detected at day 10. (B and C) The expanded V\textsubscript{d}2 T cells were rested by incubating in fresh medium for 24 hours without stimulation. The cells were pretreated with GW9662 (100 nM) for 1 hour, then washed and treated with 15d-PGJ2 (10 μM) or rosiglitazone (50 μM), and stimulated with IPP. After 4 hours stimulation, the levels of IFN-γ (B) or TNF-α (C) in cell-free supernatants were detected by antigen capture ELISA. (D) Fresh isolated PBMC were transfected with control or PPARγ-specific siRNA and cultured for 48 hours. Cells were then collected for Western blotting analyses (D); or cells were treated with 15d-PGJ2 (10 μM) or rosiglitazone (50 μM), then cultured with IPP (15 μM) plus IL2 (100 U/ml) for proliferation (E); or V\textsubscript{d}2 T cell were purified and treated with 15d-PGJ2 (10 μM) or rosiglitazone (50 μM) then stimulated with IPP for IFN-γ (F) or TNF-α (G) analyses as described. Data are representative of three independent experiments with different donors. (H) Human monocytes were treated for 16 h with rosiglitazone (10 μM) in the presence or not of GW9662 (100 nM); CD36 expression was analyzed by flow cytometry.

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important for initiating Vδ2 T cell functions, and induced B cell apoptosis [34] through PPARγ-independent effects. These findings, along with our observation that a specific PPARγ antagonist or siRNA failed to block the effects of 15d-PGJ2 or rosiglitazone in Vδ2 T cells, support the view that a T cell target other than PPARγ is altered by these treatments. The PPARγ was expressed in Vδ2 T cells (Fig. 5) and most immune cells, although non-genomic action of PPARγ ligands have been reported. The continued study of PPARγ in Vδ2 T cell and other immune cell development and function may reveal a yet undiscovered role for PPARγ in the human immune system.

We postulated that 15d-PGJ2 or rosiglitazone might affect the Vδ2 TCR signaling pathway. The δβ TCR signaling pathway has been well studied, but less is known about the γδ TCR and its downstream signaling pathway. A recent paper reported that another phosphoantigen, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate (HMB-PP), induced MEK/Erk and PL-3K/Akt-mediated signal transduction in primary Vδ2 T cells but IPP failed to induce Akt phosphorylation [59]. In the present study, we tested several key factors in the TCR signaling pathway of Vδ2 T cells; after IPP stimulation including NFκB, Erk, p38, JNK and Akt. We found that NFκB, p38 and Akt, but not Erk and JNK (data not shown for JNK), were phosphorylated constitutively in Vδ2 T cell lines. IPP-stimulation activated Erk but not JNK, and increased the phosphorylation level of Akt but not NFκB. The NFκB signaling pathway might be important to Vδ2 T cell survival as we reported previously [60]. The Erk and Akt signaling pathway might have greater impact on functional responses by Vδ2 T cells. To test a hypothesis that Erk activation is responsible for Vδ2 T cell function, we inhibited Erk using U0126, a highly selective inhibitor of MEK1/2. We found that the cytokine production and cytotoxicity functions of Vδ2 T cell were suppressed when Erk was inhibited specifically, indicating a key role for Erk in Vδ2 T cell functions. The U0126 also suppressed Vδ2 T cell proliferation, indicating that Erk activation is important for initiating Vδ2 T cell proliferation.

The drugs 15d-PGJ2 and rosiglitazone were distinct in terms of their effects on the Vδ2 TCR signaling pathway. The 15d-PGJ2 but not rosiglitazone specifically inhibited Erk activation. This might explain why only 15d-PGJ2 suppressed Vδ2 T cell effector functions. It was unclear why rosiglitazone suppressed Vδ2 T cell proliferation since it did not affect the Erk activation. Because Vδ2 T cell proliferation requires IL2, we proposed that rosiglitazone might inhibit IL2 signaling. Indeed, both 15d-PGJ2 and rosiglitazone inhibited IL2-induced STAT5 activation, a key factor in the IL2 signaling pathway, arguing that rosiglitazone effects on IL2 signaling explained the inhibition of cell proliferation.

In Vitro Proliferation Assays

PBMC (5×10^6 cells/well) were cultured in 12-well plates with complete medium, 15 μM isopentyl pyrophosphate (IPP) (Sigma) and 100 U/ml human recombinant IL-2 (Tecin, Biological Resources Branch, National Institutes of Health, Bethesda, MD). In some experiments, 15d-PGJ2 or rosiglitazone (Cayman Chemical Company, MI) were added. Fresh complete medium and 100 U/ml IL-2 were added every 3 days. γδ T cell proliferation was measured by staining for CD3 and Vδ2, then defining, by flow cytometry, the percentage of γδ T cells within the total lymphocyte population at days 0, 4, 7 and 10.

RNA Interference

Fresh isolated PBMC were transfected with control siRNA or a specific siRNA that target PPARγ mRNA (Dharmacon) using a human T cell nucleofactor kit following the manufacturer’s instructions (Amaga Biosystem Inc. USA). Cells were used after 48 hours. The impact of RNA interference was evaluated by immunoblotting for the PPARγ protein (see below).

Purifying Vδ2+ Cells and Monocytes

The Vδ2+ or CD14+ monocyte subsets were purified from fresh PBMC or PBMC expanded with IPP and IL-2 using a MultiSort Kit (MiltenyiBiotec, Auburn, CA) according to the manufacturer’s instructions. Cells were stained with PE-conjugate Vδ2 or CD14 antibodies for 10 minutes on ice. The labeled cells were washed and incubated with anti-PE MicroBeads for 15 minutes on ice, then separated in a magnetic field. We achieved 90 to 98% purity after magnetic bead separation as measured by flow cytometry.

Immunoblot Analysis

Cells (2×10^6) were lysed in gel loading buffer (Invitrogen, Carlsbad, CA); samples were boiled for 10 minutes and proteins were separated by SDS-PAGE. Proteins were transferred to nitrocellulose membranes and probed with various primary antibodies. Secondary antibodies including HRP-conjugated, anti-rabbit or anti-mouse (Cell Signaling Technology, Inc.) were visualized with enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK) and exposure to Kodak X-ray film.

Cytotoxicity Assay

A nonradioactive, fluorometric cytotoxicity assay with calcein-acetoxyethyl (calcein-AM; Molecular Probes) was used to measure cytotoxicity against Daudi B cell or TU167 squamous cell tumor lines [60]. Expanded γδ cells (effector cells) were treated with 15d-PGJ2 or rosiglitazone (Cayman Chemical Company, MI) at varying concentrations for 1 hour at 37°C. Daudi B or TU167 target cells were labeled for 15 minutes with 2 mMol/L calcein-AM at 37°C and then washed once with PBS. Cells were combined at various effector-to-target (E:T) ratios in 96-well, round-bottomed microtiter plates (Corning, NY) and incubated at 37°C in 5% CO2 for 4 hours; assays were performed in triplicate. After incubation, supernatants were transferred to a 96-well flat-bottomed microtiter plate and

Materials and Methods

PBMC and Tumor Cell Lines

Whole blood was obtained from healthy human volunteers who provided written informed consent and all protocols were approved by the Institutional Review Board at the University of Maryland, Baltimore. Total lymphocytes were separated from heparinized peripheral blood by density gradient centrifugation (Ficoll-Paque; Amersham Biosciences). Peripheral-blood mononuclear cells (PBMC) and TU167 cells (squamous cell carcinoma) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco), 2 mMol/L L-glutamine, and penicillin-streptomycin (100 U/ml and 100 mg/mL, respectively); for Daudi B cells (CCL-213; ATCC), 4.5 g/L glucose, 1.5 g/L NaHCO3, 10 mMol/L HEPES, and 1 mMol/L sodium pyruvate were added.
calcine content was measured using a Wallac Victor2 1420 multi-channel counter (405/553 nm). Percent specific lysis was calculated as: (test release—spontaneous release)/(maximum release—spontaneous release) × 100.

Flow Cytometry

Unless noted, cells were stained with fluorophore-conjugated monoclonal antibodies from BD Biosciences, San Jose, CA. Generally, 3 × 10^5–5 × 10^5 cells were washed, resuspended in 50–100 μL of RPMI 1640, and stained with mouse anti-human Vδ2-PE clone B6, mouse anti-human CD3-fluorescein isothiocyanate (FITC) clone UCHT1, mouse anti-human CD5-PE clone UCHT1, mouse anti-human CD8-PE clone CD107a-FITC clone X40, IgG1-PE clone X40, and IgG1-APC clone X40. For detecting phospho-STAT5, cells were stained with fluorophore-conjugated monoclonal antibodies from BD Biosciences, San Jose, CA. Generally, 3 × 10^5–5 × 10^5 cells were washed, resuspended in 50–100 μL of RPMI 1640, and stained with mouse anti-human Vδ2-PE clone B6, mouse anti-human CD3-fluorescein isothiocyanate (FITC) clone UCHT1, mouse anti-human CD5-PE clone UCHT1, mouse anti-human CD8-PE clone CD107a-FITC clone X40, IgG1-PE clone X40, and IgG1-APC clone X40. For detecting intracellular IL-17, cells were stained with mouse anti-human CD4 (PerCP), mouse anti-CD8 (PerCP), mouse anti-human V81 (FITC), mouse anti-human CD20 (PE), mouse anti-human CD14 (PE), mouse anti-human CD56 (PE), then fixed, permeabilized and incubated for 45 min at 4°C with mouse anti-human IL-17 (FITC or PE). Intracellular staining solutions were obtained from the Cytofix/Cytoperm Kit (BD Biosciences). For intracellular PPARγ and phospho-STAT5 staining, treated and untreated cells were fixed by adding 16% formaldehyde directly into untreated cells were fixed by adding 16% formaldehyde directly into samples and resuspended. Data for at least 1 × 10^6 cells were acquired on a FACSCalibur flow cytometer (BD Biosciences). All samples were analyzed using FlowJo software (FlowJo 8.8.2, Tree Star, San Carlos, CA). For stimulation before staining, PBMC or Vδ2 T cells were treated with IFP or PMA/fomycin for 4 hours. In some experiments, Vδ2 T cells were treated with 15d-PGJ2 or rosiglitazone (Cayman Chemical Company, MI) at varying concentrations for 1 hour at 37°C before stimulation and then washed.

Detecting Cytokines by ELISA

Human IFN-γ in culture supernatants was detected with a human IFN-γ ELISA kit (R&D Systems), according to the manufacturer’s directions. Human TNF-α in culture supernatants was detected with a human TNF-α ELISA kit (R&D Systems), according to the manufacturer’s directions.

Statistical Analysis

Differences among groups were analyzed by Student’s t test. P < 0.05 was considered to be significant.

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Author Contributions

Conceived and designed the experiments: HL CDP. Performed the experiments: HL. Analyzed the data: HL CDP. Contributed reagents/materials/analysis tools: CDP. Wrote the paper: HL CDP.

References

1. Battistini L, Selma K, Kowal C, Ohlmer J, Modlin RL, et al. (1995) Multiple sclerosis: limited diversity of the V delta 2-J delta 3 T-cell receptor in chronic active lesions. Ann Neurol 37: 198–203.
2. Selma K, Bresnan CF, Raine CS (1991) Colocalization of lymphocytes bearing gamma delta T-cell receptor and heat shock protein hsp65 oligodeoxynucleotides in multiple sclerosis. Proc Natl Acad Sci U S A 88: 6452–6456.
3. Rajan AJ, Gao YL, Raine CS, Bresnan CF (1996) A pathogenic role for gamma delta T cells in relapsing-remitting experimental allergic encephalomyelitis in the SJL mouse. J Immunol 157: 941–949.
4. Holodniy R, Engel J, K, Harper MC (1991) Polyomynosis mediated by T lymphocytes that express the gamma/delta receptor. N Engl J Med 324: 877–881.
5. Wiendl H, Malatska J, Holzworth B, Weltszin HU, Weckerle H, et al. (2002) An autoreactive gamma delta TCR derived from a polymyositis lesion. J Immunol 169: 515–521.
6. Yamashita N, Kanesaka H, Kaneo S, Takeno M, Oueda K, et al. (1997) Role of gamma delta T lymphocytes in the development of Behcet's disease. Clin Immunol 107: 241–247.
7. Bank I, Dudevani M, Laveh A (2003) Expansion of gammadelta T-cells in Behcet's disease: role of disease activity and microbial flora in oral ulcers. J Lab Clin Med 141: 33–40.
8. Holodniot L (1999) Activation of gammadelta T cells by mycobacterial antigens in rheumatoid arthritis. Microbes Infect 1: 197–202.
9. Cairo C, Arabito E, Landi F, Casati A, Brunetti E, et al. (2005) Analysis of circulating gammadelta T cells in children affected by IgE-associated and non-IgE-associated atopic eczema/dermatitis syndrome. Clin Exp Immunol 141: 116–121.
10. Robak E, Blozinski JZ, Bartkowska J, Niewiadomska H, Syxa-Redzewska A, et al. (1999) Circulating TCR gammadelta cell in the patients with systemic lupus erythematosus. Mediators Inflamm 8: 305–312.
11. Battistini L, Caccamo N, Borsellino G, Mervaglia S, Angelini DF, et al. (2005) Homing and memory patterns of human gammadelta T cells in physiopathological situations. Microbes Infect 7: 510–517.
12. Bresnan CF, Raine CS (1996) Mechanisms of immune injury in multiple sclerosis. Brain Pathol 6: 245–257.
13. Carding SR, Egan PJ (2002) Gammadelta T cells: functional plasticity and heterogeneity. Nat Rev Immunol 2: 336–345.
14. Sutton CE, Lator SJ, Sweeney GM, Berreto CF, Lavelle EC, et al. (2009) Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. Immunity 31: 331–341.
15. Martin B, Hirota K, Cua DJ, Stockinger B, Veldhoen M (2009) Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity 31: 321–330.
16. Shichita T, Sugiyama Y, Ooboshi H, Sugimori H, Nakagawa R, et al. (2009) Pivotal role of cerebral interleukin-17-producing gammadelta T cells in the delayed phase of ischemic brain injury. Nat Med 15: 946–950.
17. O’Brien RL, Roark CL, Born WK (2009) IL-17-producing gammadelta T cells. Eur J Immunol 39: 662–666.
18. Roark CL, Simonian PL, Fontenot AP, Born WK, O’Brien RL (2008) gammadelta T cells: an important source of IL-17. Curr Opin Immunol 20: 353–357.
19. Evans PS, Enders PJ, Yin G, Rackwitz TJ, Malkovsky M, et al. (2001) In vitro stimulation with a non-peptidic alkylphosphate expands cells expressing Vgamma2-Jgamma2/2Vdelta2 T-cell receptors. Immunology 104: 19–27.
20. Constant P, Davodeau F, Perez MA, Poux Y, Puzo G, et al. (1994) Pivotal role of cerebral interleukin-17-producing gammadelta T cells in the delayed phase of ischemic brain injury. Nat Med 15: 946–950.
21. Tanaka Y, Morita CT, Nieves E, Brenner MB, Bloom BR (1995) Natural and synthetic non-peptide antigens recognized by human gammadelta T cells. Nature 375: 155–158.
22. Garcia VE, Siepling PA, Gong J, Barnes PF, Uyemura K, et al. (1997) Single-cell cytokine analysis of gammadelta T cell responses to nonpeptide mycobacterial antigens. J Immunol 159: 1328–1335.
23. Lang F, Peyrat MA, Constant P, Davodeau F, David-Amenelle J, et al. (1995) Early activation of human V gamma 9 V delta 2 T cell broad cytokotoxicity and TNF production by nonpeptide mycobacterial ligands. J Immunol 154: 5885–5894.
24. Poccia F, Battistini L, Cipriani B, Mancino G, Marinetti F, et al. (1999) Phosphoantigen-reactive Vgamma9Vdelta2 T lymphocytes suppress in vitro delayed type hypersensitivity. J Immunol 162: 850–861.
25. Tikhonov I, Deetz CO, Paca R, Berg S, Lukyanenko V, et al. (2006) Human Vgamma2Vdelta2 T cells contain cytoplasmic RANTES. Int Immunol 18: 1243–1251.
26. Bukowski JF, Moira CT, Tanaka Y, Bloom BR, Rossner MB, et al. (1995) V gamma 2 delta T cell-TCR-dependent recognition of non-peptide antigens and Daudi cells analyzed by TCR gene transfer. J Immunol 154: 998–1006.

27. Alexander AA, Maniar A, Cummings JS, Hbhebeler AM, Schulze DH, et al. (2000) Isopentenyl pyrophosphate-activated CD69+ gamma (delta) T lymphocytes display potent antitumor activity toward human squamous cell carcinoma. Clin Cancer Res 14: 4232–4240.

28. Ortonne F, Donnand J, Naroeni A, Lamiard JP, Favero J (2000) V gamma delta 2 T cells impair intracellular multiplication of Brucella suis in autologous monocytes through soluble factor release and contact-dependent cytotoxic effect. J Immunol 165: 7135–7139.

29. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in 3T3-L1 adipocytes by PPARgamma 2, a lipid-activated transcription factor. Cell 79: 1147–1156.

30. Tontonoz P, Hu E, Spiegelman BM (1995) Regulation of adipocyte gene expression and differentiation by peroxisome proliferator activated receptor gamma. Curr Opin Genet Dev 5: 571–576.

31. Szelles I, Torocsik D, Nagy I (2007) PPARgamma in immunity and inflammation: cell types and diseases. Biochim Biophys Acta 171: 1014–1030.

32. Szamani I, Torocsik D, Agostini M, Nagy T, Gurnell M, et al. (2007) PPARgamma regulates the function of human dendritic cells primarily by altering lipid metabolism. Blood 110: 3271–3280.

33. Zhang X, Rodriguez-Galan MC, Suhleski JJ, Oitalo JR, Hodge DL, et al. (2004) Peroxone proliferator-activated receptor-gamma and its ligands attenuate biologic functions of human natural killer cells. Blood 104: 3276–3284.

34. Ray DM, Akbiyik F, Phipps RP (2006) Peroxisome proliferator-activated receptor gamma (PPARgamma) ligands inhibit nitrotyrosine formation and inflammatory mediator expression of CD36 in human monocytes through PPARgamma activation. J Immunol 177: 5068–5076.

35. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L, et al. (2008) The nuclear receptor PPAR gamma and immunoregulation: PPAR gamma mediates inhibition of helper T cell responses. J Immunol 180: 1364–1371.

36. Wohlert EA, Nichols FC, Nieves E, Clark RB (2007) Peroxisome proliferator-activated receptor gamma (PPARgamma) and immunoregulation: enhancement of regulatory T cells through PPARgamma-dependent and -independent mechanisms. J Immunol 178: 4129–4135.

37. Nataraian C, Bright J (2002) Peroxone proliferator-activated receptor-gamma agonists inhibit experimental allergic encephalomyelitis by blocking IL-12 production. IL-12 signaling and Th1 differentiation. Genetics Immun 3: 59–70.

38. Nataraian C, Muthian G, Barak Y, Evans RM, Bright J (2003) Peroxisome proliferator-activated receptor-gamma-deficient heterozygous mice develop an exacerbated neural antigen-induced Th1 response and experimental allergic encephalomyelitis. J Immunol 171: 5743–5750.

39. Shiojiri T, Wada K, Nakajima A, Katayama K, Shibuya A, et al. (2002) PPAR gamma ligands inhibit nitrotyrosine formation and inflammatory mediator expressions in adjunct-induced rheumatoid arthritis mice. Eur J Pharmacol 440: 231–238.

40. Saubermann L, Nakajima A, Wada K, Zhao S, Terraschi Y, et al. (2002) Peroxisome proliferator-activated receptor gamma agonist stimulates ligands contain a functional cytokine response and prevent acute colitis. Inflamm Bowel Dis 8: 496–499.

41. Wockey G, Honda K, Loveday M, Papin JP, Auseys J, et al. (2003) Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. J Exp Med 198: 411–421.

42. Hammad H, de Heer HJ, Soeille T, Angeli V, Trotein F, et al. (2004) Activation of peroxisome proliferator-activated receptor-gamma in dendritic cells inhibits the development of eosinophilic airway inflammation in a mouse model of asthma. Am J Pathol 164: 263–271.

43. Mueller C, Weaver V, Vanden Heuvel JP, August A, Cantorna MT (2003) Peroxisome proliferator-activated receptor gamma ligands attenuate immunological symptoms of experimental allergic asthma. Arch Biochem Biophys 418: 186–196.

44. Ueki S, Matsuzaki Y, Kayaba H, Oyamada H, Kanda A, et al. (2004) Peroxone proliferator-activated receptor gamma regulates eosinophil function by new therapeutic targets for allergic airway inflammation. Int Arch Allergy Immunol 134 Suppl 1: 30–36.

45. Beales PE, Liddi R, Giorgini AE, Suyone A, Procaccini E, et al. (1998) Tryptagluon prevents insulin dependent diabetes in the non-obese diabetic mouse. Eur J Pharmacol 357: 224–229.

46. Augstein P, Danson A, Heinke P, Wachlin G, Berg S, et al. (2003) Prevention of autoimmune diabetes in NOD mice by trytagluon is associated with modulation of ICAM-1 expression on pancreatic islet cells and IFN-gamma expression in splenic T cells. Biochem Biophys Res Commun 304: 378–394.

47. Pershadingha HA, Heneka MT, Saini R, Amin MM, Brooks DJ, et al. (2004) Effect of pioglutam treatment in a patient with secondary multiple sclerosis. J Neurolimmunol 1: 3.

48. Lehmann JM, Moore ED, Smith-Oliver TA, Wilkinson WO, Wilson TM, et al. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxone proliferator-activated receptor gamma (PPAR gamma). J Biol Chem 270: 12953–12956.

49. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, et al. (1995) 15-Deoxy-Delta 12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell 83: 803–812.

50. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, et al. (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 421: 744–748.

51. Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, et al. (2003) Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med 198: 1951–1957.

52. Follows GA, Dhami P, Gertgens B, Bruce AW, Campbell PJ, et al. (2006) Identifying gene regulatory elements by genomic microarray mapping of DNASel hypersensitive sites. Genome Res 16: 1310–1319.

53. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, et al. (2008) Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol 172: 146–155.

54. Klotz L, Burgdorf S, Dani I, Sajoo K, Flossdorf J, et al. (2009) The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmune immunity. J Exp Med.

55. Fenoglio D, Poggi A, Catellani S, Battaglia F, Ferrera A, et al. (2009) Vdelta1 T Cell...