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Peroxisome Proliferator-Activated Receptor γ Inhibits the Migration of Dendritic Cells: Consequences for the Immune Response[1]

Véronique Angeli,[2]* Hamida Hammad,[2‡] Bart Staels,[†] Monique Capron,* Bart N. Lambrecht,[‡] and François Trottein[3]*

The migration of dendritic cells (DCs) from the epithelia to the lymphoid organs represents a tightly regulated multistep event involved in the induction of the immune response. In this process fatty acid derivatives positively and negatively regulate DC emigration. In the present study we investigated whether activation of peroxisome proliferator-activated receptors (PPARs), a family of nuclear receptors activated by naturally occurring derivatives of arachidonic acid, could control DC migration from the peripheral sites of Ag capture to the draining lymph nodes (DLNs). First, we show that murine epidermal Langerhans cells (LCs) express PPARγ, but not PPARα, mRNA, and protein. Using an experimental murine model of LC migration induced by TNF-α, we show that the highly potent PPARγ agonist rosiglitazone specifically impairs the departure of LCs from the epidermis. In a model of contact allergen-induced LC migration, PPARγ activation not only impedes LC emigration, and their subsequent accumulation as DCs in the DLNs, but also dramatically prevents the contact hypersensitivity responses after challenge. Finally, after intratracheal sensitization with an FITC-conjugated Ag, PPARγ activation inhibits the migration of DCs from the airway mucosa to the thoracic LNs and also profoundly reduces the priming of Ag-specific T lymphocytes in the DLNs. Our results suggest a novel regulatory pathway via PPARγ for DC migration from epithelia that could contribute to the initiation of immune responses. *The Journal of Immunology,* **2003, 170: 5295–5301.**

Langerhans cells (LCs),[4] as members of the dendritic cell (DC) lineage, are part of a system of potent APCs. Located in epithelia in an immature state, they act as sentinels of the immune system (1). Upon antigenic and/or inflammatory stimuli, LCs undergo a complex process of maturation, leave the peripheral tissue, and migrate to the draining lymph nodes (DLNs) where they present Ag to naive T cells (2). The molecular mechanisms that induce and/or control LC migration have been the purpose of extensive research in the past few years. From these studies it is shown that the early production of TNF-α and IL-1β provokes the departure of LCs from the epithelium by affecting the expression of adhesion molecules and chemokine receptors and by stimulating actin-dependent movements of LCs (3–6). More recently, it has been suggested that some lipoxygenase and cyclooxygenase products of arachidonic acid, including cysteinyl leukotriene C_4 and PGE_2, potentiate chemokine-driven DC migration (7, 8). On the other hand, LC motility is negatively controlled by the later production of anti-inflammatory cytokines, such as IL-4 and IL-10 (9, 10). More recently, we demonstrated that PGD_2 also plays a part in DC trafficking by inhibiting the emigration of epidermal LCs to the DLNs (11). Since numerous fatty acids (FAs) are generated during the early phases of inflammatory/immune reactions, we investigated in the present study the possibility that the FA-activated nuclear receptors of the peroxisome proliferator-activated receptor (PPAR) subfamily may be involved in the control of LC/DC migration and in the subsequent induction of the immune response.

PPARs are activated by a wide array of endogenous polyunsaturated FA derivatives, oxidized FAs, and phospholipids found in oxidized low density lipoproteins and also by a range of synthetic compounds, including antidiabetic thiazolidinediones and hypolipidemic fibrates (12–16). In response to ligand activation, PPARs heterodimerize with retinoid X receptors and activate the expression of target genes containing peroxisome proliferator-responsive elements (17). After activation, PPARs can also interfere with different intracellular signaling pathways involved in gene expression or protein functionality, thus impacting on diverse physiological functions such as lipid metabolism (18), glucose homeostasis (19), and cell growth, differentiation, and motility (20, 21). More recently, their roles in the control of the inflammatory response have been reported (22–24). Three subtypes of PPARs have been described in humans and rodents: PPARα, -δ (β), and -γ. PPARα is highly expressed in cells with high catabolic rates of FAs, such as liver, muscle, heart, and skin (25, 26). PPARδ is ubiquitously expressed and plays a role in embryonic development and adipocyte physiology (26, 27), whereas PPARγ is predominantly expressed

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[4] Abbreviations used in this paper: LC, Langerhans cell; CHS, contact hypersensitivity; DC, dendritic cell; DLN, draining lymph node; FA, fatty acid; i.d., intradermal; KC, keratinocyte; MAP, mitogen-activated protein; PPAR, peroxisome proliferator-activated receptor; RSG, rosiglitazone; Tg, transgenic.

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in adipose tissue, colon, and vascular wall (28). Interestingly, PPARα and PPARγ have been shown to be expressed in organs of the immune system (26, 29), more precisely in monocytes/macrophages (30–32), and in B and T lymphocytes (33–36). More recently, we and others have shown that PPARγ, but not PPARα, is present in DCs (37–39). Altogether this suggests that PPARs could be involved in innate/adaptive immunity (29, 40–42).

In the present study we tested the hypothesis that PPAR members may be involved in the induction of the immune response by controlling the migration of DCs. First, we show that PPARγ, but not PPARα, is expressed in murine LCs and that PPARγ activation inhibits the TNF-α-induced migration of LCs from the epidermis to the DLNs. Moreover, in a model of contact allergen-induced LC migration, we show that PPARγ activation not only inhibits LC emigration, but also drastically prevents the contact hypersensitivity (CHS) responses after challenge. Finally, using another model of sensitization based on the intratracheal instillation of FITC-conjugated Ag, PPARγ activation significantly reduces the migration of Ag-loaded DCs from the airway mucosa to the thoracic LNs and also dramatically decreases the proliferation of Ag-specific T cells and the production of cytokines by DLN cells. Taken together, we speculate that the local production of PPARγ activators, for instance arachidonic acid derivatives, in the peripheral sites during inflammatory/immune reactions may be important in the regulation of immune responses.

Materials and Methods

Reagents and Abs

All reagents were purchased from Sigma-Aldrich (St. Quentin-Fallavier, France) unless otherwise indicated. Recombinant murine TNF-α (sp. act., $\geq 5 \times 10^7$ U/mg) was purchased from R&D Systems (Abingdon, U.K.). FITC-conjugated OVA and CFSE were obtained from Molecular Probes (Eugene, OR). Wy14643 was purchased from Biomol (Plymouth, PA), and rosiglitazone (RSG) was provided by Dr. A. Bril (GlaxoSmithKline, Rennes, France). GW501516 and GW9662 were gifts from Dr. P. Brown (GlaxoSmithKline, Research Triangle Park, NC). Affinity-purified rabbit polyclonal Abs specific for PPARα and PPARγ have been previously described (31, 37). The anti-PPARγ Ab used for FACS analysis was obtained from Calbiochem-Novabiochem (La Jolla, CA). The anti-I-A^d/I-E^d mAbs (clone M5/114, rat IgG2b) were provided by Drs. A. Ager (National Institute for Medical Research, London, U.K.). Biotin-conjugated anti-CD11c (hamster IgG) and PE-conjugated anti-I-A^d/I-E^d (rat IgG2b) mAbs were purchased from BD PharMingen (San Diego, CA). APC-labeled anti-murine CD4 and PE-conjugated anti-clonotypic OVA TCR mAbs (KJ1-26) were obtained from BD PharMingen and Caltag Laboratories (Burlingame, CA), respectively.

Mice

Female BALB/c mice (6–8 wk old) were purchased from Iffa-Credo (L'Arbresle, France) and kept in a specific pathogen-free facility. The OVA-TCR transgenic (Tg) mice (DO11-10) (43) were bred at Erasmus Medical Center (Rotterdam, The Netherlands).

Cell cultures

The LC line XS52 has been established from mouse epidermis and presents the phenotypic and functional features of immature epidermal LCs (44, 45). XS52 was cultured in RPMI containing 10% (v/v) heat-inactivated FCS in the presence of 2 ng/ml GM-CSF (BioSource, Camarillo, CA) and 10% (v/v) NS47 fibroblast supernatant as previously described (43). The mouse keratinocyte (KC) line Pam212 was cultured in Eagle's MEM supplemented with 10% FCS and 0.05 mM $CaCl_2$ as recommended (46).

Preparation of epidermal cells and FACS analysis

Epidermal cells containing 1–3% LCs were prepared from ear epidermis by standard trypsinization (47). Freshly isolated epidermal cells were first stained with PE-conjugated anti-I-A^d/I-E^d mAb. Cells were fixed with 2% paraformaldehyde and permeabilized with 0.1% saponin/1% BSA in PBS for the detection of intracellular PPARγ with rabbit polyclonal anti-PPARγ Abs, followed by FITC-conjugated anti-rabbit mAb (Clinisciences, Montrouge, France).

RT-PCR and Western blot analysis

Total RNA was obtained using TRIzol reagent (Life Technologies, Grand Island, NY) and was reverse transcribed using random hexamer primers and SuperScript reverse transcriptase (Life Technologies). Amplification by PCR was performed with the primers shown in Table I. Total protein was extracted with SDS sample buffer, resolved by SDS-PAGE, and transferred to nitrocellulose filters. Proteins were analyzed by Western blot with affinity-purified rabbit anti-PPARγ Abs and HRP-labeled goat anti-rabbit Ig (Sanofi Diagnostics Pasteur, Marnes, France).

Intradermal administration of reagents

Mice were intradermally (i.d.) injected with 30 μl of TNF-α (50 ng, reconstituted in sterile PBS containing 0.1% (w/v) BSA) into both ear pinnae with $27^{3/4}$-gauge stainless steel needles. Fifteen minutes before TNF-α treatment, 30 μl of Wy14643 (5 and 50 μM), RSG (1, 10, and 100 μM), GW501516 (5 and 50 nM), or vehicle (DMSO) was injected i.d. into the ear. Epidermal sheets were analyzed 1 h after injection of TNF-α, a time previously shown to be optimal for LC emigration (11). To test the specificity of the PPARγ agonist used, the PPARγ antagonist GW9662 (10 μM) was injected i.d. into the ear 15 min before PPARγ agonist treatment.

Quantification of epidermal LCs.

The preparation and analysis of epidermal sheets were performed exactly as previously described (11, 48). Briefly, epidermal sheets were fixed in 2% paraformaldehyde, washed, and incubated with anti-MHC class II Abs for 90 min. Biotinylated conjugated goat anti-rat Ig was then added for an additional 30 min. In the final step, sheets were developed with 3-amino 9-ethyl carbazol, washed, and mounted onto glass slides in Immumount (Shandon, Pittsburgh, PA) for immunohistochemical analysis. LCs were enumerated by counting MHC class II-positive cells. Epidermal sheets were prepared from each experimental group, and for each sheet 10 random fields were examined. Cell frequency was converted to LC per square millimeter, and results were expressed as the mean ± SD.

Induction and elicitation of CHS responses

Mice were sensitized by painting 10 μl of a 0.5% (w/v) solution of FITC prepared in acetone/dibutylphtalate (solvent; 1:1, v/v) on the total surface of the left ear. Thirty microliters of RSG (10 μM) or DMSO (as a control) was injected i.d. 15 min before and 5 h after sensitization. CHS was elicited

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Table I. *Sequences of primers used for PCR amplification of cDNA, product sizes, and PCR cycle numbers*

Gene	Primer	Sequence	Size (bp)	Cycle
PPARα	5′ 3′	5′-CCAAGTCACCTTGCTAAAGTACGGTGT 5′-AGGAAGGTGTCATCTGGATGGTTGCTC	330	36
PPARβ	5′ 3′	5′-CTCAATGACCAGGTGACCCTCCTC 5′-GGGAAGAGGTACTGGCTGTCAGG	367	36
PPARγ	5′ 3′	5′-TGGTGTCCATGAGATCATCTACACG 5′-TGCACGTGCTCTGTGACGATCTGCCT	386	36
β-Actin	5′ 3′	5′-GCTGCTCACCGAGGCCCCCCTGAAC 5′-CTTTAGCACGCACTGTAATTCCTC	334	28

5 days after sensitization by painting the dorsal and ventral surfaces of the right ear with 10 μl of 0.5% FITC (49). Ear thickness was measured using an engineer's micrometer (Mitutoyo, Kawasaki, Japan) 24 h after challenge. Results are expressed as ear swelling, which was calculated by subtracting the thickness of the ear before challenge from the thickness after challenge. In experiments where elicitations were not required, mice were killed 18 h (for the determination of epidermal LC density) or 24 h after sensitization to determinate the number of migrating FITC-positive DCs in the DLNs. For this purpose, single-cell suspensions were prepared from auricular LNs, and DCs were enriched by centrifugation on a 14.5% (w/v) metrizamide gradient. DCs were then stained with the biotin-conjugated anti-CD11c mAb, followed by PE-streptavidin. The percentage of CD11c$^+$, FITC$^+$ DLN cells was determined on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA). Data were analyzed using CellQuest software.

Intratracheal sensitization

For instillation, mice were anesthetized by i.p. injection of avertin. Eighty microliters of FITC-OVA solution (1 mg/ml), containing, or not, RSG (5, 50, and 500 μM), was administered intratracheally under direct vision through the opening vocal cords using an 18-gauge polyurethane catheter connected to the outlet of a micropipette as previously described (50). Control mice received 80 μl of PBS.

Quantification of FITC$^+$ DCs in the thoracic LNs

One day after FITC-OVA instillation, animals were killed by i.p. injection of a lethal dose of avertin, and thoracic LN cells were prepared by collagenase/DNase/EDTA treatment as previously described (51). LN cell suspensions (95% viability) were stained using a combination of anti-MHC class II and anti-CD11c Abs (DC markers) and were examined by flow cytometry. The cells were analyzed on bivariate plots of MHC class II vs CD11c and were examined for FITC positivity. Dead cells and debris were excluded using propidium iodide.

Effect of PPAR agonist on T cell proliferation in the thoracic LNs

Because the frequency of OVA-specific T cells is very low in immunized animals, naive T cells purified from D011.10 OVA-TCR Tg mice were adoptively transferred into recipient mice. Briefly, pooled peripheral DLNs (inguinal and mesenteric) and spleen were harvested from DO11.10 mice and homogenized, and after RBC lysis, cell suspensions were labeled with CFSE as previously described (52). Two days before FITC instillation, 10×10^6 viable cells were injected i.v. in the lateral tail vein of each mouse (day -2). On day 0 mice received an intratracheal injection of FITC-OVA (1 mg/ml) in the absence or the presence of RSG (500 μM). On day 4 mediastinal LNs were collected, homogenized, and stained for the presence of KJ1-26$^+$CD4$^+$ reactive OVA-specific T cells. Some of the LN cells (2×10^5 cells/well in triplicate) were resuspended in RPMI 1640 containing 5% FCS and antibiotics and placed in 96-well plates. Four days later supernatants were harvested and analyzed for the presence of IL-4, IL-5, IL-10, and IFN-γ by ELISA (BD Biosciences).

Statistical analysis

The statistical significance of differences between experimental groups was calculated using Student's t test.

Results

PPARγ, but not PPARα, is expressed in murine epidermal LCs

We first determined by RT-PCR the expression of PPARs in murine LCs. Since purification of fresh LCs from murine epidermis is extremely difficult to perform, XS52, an LC line previously shown to present phenotypic and functional features of immature LC (44, 45), was used. We also analyzed PPAR expression in freshly isolated epidermal cells (97% KCs and 3% LCs, as determined by FACS analysis; not shown) and in the KC line Pam212. As shown in Fig. 1*A*, RT-PCR analysis demonstrated the presence of PPARδ and PPARγ, but not PPARα, mRNAs in XS52. In accordance with a recent study (53), we detected mRNA transcripts for all PPAR subtypes in epidermal cells and in the KC line Pam212. We then investigated the expression of PPARα and PPARγ in the LC and KC lines by Western blotting. Consistent with RT-PCR analysis, we could not detect PPARα protein in XS52, whereas it was expressed in Pam212 (not shown). The use of specific Abs also revealed the presence of PPARγ protein in XS52 and Pam212 cells (Fig. 1*B*). To confirm the later result, flow cytometric analysis of permeabilized, freshly isolated, epidermal cells was performed. As shown in Fig. 1*C*, PPARγ protein was detected in KCs (class II$^-$) and LCs (class II$^+$). Taken together, these data indicate that PPARα is present in KCs, but not in LCs (at least in XS52), whereas PPARγ is expressed in both KCs and LCs.

PPARγ activation inhibits TNF-α-induced migration of LCs

We next investigated whether PPAR activation could alter LC migration in a system known to promote a strong LC departure to the DLNs (54). For this purpose mice were treated with increasing doses of the highly selective synthetic PPARγ (RSG), PPARα (Wy14643), or PPARδ (GW501516) agonists (16, 19, 55) and subsequently injected into ear pinnae with TNF-α. After checking that activation of PPAR members does not modify the extent of CMH class II expression on LCs in vitro, the capacity of LCs to emigrate from the skin was assessed 1 h after TNF-α injection by counting the frequency of MHC class II-positive cells in the epidermis. As shown in Fig. 2*A*, TNF-α caused a drastic reduction in LC frequency compared with that in control mice (carrier). By contrast,

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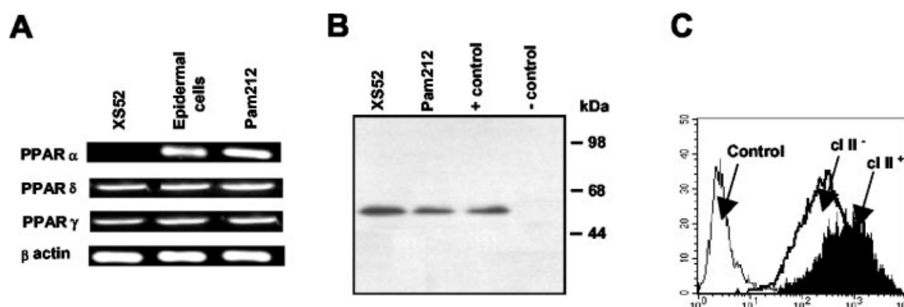

FIGURE 1. Expression of PPARγ in LCs. *A*, mRNA expression of PPARα, PPARδ, and PPARγ, as analyzed by RT-PCR. As positive controls for PPAR mRNA expression, cDNA from mouse adipocyte tissue or liver was used (not shown). *B*, Western blot analysis of PPARγ protein expression in cultured XS52 and Pam212 (10^6 cells/lane). Western blots were probed with affinity-purified rabbit anti-PPARγ Abs at 2 μg/ml. The identity of the 55-kDa band is confirmed by comigration with a band seen in in vitro produced PPARγ protein (+control), but not in the control in vitro translation products ($-$control). The affinity-purified rabbit Abs used as a negative control did not reveal any reactivity (not shown). The sizes are indicated in kilodaltons. *C*, Flow cytometric analysis of PPARγ in epidermal cells. Freshly isolated epidermal cells were exposed to PE-labeled anti-MHC class II Abs, and after permeabilization, cells were stained with anti-PPARγ Abs or species-matched control polyclonal Abs, followed by the anti-rabbit, FITC-labeled mAb. MHC class II$^-$ (cl II$^-$) and MHC class II$^+$ (cl II$^+$) were electronically gated and analyzed for PPARγ expression by FACS.

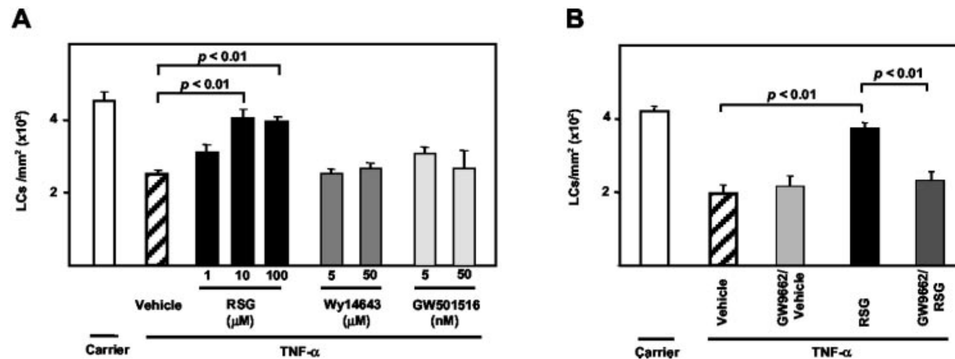

FIGURE 2. Effect of PPAR activation on TNF-α-induced LC migration in vivo. *A*, Mice were i.d. injected with 30 μl of RSG (1, 10, or 100 μM), Wy14643 (10 or 50 μM), or GW501516 (2.5 and 25 nM) 15 min before being injected with PBS/BSA (carrier) containing, or not, 50 ng of TNF-α into both ear pinnae. Ears were removed 1 h later, epidermal sheets were prepared, and the number of LC per square millimeter was determined by immunohistochemistry (anti-MHC class II staining). Of note, the injection of the carrier in the absence or the presence of the PPAR agonists used does not modify the number of LCs in the epidermis (not shown). *B*, Mice were first injected with 30 μl of the PPARγ antagonist GR9662 (10 μM) 15 min before RSG (10 μM) and TNF-α treatment. The experiment shown is representative of four experiments ($n = 5$), and values are the mean ± SD. Significant differences are shown.

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RSG treatment inhibited, in a dose-dependent manner, TNF-α-induced LC departure, whereas Wy14643 and GW501516 had no effect. Optimal inhibition (68%) was achieved at 10 μM. These results indicate that RSG interferes with TNF-α to inhibit LC migration from the epidermis to the DLNs. To confirm that these effects are mediated via PPARγ, mice were pretreated with GW9662, a highly selective and irreversible PPARγ antagonist (56), 15 min before RSG and TNF-α application. As represented in Fig. 2*B*, GW9662 alone neither enhanced nor blocked LC emigration from the epidermis, suggesting that endogenous activation of PPARγ in LCs does not limit their mobilization in response to TNF-α. On the other hand, GW9662 pretreatment reverses almost entirely (by 80%) the inhibitory action of RSG on TNF-α-induced LC migration.

RSG inhibits CHS responses elicited by FITC

To confirm and extend our findings, we tested the effect of RSG in a model of contact sensitization induced by the hapten FITC (57). Compared with unsensitized mice (solvent), the number of LCs was reduced in the epidermis of sensitized mice 18 h after FITC painting (Fig. 3*A*). In contrast, LC migration was significantly impaired in RSG-treated mice compared with sensitized control mice (vehicle). As assessed by flow cytometry, this defect in LC departure was associated with a drastic reduction in the number of CD11c$^+$, FITC$^+$ cells in DLNs 24 h after sensitization (Fig. 3*B*). Of note, pretreatment with the PPARγ antagonist GW9662 reversed the inhibitory effect of RSG on LC emigration and DC accumulation in DLNs (Fig. 3, *A* and *B*). Finally, to investigate whether activation of PPARγ during the sensitization phase results in an altered development of LC-dependent immune response and, as a consequence, reduced pathology, the CHS response was measured 5 days after FITC challenge. When expressed as ear swelling, RSG-treated mice demonstrated a profoundly reduced CHS response (80% inhibition) compared with control mice (Fig. 3*C*).

RSG inhibits the migration of lung DCs to thoracic LNs

As RSG alters the hapten-induced departure of LCs from skin, we next investigated the possibility that it might impact on the rapid steady state migration of DCs from a mucosal site such as the lung. To test this hypothesis, FITC-conjugated OVA was administered intratracheally into mice together with increasing concentrations of RSG, and 24 h later thoracic LNs were analyzed by flow cytometry. As shown in Fig. 4, a high number of MHC class II$^+$, CD11c$^+$ (DCs)/FITC$^+$ cells was detected in thoracic LNs 24 h after instillation of FITC-OVA. Interestingly, RSG dose-dependently decreased the number of FITC$^+$ DCs in thoracic LNs. As the maximum inhibitory effect was obtained with 500 μM RSG

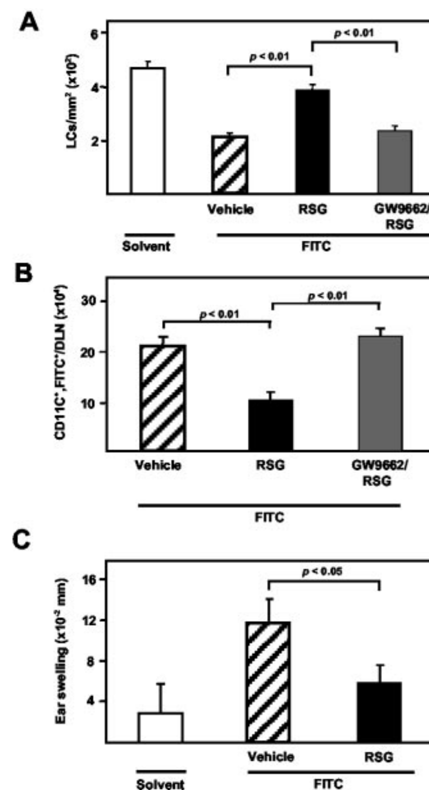

FIGURE 3. Effect of RSG on the FITC-induced migration of LCs and on CHS responses. Mice were injected i.d. into ear pinnae with 30 μl of RSG (10 μM) 15 min before and 5 h after FITC topical application. Epidermal LC density was analyzed 18 h after FITC painting (*A*), and the number of CD11c$^+$, FITC$^+$ cells present in the DLNs was determined 24 h after FITC application (*B*). *A* and *B*, In some cases mice were pretreated with 30 μl of GW9662 (10 μM) 15 min before RSG treatment. *C*, Five days after sensitization mice were challenged, and 24 h later ear thickness was measured. Results are expressed as the mean ± SD and are representative of three independent experiments ($n = 7$). Significant differences are shown.

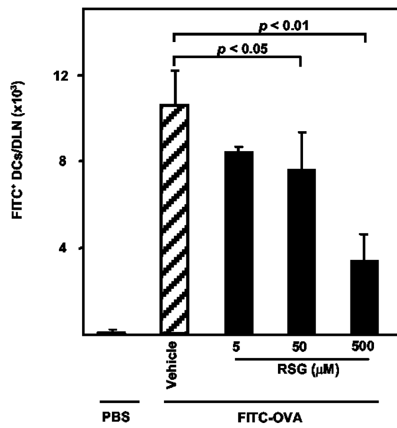

FIGURE 4. Effect of RSG on the migration of lung DCs to thoracic LNs. On day 0 mice were injected intratracheally with FITC-OVA alone or in the presence of increasing doses of RSG (5, 50, or 500 μM). On day 1 thoracic LNs were collected and analyzed by flow cytometry on bivariate plots of MHC class II vs CD11c and examined for FITC positivity. Dead cells and debris were excluded using propidium iodide. Results are expressed as the mean number of $FITC^+$ DCs (MHC class II^+, $CD11c^+$) ± SD (10–12 mice/group). This experiment is representative of three. Significant differences are indicated.

(68% inhibition), additional experiments were performed with this dose.

RSG impairs OVA-induced T cell proliferation and cytokine production in thoracic LNs

Since PPARγ activation seems important in controlling the migration of lung DCs, it may also impact on the development of the primary immune response elicited by Ag sensitization. This hypothesis was tested by examining in vivo the activation of Ag-specific T cells in the thoracic LNs of mice sensitized intratracheally with FITC-OVA in the absence or the presence of 500 μM RSG (Fig. 5*A*). Since in naive BALB/c mice, the precursor frequency of Ag-specific T cells is low, CFSE-labeled $CD4^+$ T cells from DO11.10 mice were adoptively transferred into naive syngeneic mice 2 days before sensitization, and their proliferation was studied 4 days after sensitization. As expected, flow cytometric analysis ($CFSE^+$, $CD4^+$, KJ1-26^+) of thoracic LNs showed that T cell division did not occur in the absence of sensitization (not shown). On the contrary, in RSG- and vehicle-treated sensitized mice, dividing cells were seen and had undergone up to eight divisions 4 days after OVA sensitization (Fig. 5*A*). However, in RSG-treated mice, although the maximal number of divisions reached by some daughter cells was not affected, the size of the T cell pool dividing in response to OVA was greatly reduced, leading to a decreased number of activated T cells.

It was next investigated whether RSG treatment could affect the production of cytokines by thoracic LN cells from OVA-sensitized mice (Fig. 5*B*). In this case, 4 days after OVA sensitization, thoracic LN cells were collected and cultured for another 4 days in the absence of exogenous OVA. Supernatants were then tested for the production of IFN-γ, IL-4, IL-5, and IL-10. As shown in Fig. 5*B*, compared with control mice, DLN cells from RSG-treated mice secreted reduced amounts of all cytokines tested.

Discussion

Epithelia, including the epidermal layer of the skin and lung epithelium, are sites of active arachidonic acid metabolism, particularly during inflammation or immune reactions. For instance, among the cyclooxygenase/lipoxygenase-derived products, leukotriene B_4, hydroxyeicosatetranoic acids, and PGD_2, the precursor of the cyclopentenone 15d-PGJ_2, are the major eicosanoids produced in epithelia. These compounds probably represent natural ligands for PPARs (12–15) and may therefore impact the function of peripheral immune cells by controlling gene expression or affecting cell motility. Our data demonstrate a novel function for PPARγ in the control of DC emigration and in the induction of the immune response.

In the present report we first show that PPARγ is expressed in epidermal LCs. Indeed, high amounts of PPARγ were found by RT-PCR and Western blot analysis in the immature epidermal LC line XS52. Flow cytometric analysis of freshly isolated epidermal cells confirmed the expression of PPARγ protein in LCs. Recently, we have demonstrated that PPARγ is functional in murine and human DCs and that it may affect the immune response by inhibiting the release of IL-12 (37, 38), a potent Th1-driving factor (58), in mature DCs. In another report PPARγ has been shown to alter the stimulatory function of human DCs (39). In the present study we evaluated the influence of PPARγ activation on the in vivo migratory properties of LCs/DCs, a key event implicated in the

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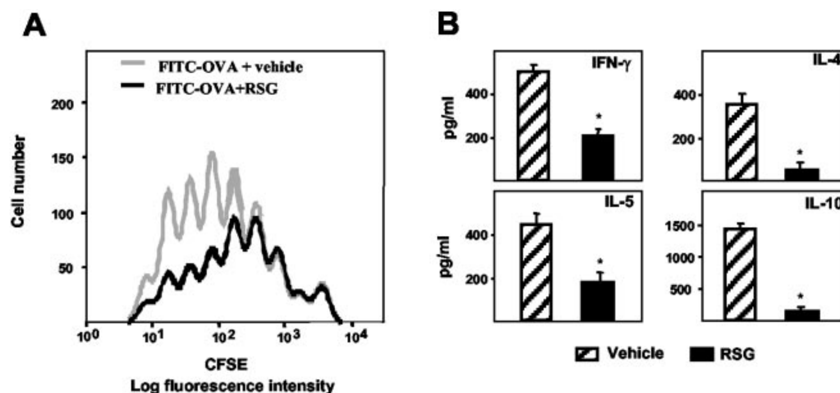

FIGURE 5. Effect of RSG treatment on the local response of T cells following intratracheal sensitization with OVA. Two days before sensitization, CFSE-labeled T cells from OVA TCR Tg mice were adoptively transferred to recipient mice. On day 0 mice were injected intratracheally with FITC-OVA (1 mg/ml) in the absence or the presence of 500 μM RSG. DLN cells were recovered on day 4. *A*, DLN cells were analyzed by FACS according to their scatter characteristics, expression of CD4, KJ1-26, and propidium iodide exclusion. Cell division in T cells corresponds to sequential halving of CFSE fluorescent (*x*-axis), and cell number is reported on the *y*-axis. Results show one plot/condition representative of 8–10 mice/group. *B*, LN cells were cultured for 4 days in 96-well plates, and cytokine production was quantified by ELISA. Results are representative of three independent experiments and are shown as the mean ± SD of 8–10 mice/group. *, $p < 0.01$.

initiation of the immune response. We show that PPARγ activation specifically inhibits the TNF-α-induced departure of LCs from the epidermis (optimal RSG concentration, 10 μM). Moreover, using a model of contact allergen-induced LC migration, the potent PPARγ agonist RSG impairs LC migration out of the skin and subsequent accumulation of DCs in DLNs. Importantly, this defect in LC mobilization after hapten sensitization was associated with a defective development of the local immune response, as assessed by CHS responses after challenge.

We next studied the possibility that PPARγ activation could also play a role in the spontaneous migration of intramucosal DCs (59) after intratracheal injection of FITC-OVA. Indeed, the conjugation of FITC to OVA protein eliminates the irritant effect of FITC (50) and allows to study the migration of endogenous airway DCs under steady state conditions. We found that the migration of lung DCs was also reduced, in a dose-dependent manner, by RSG (optimal concentration: 500 μM). It is likely that the difference in the optimal doses to inhibit the migration of lung DCs vs epidermal LCs is probably due to the higher surface of the epithelium in the lungs vs in the skin. Because the kinetics of LC/DC activation impact on the outcome of the immune response (60), the possibility was next investigated that, by inhibiting or delaying the migration of DCs to the DLNs, activation of PPARγ may affect the intensity and/or quality of the immune response. RSG treatment strongly impaired T cell proliferation in the DLNs and highly reduced the levels of both type 1 and type 2 cytokines produced by T cells. Although we cannot rule out the possibility that RSG also affects the viability and the maturation process of DCs in vivo, this indicates that in this system inhibition of DC migration impacts, in a quantitative manner, the outcome of the immune response.

To date, various anti-inflammatory compounds have been described to affect the in vivo migration of LCs/DCs, particularly by preventing the release of inflammatory cytokines, such as TNF-α or IL-1β (9, 10). More recently, we have described a mechanism blocking LC migration via PGD_2 interfering, through a cAMP-mediated pathway, with the TNF-α-induced signals implicated in LC departure (11). Although the role of PPARγ in the inhibitory effects exerted by PGD_2 cannot be ruled out, activation of the membrane-bound D prostanoid receptor is important in this phenomenon (11). In the present study the PPARγ-mediated mechanism that leads to the retention of LCs/DCs in epithelia has not been investigated. PPARγ activation induces the transcription of genes and also interferes negatively with signal transduction pathways such as NF-κB, AP-1, and mitogen-activated protein (MAP) kinases, to affect the synthesis of many genes involved in cell function. In our models the inhibitory action of PPARγ on DC migration may be linked to its ability to positively or negatively control the expression of genes involved in DC motility, such as inflammatory cytokines, chemokines, adhesion molecules, or chemokine receptors. In particular, the effect of PPARγ on reducing/preventing the expression of CCR7, a key chemokine receptor involved in DC motility, has been described (39) and may be involved in the process. Similarly, the recently described inhibitory effect of PPARγ on the expression and gelatinolytic activity of matrix metalloproteinase 9 (32), an enzyme recently shown to be implicated in DC migration (61), may play a part in the observed effect. Finally, because the inhibitory effect of PPARγ on LC/DC blockade is rapid (within 1 h), another more likely possibility is that PPARγ inhibits DC migration without a change in gene expression by interfering with intracellular pathways that are directly implicated in cellular movement. At present relatively little is known about the intracellular signaling pathways involved in DC movement and directed migration to DLNs. In other cells, signaling proteins and second messengers implicated in migration include increased intracellular calcium and activation of calcium/calmodulin-dependent kinase II, phosphatidylinositol 3-kinase, focal adhesion kinases, and NF-κB. Moreover, the MAP kinases are suggested to be key signaling intermediates mediating cell (62), including DC (63), migration. In neutrophils, it was reported that activated MAP kinase phosphorylates and thereby enhances myosin light-chain kinase activity, which, in turn, leads to myosin light chain phosphorylation and subsequent cell movement (62). Whether PPARγ impacts DC migration by targeting these signaling molecules and/or by directly inhibiting the activity of proteins involved in the modulation of the cytoskeleton is an open question that deserves further investigation.

Taken together, our data demonstrate for the first time that in vivo PPARγ activation inhibits cell motility and suggest that during inflammatory and/or immune responses, PPARγ activation by FA-derived ligands may play a role in innate/adaptive immunity by affecting DC migration and their subsequent accumulation in lymphoid organs. Therefore, our results underline a novel regulatory pathway for DC migration that could contribute to the initiation and modulation of immune responses. Our findings may also have important consequences in the improvement of therapeutic treatments that aim to control, in a positive or negative manner, the induction of the immune response. Moreover, since agonists of PPARγ are used for the treatment of type II (noninsulin-dependent) diabetes and are also considered as potential therapeutic agents against other diseases (64, 65), additional work is clearly required to delineate more precisely the impact of such treatment on the outcome of the immune response.

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