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Activation of Peroxisome Proliferator-Activated Receptor-γ in Dendritic Cells Inhibits the Development of Eosinophilic Airway Inflammation in a Mouse Model of Asthma

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Peroxisome proliferator-activated receptors (PPARs) are activated by an array of polyunsaturated fatty acid derivatives, oxidized fatty acids, and phospholipids and are proposed to be important modulators of immune and inflammatory responses. Recently, we showed that activation of PPAR-γ alters the maturation process of dendritic cells (DCs), the most potent antigen-presenting cells. In the present report, we investigated the possibility that, by targeting DCs, PPAR-γ activation may be involved in the regulation of the pulmonary immune response to allergens. Using a model of sensitization, based on the intratracheal transfer of ovalbumin (OVA)-pulsed DCs, we show that rosiglitazone, a selective PPAR-γ agonist, reduces the proliferation of Ag-specific T cells in the draining mediastinal lymph nodes but, surprisingly enough, dramatically increases the production of the immunoregulatory cytokine interleukin (IL)-10 by T cells, as compared to control mice sensitized with OVA-pulsed DCs. After aerosol challenge, the recruitment of eosinophils in the bronchoalveolar lavage fluids was strongly reduced compared to control mice. Finally, T cells from the mediastinal lymph nodes produced higher amounts of IL-10 and interferon-γ. Inhibition of IL-10 activity with anti-IL-10R antibodies partly restored the inflammation. The specificity of the phenomenon was confirmed by treating OVA-pulsed DCs with ciglitazone, another PPAR-γ agonist, and by using GW9662, a PPAR-γ antagonist. Our data suggest that PPAR-γ activation prevents induction of Th2-dependent eosinophilic airway inflammation and might contribute to immune homeostasis in the lung. ***(Am J Pathol 2004, 164:263–271)***

Dendritic cells (DCs) are powerful antigen-presenting cells with a unique capacity to stimulate naïve T cells.[1] In the airways, immature lung DCs are ideally placed to sample inhaled antigens.[2] After they acquire antigens in the periphery, DCs migrate to the draining lymph nodes (LNs) where they localize in the T cell-rich area and initiate immune responses. However, some evidence also suggests that tissue-resident DCs take up tissue antigens, migrate to the afferent LNs even in the absence of inflammatory conditions,[3] and contribute to the maintenance of tolerance.[4,5]

The signals that control DC migration from the periphery to the LNs are not fully understood. During inflammation, it is known that tumor necrosis factor-α and/or interleukin (IL)-1β provoke the departure of DCs from the periphery to the draining LNs by affecting the expression of adhesion molecules and chemokine receptors (particularly CCR7), by increasing the synthesis of metalloproteinases and by stimulating actin-dependent movements.[6–10] In addition to inflammatory cytokines and chemokines, other factors such as fatty acid derivatives also play a critical role in DC migration. For instance, LTC_4 and PGE_2, two major lipoxygenase and cyclooxygenase products of arachidonic acid, have been shown to promote the chemokine-driven DC migration.[11,12] On the other hand, by activating the D prostanoid receptor 1 (DP1), PGD_2 has an opposite action by preventing the departure of DCs from the skin and from the lung to the draining LNs.[13,14] PGD_2 is further metabolized into the cyclopentenone prostaglandin 15-deoxy-Δ(12,14)-prostaglandin-J_2 (15d-PGJ_2), a peroxisome proliferator-activated receptor (PPAR)-γ agonist. PPARs are nuclear eicosanoid receptor transcription factors that regulate adipocyte differentiation and metabolism.[15] Three PPAR isoforms (α, β, and γ) have been identified. PPAR-γ is expressed in adipocytes, in the vascular wall,[16] and in cells of the immune system such as monocytes/macrophages,[17,18] B and T cells,[19,20] and

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DCs.[21,22] Moreover, it has been proposed that PPAR-γ may possess anti-inflammatory properties.[23–25] We have recently shown that PPAR-γ, a fatty acid-activated nuclear receptor, inhibits the tumor necrosis factor-α-induced migration of epidermal Langerhans cells and reduces the spontaneous migration of lung DCs.[13,26] These findings have encouraged different laboratories to investigate the role of PPAR-γ as therapeutic targets for immune-mediated diseases. PPAR-γ agonists such as the thiazolidinedione class of anti-diabetic drugs have been shown to suppress experimental 2,4,6-trinitro-benzene sulfonic acid (TNBS) induced colitis and experimental allergic encephalitis.[27–29] Very recently, an effect of PPAR-γ agonists on lung allergic responses has also been observed.[30,31]

In this study, using a model of sensitization based on the intratracheal transfer of ovalbumin (OVA)-pulsed DCs,[32,33] we show that the PPAR-γ agonist rosiglitazone (RSG) inhibits the migration of antigen-loaded DCs in the mediastinal lymph nodes (MLNs) and reduces, in a quantitative and qualitative manner, the T-cell response in the MLNs. After challenge, a marked shift in the immune response [increased interferon (IFN)-γ and IL-10] was observed in the MLNs and was accompanied by a significant decrease in airway eosinophilia. These data suggest that activation of PPAR-γ in lung DCs might be important in the regulation of airway inflammatory diseases such as asthma.

Materials and Methods

Reagents and Antibodies

Reagents

OVA was from Worthington Biochemical Corp (Lakewood, NJ). At the dose we used in our experiments, the endotoxin level of OVA measured by a limulus-amebocyte lysate assay (Biowhittaker, Verviers, Belgium) was <0.001 μg. RSG was kindly provided by Dr. A. Bril (Glaxo Smithkline, Rennes). The PPAR-γ agonist ciglitazone and the PPAR-γ antagonist GW9662 were from Cayman (Ann Arbor, MI). Carboxyfluorescein diacetate succinimidyl ester (CFSE) was from Molecular Probes (Eugene, OR). The fluorescein isothiocyanate (FITC)-conjugated anti-I-Ad/I-Ed (M5/114.5.2), Cy-chrome-labeled anti-CD3 (145.2C11), and anti-CD45R (RA3-6B2), and APC-labeled anti-CD4 (RM4-5) and anti-CD11c (HL3) were all from Pharmingen (Heidelberg, Germany). The phycoerythrin (PE)-conjugated anti-CCR3 and KJ1-26 (clonotypic OVA-TCR) were from R&D (Abingdon, UK) and Caltag Laboratories (Burlingame, CA). The polyclonal rabbit anti-PPAR-γ was from Calbiochem (San Diego, CA) and the secondary FITC-labeled goat anti-rabbit was from Nordic Laboratories (Aalborg, Denmark). The CCL19-Fc was a gift from J. Cyster (University of San Francisco, San Francisco, CA) and the secondary FITC-labeled anti-human IgG Fab fragments were from Jackson Immunoresearch Laboratories (West Grove, PA).

Mice

BALB/c mice (6 to 8 weeks old) were purchased from Harlan (Zeist, The Netherlands). OVA-TCR transgenic mice (DO11.10) on a BALB/c background were bred at the Erasmus University (Rotterdam, The Netherlands).[34] All experiments were performed according to institutional guidelines of the animal ethics committee at Erasmus Medical Center.

Generation and Antigen Pulsing of Bone Marrow DCs

DCs were prepared as previously described.[35] Briefly, bone marrow was flushed with RPMI 1640 (Invitrogen, Paisley, UK) from femurs and tibiae of BALB/c mice. Cells were washed, enumerated, and plated in bacteriological 100-mm-diameter Petri dishes. Cell-culture medium (TCM) was RPMI 1640 supplemented with gentamicin (60 μg/ml), 2-mercaptoethanol (5×10^{-5} mol/L) and 5% fetal calf serum (Biocell Laboratories). At day 0 of the culture, the cells were seeded at a concentration of 2×10^6/dish in medium containing rm GM-CSF (200 IU/ml) (kindly provided by K. Thielemans, University of Brussels, Belgium). At day 3, TCM containing 200 IU/ml rm GM-CSF was added. At days 6 and 8, half of the medium was collected, centrifuged, and the pellet was resuspended in TCM containing 200 IU/ml of recombinant murine GM-CSF.

At day 9 of the culture, DCs were pulsed overnight with 100 μg/ml of OVA containing the vehicle (dimethyl sulfoxide) in which RSG was resuspended (OVA-DCs), or with medium alone as a control. To stimulate PPAR-γ, some plates were also treated with 10 μmol/L of RSG (RSG/OVA-DCs) or 10 μmol/L of ciglitazone added 30 minutes before addition of OVA. To confirm the specificity of the phenomenon, in some experiments, we also pretreated DCs with 10 μmol/L of the PPAR-γ antagonist GW9662 for 30 minutes before the addition of RSG. After antigen pulsing overnight, nonadherent DCs were collected, washed to remove free OVA or RSG, and resuspended in phosphate-buffered saline (PBS) at a concentration of 12.5×10^6 cells/ml. The viability of the DCs after treatment with the agonists or with the antagonist was $>99\%$ as assessed by trypan blue exclusion.

The phenotype of DCs was determined by staining for 30 minutes with CD11c-APC, MHCII-FITC, in combination with CD40-PE, CD80-PE, and CD86-PE dissolved in PBS containing 0.5% bovine serum albumin and 0.01% sodium azide. To detect CCR7 expression, DCs were first stained with CCL19-Fc for 30 minutes, washed, and incubated for another 30 minutes with anti-human IgG. DCs were washed and analyzed by flow cytometry on a FACScalibur (BD).

Intratracheal Injection of DCs

Mice were anesthetized with avertin (2% v/v in PBS) and 80 μl of the cell suspension (1×10^6 DCs) was instilled through the opening vocal cords.

Migration of DCs

At day 10 of the culture, unpulsed DCs and OVA-pulsed DCs treated or not with RSG were collected, washed, and labeled with CFSE as previously described.[33] One million DCs were instilled into the trachea of naïve BALB/c mice. Twenty-four hours later, MLNs were collected and minced using scissors. The LNs were then incubated for 1 hour at 37°C in RPMI 1640 containing 5% fetal calf serum, 1 mg/ml collagenase type 2 (Worthington), and 0.02 mg/ml DNase I (Sigma, Zwijndrecht, The Netherlands), according to a modified protocol.[36] LN cells were resuspended in PBS containing 10 mmol/L of ethylenediaminetetraacetic acid and centrifuged. $CFSE^+$ DCs were detected by flow cytometry on a FACScalibur. Cell viability was determined by trypan blue and was >95%.

Effect of RSG on the Activation of OVA-Specific Naïve T Cells in a Primary Immune Response

Because the frequency of OVA-specific T cells is very low in immunized animals, the primary activation of a naïve T cell is difficult to detect. To avoid this problem, a detectable number of naïve T cells purified from DO11.10 mice were adoptively transferred into BALB/c mice. Briefly, LNs and spleen were collected from DO11.10 mice and smashed. After red blood cell lysis, cells were labeled with CFSE. Cells were enumerated and dead cells, stained for trypan blue, were excluded. Cells (10×10^6) were injected intravenously in the lateral tail vein of BALB/c mice (day −2). On day 0, the mice received an intratracheal injection of OVA-DC, RSG/OVA-DC, or control unpulsed DCs. On day 4, MLNs were collected, homogenized, and stained for the presence of $KJ1\text{-}26^+$ $CD4^+$ reactive OVA-specific T cells. Some of the LN cells (200,000 cells/well in triplicates) were resuspended in RPMI 1640 containing 5% fetal calf serum and antibiotics and placed in 96-well plates. Four days later, supernatants were harvested and analyzed for the presence of Th1 (IFN-γ) and Th2 (IL-4, IL-5, and IL-10) cytokines by enzyme-linked immunosorbent assay (BD Pharmingen).

Effect of PPAR-γ Agonists on the Potential of DCs to Prime for Eosinophilic Airway Inflammation

On day 0, BALB/c mice were injected intratracheally with unpulsed DCs, OVA-DCs treated or not with ciglitazone (ciglitazone/OVA-DCs), or with RSG (RSG/OVA-DCs). To confirm the specificity of the phenomenon, RSG/OVA-DCs were also treated or not with the antagonist GW9662 (GW9662/RSG/OVA-DCs). In some experiments, mice received 250 μg of blocking anti-IL-10R antibodies or control antibodies (BD) 1 day before secondary challenge. From days 10 to 13, mice were exposed to 30-minute OVA aerosols (Grade III, Sigma). Mice were sacrificed 24 hours after the last aerosol. Bronchoalveolar lavage (BAL) was performed with 3×1 ml of Ca^{2+}- and Mg^{2+}-free HBSS (Invitrogen) supplemented with 0.1 mmol/L of sodium ethylenediaminetetraacetic acid. The BAL fluid was centrifuged; the cells were resuspended in HBSS, and enumerated in a hemocytometer. After washing, cells were stained for 30 minutes with anti-I-Ad/I-Ed FITC (macrophages), anti-CCR3 PE (eosinophils), anti-CD3 cy-chrome, anti-B220 cy-chrome (T and B cells, respectively), and anti-CD11c APC (macrophages) in PBS containing 0.5% bovine serum albumin and 0.01% sodium azide. Cells were washed and analyzed by flow cytometry as previously described.[37]

Airway Histology

After BAL was performed, 1 ml of fixative was gently infused through the catheter. The lungs were resected and embedded in paraffin. Four-μm sections were performed and stained with May-Grunwald Giemsa.

Cytokine Measurements in MLNs in a Secondary Response

MLNs were removed, homogenized, and resuspended in RPMI 1640 containing 5% fetal calf serum and antibiotics before enumeration. *Ex vivo* production of cytokines by T cells collected from the MLNs was measured after restimulation of 2×10^6 cells/ml with 10 μg/ml of OVA for 4 days.

Statistical Analysis

For all experiments, the difference between the groups was calculated using the Mann-Whitney *U*-test for unpaired data. Differences were considered significant if P was <0.05.

Results

We have previously established a model of adoptive transfer of bone marrow-derived DCs (BM-DCs) pulsed with OVA into the trachea of naïve mice. This model generates a primary OVA-specific immune response in the lung draining MLNs[33] that leads to Th2 priming. When mice are challenged with OVA aerosols, a Th2-dependent eosinophilic airway inflammation develops.[32] This model was used to test the effect of RSG.

RSG Impairs OVA-Induced BM-DCs Maturation

We have previously shown that mouse spleen DCs taken from Flt-3L-treated mice express the PPAR-γ.[21] By staining permeabilized DCs with a polyclonal rabbit anti-PPAR-γ antibody, we confirmed that BM-DCs also express PPAR-γ (Figure 1). We next investigated whether RSG, a PPAR-γ agonist, could alter the OVA-induced maturation of BM-DCs. Compared to unpulsed DCs, the expression of the co-stimulatory molecules CD40, CD80, and CD86, but also of MHC II was increased in OVA-DCs (100 μg OVA/ml), a phenomenon probably caused by trace amounts of LPS in commercially available OVA. However, PPAR-γ activation by RSG treatment did not

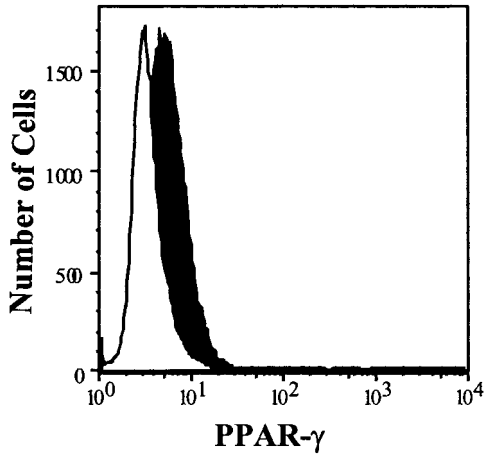

Figure 1. PPAR-γ expression in BM-DCs. DCs were fixed, permeabilized, and stained for PPAR-γ expression (**black histogram**). **White histogram** represents fluorochrome-matched isotype control mAbs.

modify the expression of these markers on the cells (data not shown). Compared to unpulsed DCs, OVA-DCs express a higher amount of CCR7, as assessed by CCL19-Fc binding (Figure 2). However, compared to untreated OVA-pulsed DCs, CCR7 expression was down-regulated in cells treated with RSG. These data suggest that PPAR-γ activation alters DC maturation by affecting the expression of CCR7, a chemokine receptor involved in DC emigration, but not the synthesis of costimulatory molecules and MHC II.

Activation of PPAR-γ Inhibits the Migration of OVA-DCs in Vivo

Because the treatment of OVA-pulsed DCs with RSG decreased the expression of CCR7, we next hypothesized that PPAR-γ activation might affect the migratory capacities of DCs to the MLNs. As shown in Figure 3, when injected into the trachea, CFSE-labeled OVA-DCs could be detected in the MLNs 24 hours after the instillation.[32,37] However, the treatment of OVA-DCs with RSG reduced their capacity to reach the MLNs, as compared to mice injected with OVA-DCs. These data were confirmed *in vitro* in chemotaxis assays performed in transwells, where the migration of OVA-DCs treated with RSG at the dose of 10 μmol/L in response to the CCR7 ligand MIP-3β was highly reduced as compared to OVA-DCs. Treatment of OVA-DCs with 1 μmol/L of RSG did not modify the migration as compared to untreated OVA-DCs (data not shown). These data suggest that the activation of PPAR-γ interferes with the CCR7-mediated migratory capacities of DCs. Because only the dose of 10 μmol/L of RSG altered DC migration *in vitro*, all further experiments were performed with this single dose of RSG.

Activation of PPAR-γ Affects T-Cell Proliferation and Differentiation in the MLNs

As PPAR-γ activation affects the migration of OVA-pulsed DCs to the MLNs, we next investigated whether it could

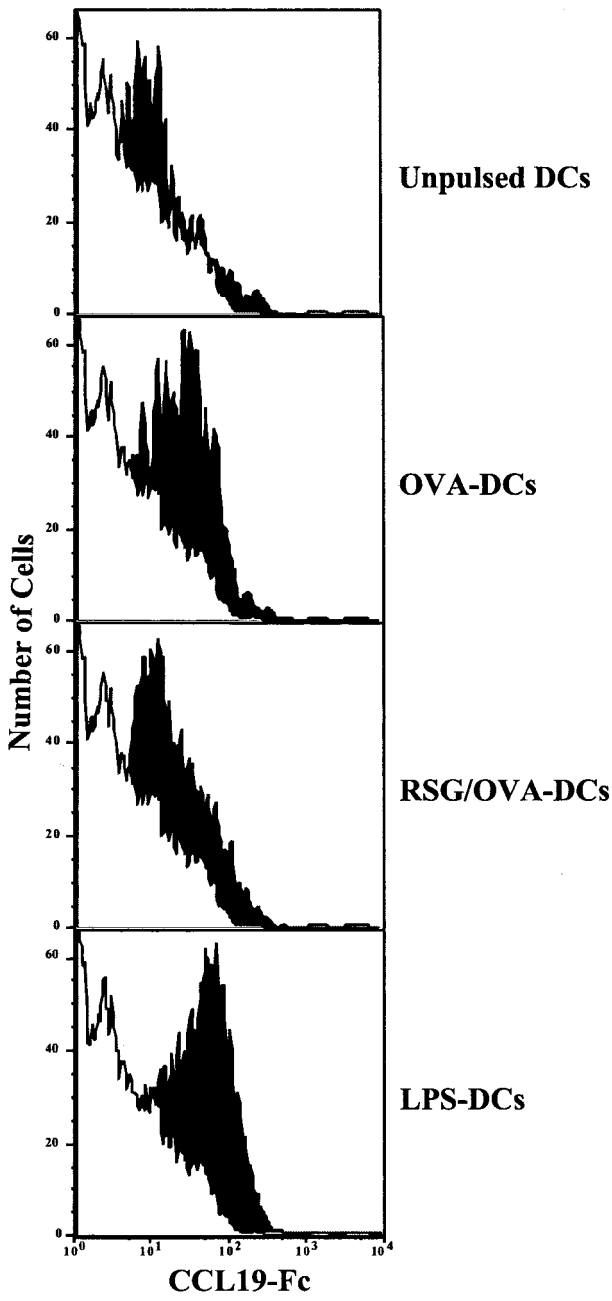

Figure 2. Effect of RSG treatment on CCR7 expression by DCs. BM-DCs were pulsed or not overnight with 100 μg/ml of OVA in the presence or in the absence of 10 μmol/L of RSG. LPS at the dose of 500 ng/ml was also used as a positive control. Cells were incubated with CCL19-Fc for 30 minutes before addition of PE-labeled anti-human IgG (**black histograms**). **White histogram** represents fluorochrome-matched isotype control mAbs.

also impact the activation of naïve T cell by DCs within the MLNs. To this end, naïve T cells from DO11.10 mice were labeled with CFSE and adoptively transferred, on day −2, into BALB/c mice. On day 0, these mice received an intratracheal administration of OVA-DCs, RSG/OVA-DCs, or unpulsed DCs. Flow cytometry was used to track cell division of CFSE-labeled T cells in MLNs. Figure 4 shows that 4 days after transfer of OVA-DCs, some transgenic T cells had already undergone seven divisions. In mice that received RSG/OVA-DC, some T cells also reached the

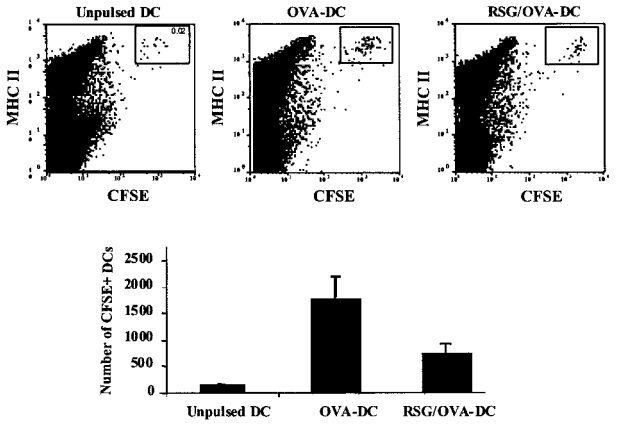

Figure 3. RSG treatment inhibits the migration of OVA-pulsed DCs. BM-DCs were pulsed or not with OVA overnight in the presence or in the absence of RSG (RSG/OVA-DCs). The next day, DCs were labeled with CFSE and 1×10^6 OVA-DCs, RSG/OVA-DCs, or unpulsed DCs were instilled into the trachea of naïve BALB/c mice. Twenty-four hours later, the presence of migrating CFSE-labeled DCs was investigated in the MLNs. **Top**: Plots show one representative mouse of four. The histogram represents the mean number of $CFSE^+$ DCs ± SEM from five mice per group.

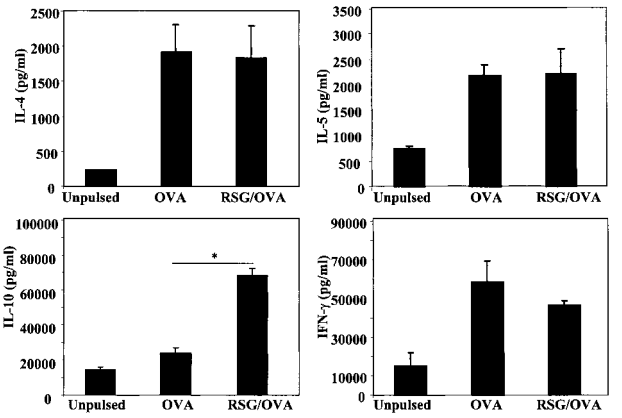

Figure 5. RSG-treated DCs modify the pattern of cytokine production of naïve T cells. On day −2, BALB/c mice received a cohort of CFSE-labeled OVA-specific naïve T cells. On day 0, mice were administered intratracheally with OVA-DCs, RSG/OVA-DCs, or with unpulsed DCs. Four days later, T cells from MLNs were collected and incubated for another 4-day period at 37°C. Supernatants were harvested and assayed for the presence of IL-4, IL-5, IL-10, and IFN-γ. Results are represented as mean ± SEM from 10 to 12 mice per group. *, $P < 0.05$.

seventh division peak, but the total number of naïve T cells activated and recruited into divisions was lower as compared to the group immunized with OVA-DCs. As expected, in mice immunized with unpulsed DCs, naïve T cells failed to divide (data not shown).

As PPAR-γ activation impacts the activation of OVA-specific naïve T cells in the MLNs, we next investigated whether the cytokine profile of T cells obtained from the MLNs was also changed. At day 4, cells from MLNs of mice that received OVA-DC, RSG/OVA-DCs, or unpulsed DCs were plated at 37°C for another 4-day period in the absence of OVA. The supernatants were then collected and assayed for the presence of IL-4, IL-5, IL-10, and IFN-γ. As expected, in mice that received OVA-DCs, T cells produced more IL-4, IL-5, IL-10, and IFN-γ than the cells of mice that were injected with unpulsed DCs (Figure 5). However, when mice were instilled with RSG/OVA-DCs, the production of IL-4, IL-5, and IFN-γ was identical but the level of the immunoregulatory cytokine IL-10 was dramatically enhanced (2.9-fold increase) as compared to the mice immunized with OVA-DCs. These results show that activation of PPAR-γ affects T-cell activation and modifies the nature of the primary immune response in the MLNs.

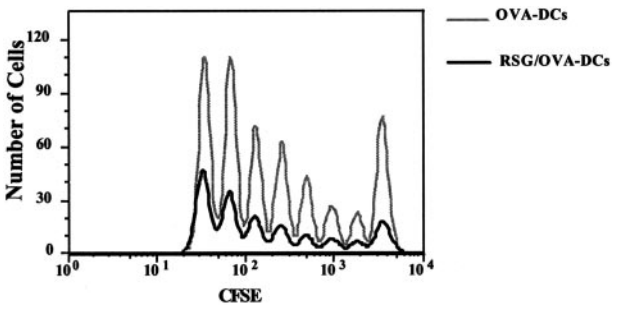

Figure 4. RSG impairs T-cell proliferation in the MLNs. On day −2, BALB/c mice received a cohort of CFSE-labeled OVA-specific naïve T cells. On day 0, mice were administered intratracheally with OVA-DCs, RSG/OVA-DCs, or with unpulsed DCs. Four days later, the proliferation of $CD4^+$ KJ1–26^+ $CFSE^+$ T cells was analyzed by flow cytometry. Results show one representative experiment of 10 to 12 mice per group. *, $P < 0.05$.

Activation of PPAR-γ Reduces Eosinophilic Inflammation Induced by DCs

As PPAR-γ activation in DCs induced a T-cell response in the MLNs with maintained levels of IL-4, IL-5, and IFN-γ, but increased levels of IL-10, we next studied whether PPAR-γ stimulation in DCs could modify their inherent capacity to prime for eosinophilic airway inflammation on secondary challenge to OVA aerosol. For this purpose, mice were immunized as described before and re-exposed to OVA aerosols 10 days later.[32,37] BAL fluids were collected 24 hours after the last OVA aerosol exposure. As expected, in mice that received unpulsed DCs, only a few inflammatory cells, mainly macrophages, were observed in the BAL (Figure 6). In contrast, in mice immunized with OVA-DCs, a strong cell recruitment occurred as confirmed by the high number of lymphocytes and eosinophils in the BAL fluid of these mice. However, when mice received OVA-DCs treated with RSG (RSG/OVA-DCs) or with ciglitazone, another PPAR-γ agonist (ciglitazone/OVA-DCs), the number of inflammatory cells including lymphocytes and eosinophils was significantly reduced as compared to mice immunized with OVA-DCs. To confirm the specificity of the phenomenon, in some experiments, mice also received RSG/OVA-DCs pretreated with the PPAR-γ antagonist GW9662. In mice that received GW9662-treated DCs, the eosinophilia was restored and was comparable to the one present in mice that received OVA-DCs. Because RSG-treated DCs induced a T-cell response with high levels of IL-10, the importance of this cytokine in the establishment of airway eosinophilia was investigated. For this purpose, some mice that received RSG/OVA-DCs on day 0 were injected intraperitoneally with 250 μg of blocking anti-IL-10Rα antibodies 1 day before the first aerosol exposure. As

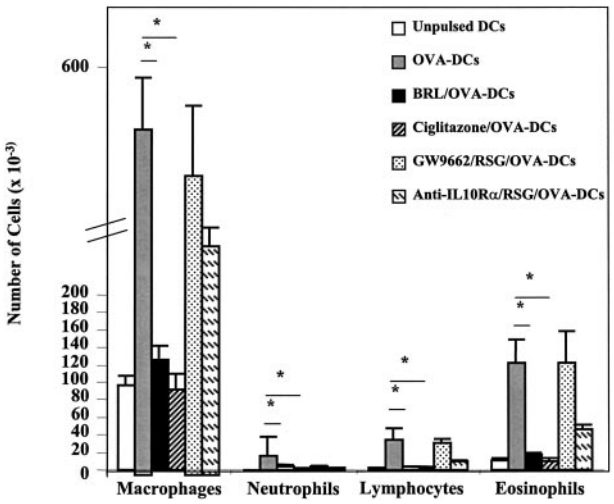

Figure 6. RSG-treated DCs modify the cellular composition of BAL. On day 0, mice received an intratracheal injection of OVA-DCs, ciglitazone/OVA-DCs, RSG/OVA-DCs treated with the PPAR-γ antagonist GW9662 (GW9662/RSG/OVA-DCs), or unpulsed DCs. From days 10 to 13, mice were exposed to OVA aerosols. To test the role of IL-10 in RSG-induced effects, some mice that received RSG/OVA-DCs on day 0 were injected with anti-IL-10Rα (IL-10Ra/RSG/OVA-DCs) 24 hours before aerosol exposure. Twenty-four hours after the last aerosol, BAL was performed. Results are expressed as mean ± SEM from 8 to 10 mice per group. *, $P < 0.05$.

shown in Figure 6, the pretreatment of mice with anti-IL-10Rα could partially restore the BAL eosinophilia as compared to mice injected with RSG/OVA-DCs, suggesting that the effects induced by RSG-treated DCs were partly mediated through an IL-10-dependent mechanism.

Because the activation of PPAR-γ reduced the number of inflammatory cells in the BAL, we next looked at the inflammation at the tissue level. Histological analysis of the lungs of mice injected with OVA-DCs and re-exposed to OVA aerosols revealed strong perivascular and peribronchial inflammatory lesions (Figure 7) composed mainly of eosinophils and mononuclear cells. These changes were absent from the lungs of mice injected with unpulsed DCs (data not shown).[32] Interestingly, in the lungs of mice injected with RSG/OVA-DCs, less inflammatory cells were present around the bronchi or around the vessels of the lungs compared to mice immunized with OVA-DCs.

Activation of PPAR-γ Impairs Cytokine Production in MLNs

To study the mechanism of inhibition of eosinophilic airway inflammation, we quantified the production of cytokines by T cells from MLNs. To this end, MLNs were collected 24 hours after the last aerosol exposure, homogenized, and cells were restimulated *in vitro* for 4 days with OVA. Supernatants were harvested and assayed for the presence of Th1/Th2 cytokines. In mice that received an intratracheal injection of OVA-pulsed DCs, the restimulation of MLNs with OVA led to a strong Th2 response with an up-regulation of the production of IL-4 and IL-5, as compared to control mice that received unpulsed DCs (Figure 8). Interestingly, in mice injected with RSG/OVA-DCs, the levels of IL-4 and IL-5 produced by MLN T cells were similar to those observed in the OVA-DC group. However, the levels of IL-10 but also of IFN-γ were higher in mice injected with RSG/OVA-DCs than in mice that received OVA-DCs.

Discussion

Migration of DCs from the periphery to the draining LN is a critical step for the induction of immunity. However, the signals controlling the migration of DCs to the nodes and controlling immunity are still poorly understood. Recently the role of lipid mediators such as polyunsaturated fatty acids, leukotrienes, and prostaglandins has received a lot of attention as inflammatory mediators with immunomodulatory potential on DCs and T-cell activation. Many of these lipid mediators such as the α-linoleic acid, γ-linoleic acid, arachidonic acid, 9-hydroxyoctadeca-9Z, 11E-dienoci acid (9-HODE), 15d-PGJ_2 are agonists of PPAR-γ, a nuclear receptor widely expressed on cells of the immune system, including DCs. Here we show that PPAR-γ activation by a selective agonist, namely RSG, impairs the emigration of intratracheally injected BM-DCs from the lung to the MLNs, probably by reducing the expression of the CCR7 receptor necessary for migration to the draining nodes. In addition, RSG-treated DCs failed to respond to the CCR7 ligands CCL19 and CCL21 in a chemotaxis assay performed *in vitro* (data not shown). PPAR-γ activation has also been reported to affect human DC maturation by reducing different costimulatory molecules,[22,38] although in our hands, no effect on the expression of CD40, CD80, or CD86 was observed (data not shown). Differences in the mode of stimulation or in the origin of DCs might explain this discrepancy.[21]

In the next step, we then investigated whether the intratracheal injection of OVA-DCs treated with RSG could affect the proliferation of adoptively transferred naive OVA-specific T cells. In mice injected with RSG/OVA-DCs, the number of cell divisions was not affected but the number of OVA-specific T cells recruited into divisions was decreased as compared to mice instilled with OVA-DCs. Therefore, treatment of DCs with RSG does not alter the antigen capture capability nor the capacity to activate naïve T cells, but merely reduces the strength of the induced T-cell response. The lower expression of CCR7 on DCs treated with RSG might limit the number of antigen-presenting cells reaching the thoracic nodes, which might, in turn, affect the outcome of the primary immune response as already reported in *plt* mice[39] or CCR7-deficient mice.[40] Interestingly, the pattern of cytokines produced by the T cells activated by RSG/OVA-DCs was different from the one induced by OVA-DCs. As compared to mice injected with OVA-DC, the production of the immunoregulatory cytokine IL-10 was dramatically increased in mice that received RSG/OVA-DCs, whereas the levels of IL-4, IL-5, and IFN-γ were similar in both groups. These results are in discrepancy with another study in which we showed that RSG induced a general decrease in the cytokine production induced by FITC-OVA.[26] In the latter study, RSG was

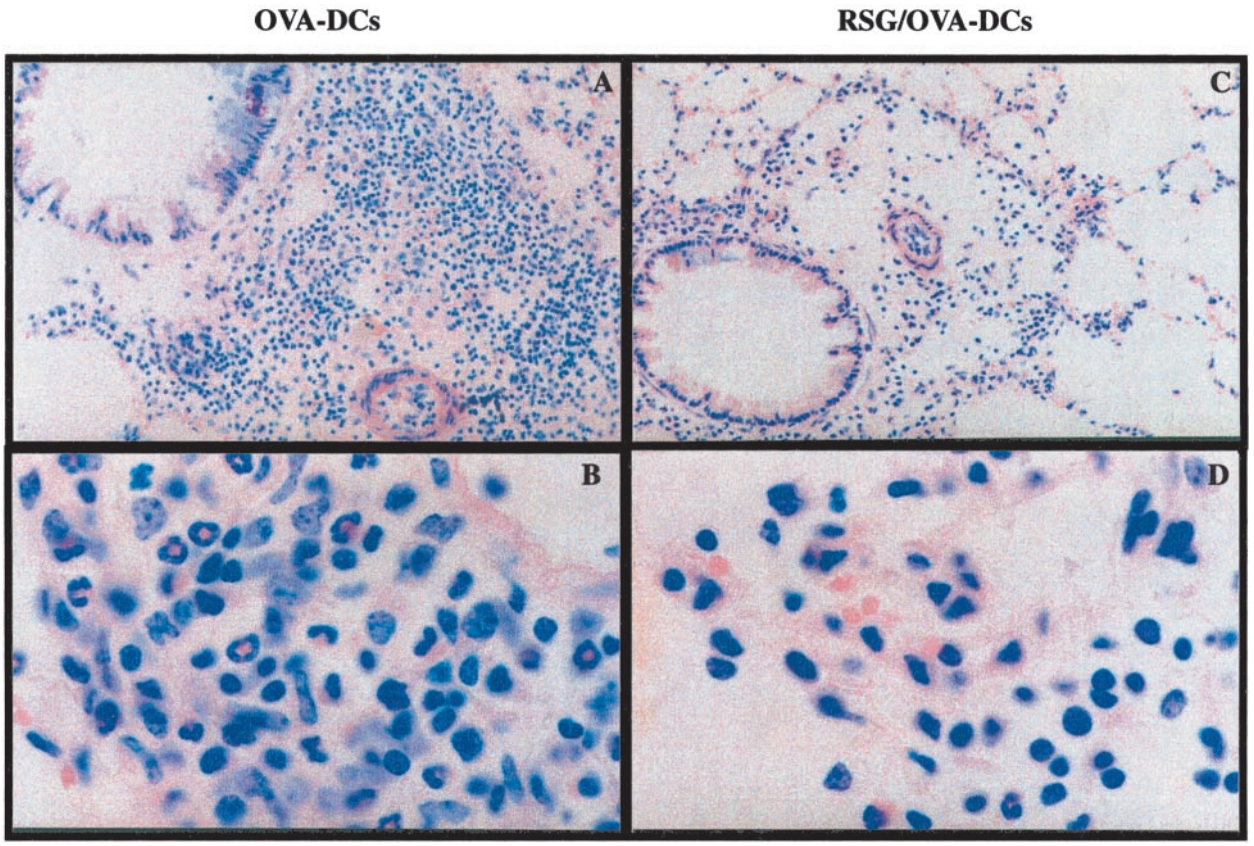

Figure 7. RSG-treated DCs reduce the pulmonary inflammatory response in the lung. On day 0, mice received an intratracheal injection of OVA-DCs, RSG/OVA-DCs, or unpulsed DCs. From days 10 to 13, mice were exposed to OVA aerosols. Four-μm sections of the lungs were stained with May-Grunwald Giemsa. Mice that received OVA-DCs revealed strong perivascular and peribronchial inflammatory lesions composed mainly of eosinophils and mononuclear cells. These features were strongly reduced in the lungs of mice injected with RSG/OVA-DCs. Original magnifications: ×20 (**A**, **C**); ×100 (**B**, **D**).

introduced directly into the trachea of mice and therefore one cannot rule out an indirect effect of RSG on airway DCs. Here, RSG was given *in vitro* to DCs and washed away before instillation. The effects induced might strictly be because of direct modifications of DC functions by

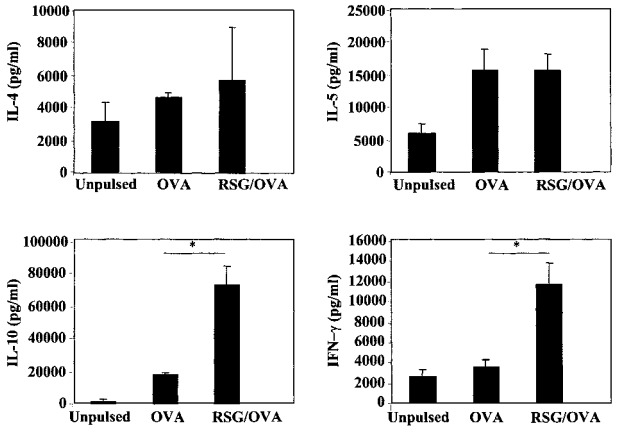

Figure 8. RSG-treated DCs modify the pattern of cytokine production of T cells after re-exposure of the mice to OVA aerosols. On day 0, mice received an intratracheal injection of OVA-DCs, RSG/OVA-DCs, or unpulsed DCs. From days 10 to 13, mice were exposed to OVA aerosols. Twenty-four hours after the last aerosol, cells from MLNs were collected and restimulated for 4 days in the presence of OVA. The supernatants were harvested and assayed for the presence of IL-5, IL-10, and IFN-γ. Results show the mean ± SEM from 8 to 10 mice per group. *, $P < 0.05$.

PPAR-γ agonists. We have previously shown that OVA-pulsed BM-DCs injected into the trachea lead to Th2 immunity that primes for the development of eosinophilic airway inflammation on rechallenge with OVA aerosols.[32,37] When the mice were instilled with RSG/OVA-DCs and exposed to OVA aerosols 10 days later, T cells from the LN restimulated *in vitro* with OVA produced the same amounts of IL-4 and IL-5 but higher levels of IL-10 but also of IFN-γ as compared to mice that received untreated OVA-DCs. In mice that received RSG-treated DCs, the number of eosinophils in the BAL and lung inflammation was dramatically reduced as compared to mice injected with OVA-DCs. As the levels of IL-5 during the secondary immune response are not affected by the treatment of DCs with RSG, we can hypothesize that the decreased eosinophilia might be because of the high levels of IL-10 produced in these mice. IL-10 by accelerating the eosinophil death[41] may play an important regulatory role in airway allergic responses as already reported.[42,43] Our data suggest that RSG-treated DCs can induce the generation of a population of IL-10- and IFN-γ-producing T cells that could suppress some of the features of asthma. These T cells have similarities with Tr1 cells that also secrete high levels of IL-10 and IFN-γ.[44] More recently, it was shown that such regulatory T cells could also inhibit Th2-specific responses *in vivo*.[45,46] However, further experiments aimed at charac-

terizing phenotypically and functionally the T cells in MLNs of mice injected with RSG/OVA-DCs remain to be performed. The production of IL-10 induced by RSG-treated DCs was physiologically relevant as inhibition of signaling through IL-10R with blocking antibodies partially restored inflammation. Thus, from our data, it seems that RSG-treated DCs can improve the features of asthma when given during the sensitization phase. To test the effect of RSG on an ongoing Th2 response, some mice were injected with OVA-DCs and fed or not with RSG 30 minutes before each OVA aerosol. RSG failed to reduce eosinophilic airway inflammation but inhibited lymphocytic and neutrophilic inflammation (data not shown). These results are in contradiction with those from Trifilieff and colleagues[31] in which they show that another PPAR-γ agonist (GI262570) could down-regulate eosinophilic airway inflammation. The reasons of such differences between our results might be because of the route of administration of each compound (gavage *versus* intranasally).

It remains to be proven whether endogenous activators of PPAR-γ such as polyunsaturated fatty acids, eicosanoids, and cyclopentenone prostaglandins (PGs) contribute to down-regulation of sensitization to inhaled allergens. Alveolar macrophages have been shown to suppress the activation of T cells in the lung through inhibitory effects on airway and interstitial DCs, mediated by nitric oxide, transforming growth factor-β, IL-10, or IL-1RA.[47] Alveolar macrophages are a rich source of 15d-PGJ_2, a major PGD_2 metabolite, which could down-regulate DC function under steady-state conditions through PPAR-γ activation. Similarly, at the resolution of inflammation, there is high-level expression of the inducible COX-2 enzyme, generating mainly PGD_2 rather than PGE_2.[48] It is likely that metabolism of PGD_2 would subsequently dampen the immune response by ligation of PPAR-γ in DCs. Finally, there is an association between altered levels of plasma polyunsaturated fatty acids and the risk to develop atopy, although it remains to be proven that PPAR-γ stimulation of DCs would be implicated as a mechanism to reduce sensitization to inhaled allergens.[49] The immunomodulatory capacity of PPAR-γ agonists to inhibit sensitization to inhaled allergens clearly deserves more attention.

This is another example of the extraordinary capacity of PPAR-γ agonists to serve as anti-inflammatory compounds.[25,27–29] It was previous hypothesized that PPAR-γ agonists might be good tools for therapeutic intervention in asthma, because of their inhibiting effects on chemokine release by bronchial epithelial cells.[50] Our data suggest that they have additional suppressing effects on airway DCs, known to be important in sensitization to inhaled antigen and for maintaining established eosinophilic airway inflammation.[51]

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References

1. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 1998, 392:245–252
2. Lambrecht BN: Allergen uptake and presentation by dendritic cells. Curr Opin Allergy Clin Immunol 2001, 1:51–59
3. Heath WR, Carbone FR: Cross-presentation, dendritic cells, tolerance and immunity. Annu Rev Immunol 2001, 19:47–64
4. Steinman RM, Turley S, Mellman I, Inaba K: The induction of tolerance by dendritic cells that have captured apoptotic cells. J Exp Med 2000, 191:411–416
5. Dhodapkar MV, Steinman RM, Krasovsky J, Munz C, Bhardwaj N: Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. J Exp Med 2001, 193:233–238
6. Cyster JG: Chemokines and cell migration in secondary lymphoid organs. Science 1999, 286:2098–2102
7. Cyster JG: Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. J Exp Med 1999, 189:447–450
8. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT: A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci USA 1998, 95:258–263
9. Ngo VN, Tang HL, Cyster JG: Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells. J Exp Med 1998, 188:181–191
10. Gunn MD, Kyuwa S, Tam C, Kakiuchi T, Matsuzawa A, Williams LT, Nakano H: Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. J Exp Med 1999, 189:451–460
11. Robbiani DF, Finch RA, Jager D, Muller WA, Sartorelli AC, Randolph GJ: The leukotriene C(4) transporter MRP1 regulates CCL19 (MIP-3beta, ELC)-dependent mobilization of dendritic cells to lymph nodes. Cell 2000, 103:757–768
12. Scandella E, Men Y, Gillessen S, Forster R, Groettrup M: Prostaglandin E2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. Blood 2002, 100:1354–1361
13. Angeli V, Faveeuw C, Roye O, Fontaine J, Teissier E, Capron A, Wolowczuk I, Capron M, Trottein F: Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. J Exp Med 2001, 193:1135–1147
14. Hammad H, de Heer HJ, Soullié T, Hoogsteden HC, Trottein F, Lambrecht BN: Prostaglandin D2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. J Immunol 2003, 171:3936–3940
15. Oberfield JL, Collins JL, Holmes CP, Goreham DM, Cooper JP, Cobb JE, Lenhard JM, Hull-Ryde EA, Mohr CP, Blanchard SG, Parks DJ, Moore LB, Lehmann JM, Plunket K, Miller AB, Milburn MV, Kliewer SA, Willson TM: A peroxisome proliferator-activated receptor gamma ligand inhibits adipocyte differentiation. Proc Natl Acad Sci USA 1999, 96:6102–6106
16. Chinetti G, Fruchart JC, Staels B: Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 2000, 49:497–505
17. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B: Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages. J Biol Chem 1998, 273:25573–25580
18. Marx N, Sukhova G, Murphy C, Libby P, Plutzky J: Macrophages in human atheroma contain PPARgamma: differentiation-dependent peroxisomal proliferator-activated receptor gamma (PPARgamma) expression and reduction of MMP-9 activity through PPARgamma activation in mononuclear phagocytes in vitro. Am J Pathol 1998, 153:17–23
19. Yang XY, Wang LH, Chen T, Hodge DR, Resau JH, DaSilva L, Farrar WL: Activation of human T lymphocytes is inhibited by peroxisome proliferator-activated receptor gamma (PPARgamma) agonists. PPARgamma co-association with transcription factor NFAT. J Biol Chem 2000, 275:4541–4544
20. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L, Padula SJ: The nuclear receptor PPAR gamma and immunoregulation:

pPAR gamma mediates inhibition of helper T cell responses. J Immunol 2000, 164:1364–1371
21. Faveeuw C, Fougeray S, Angeli V, Fontaine J, Chinetti G, Gosset P, Delerive P, Maliszewski C, Capron M, Staels B, Moser M, Trottein F: Peroxisome proliferator-activated receptor gamma activators inhibit interleukin-12 production in murine dendritic cells. FEBS Lett 2000, 486:261–266
22. Gosset P, Charbonnier AS, Delerive P, Fontaine J, Staels B, Pestel J, Tonnel AB, Trottein F: Peroxisome proliferator-activated receptor gamma activators affect the maturation of human monocyte-derived dendritic cells. Eur J Immunol 2001, 31:2857–2865
23. Jiang C, Ting AT, Seed B: PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 1998, 391:82–86
24. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK: The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 1998, 391:79–82
25. Daynes RA, Jones DC: Emerging roles of PPARs in inflammation and immunity. Nat Rev Immunol 2002, 2:748–759
26. Angeli V, Hammad H, Staels B, Capron M, Lambrecht BN, Trottein F: Peroxisome proliferator-activated receptor gamma inhibits the migration of dendritic cells: consequences for the immune response. J Immunol 2003, 170:5295–5301
27. Desreumaux P, Dubuquoy L, Nutten S, Peuchmaur M, Englaro W, Schoonjans K, Derijard B, Desvergne B, Wahli W, Chambon P, Leibowitz MD, Colombel JF, Auwerx J: Attenuation of colon inflammation through activators of the retinoid X receptor (RXR)/peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimer. A basis for new therapeutic strategies. J Exp Med 2001, 193:827–838
28. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD: A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999, 104:383–389
29. Niino M, Iwabuchi K, Kikuchi S, Ato M, Morohashi T, Ogata A, Tashiro K, Onoe K: Amelioration of experimental autoimmune encephalomyelitis in C57BL/6 mice by an agonist of peroxisome proliferator-activated receptor-gamma. J Neuroimmunol 2001, 116:40–48
30. Woerly G, Honda K, Loyens M, Papin JP, Auwerx J, Staels B, Capron M, Dombrowicz D: Peroxisome proliferator-activated receptors {alpha} and {gamma} down-regulate allergic inflammation and eosinophil activation. J Exp Med 2003, 198:411–421
31. Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E, Whittaker P: PPAR-alpha and -gamma but not -delta agonists inhibit airway inflammation in a murine model of asthma: in vitro evidence for an NF-kappaB-independent effect. Br J Pharmacol 2003, 139:163–171
32. Lambrecht BN, De Veerman M, Coyle AJ, Gutierrez-Ramos JC, Thielemans K, Pauwels RA: Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. J Clin Invest 2000, 106:551–559
33. Lambrecht BN, Pauwels RA, Fazekas De St Groth B: Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. J Immunol 2000, 164:2937–2946
34. Murphy KM, Heimberger AB, Loh DY: Induction by antigen of intrathymic apoptosis of CD4+CD8+TCRlo thymocytes in vivo. Science 1990, 250:1720–1723
35. Lutz MB, Kukutsch N, Ogilvie AL, Rossner S, Koch F, Romani N, Schuler G: An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. J Immunol Methods 1999, 223:77–92
36. Vremec D, Shortman K: Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. J Immunol 1997, 159:565–573
37. van Rijt LS, Prins JB, Leenen PJ, Thielemans K, de Vries VC, Hoogsteden HC, Lambrecht BN: Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C(neg) bone marrow precursors in a mouse model of asthma. Blood 2002, 100:3663–3671
38. Nencioni A, Grunebach F, Zobywlaski A, Denzlinger C, Brugger W, Brossart P: Dendritic cell immunogenicity is regulated by peroxisome proliferator-activated receptor gamma. J Immunol 2002, 169:1228–1235
39. Mori S, Nakano H, Aritomi K, Wang CR, Gunn MD, Kakiuchi T: Mice lacking expression of the chemokines CCL21-ser and CCL19 (plt mice) demonstrate delayed but enhanced T cell immune responses. J Exp Med 2001, 193:207–218
40. Forster R, Schubel A, Breitfeld D, Kremmer E, Renner-Muller I, Wolf E, Lipp M: CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 1999, 99:23–33
41. Takanaski S, Nonaka R, Xing Z, O'Byrne P, Dolovich J, Jordana M: Interleukin 10 inhibits lipopolysaccharide-induced survival and cytokine production by human peripheral blood eosinophils. J Exp Med 1994, 180:711–715
42. Zuany-Amorim C, Creminon C, Nevers MC, Nahori MA, Vargaftig BB, Pretolani M: Modulation by IL-10 of antigen-induced IL-5 generation, and CD4+ T lymphocyte and eosinophil infiltration into the mouse peritoneal cavity. J Immunol 1996, 157:377–384
43. Zuany-Amorim C, Haile S, Leduc D, Dumarey C, Huerre M, Vargaftig BB, Pretolani M: Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. J Clin Invest 1995, 95:2644–2651
44. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG: A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. Nature 1997, 389:737–742
45. Akbari O, Freeman GJ, Meyer EH, Greenfield EA, Chang TT, Sharpe AH, Berry G, DeKruyff RH, Umetsu DT: Antigen-specific regulatory T cells develop via the ICOS ICOS-ligand pathway and inhibit allergen-induced airway hyperreactivity. Nat Med 2002, 8:1024–1032
46. Cottrez F, Hurst SD, Coffman RL, Groux H: T regulatory cells 1 inhibit a Th2-specific response in vivo. J Immunol 2000, 165:4848–4853
47. Bilyk N, Holt PG: Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations. Immunology 1995, 86:231–237
48. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 1999, 5:698–701
49. Yu G, Bjorksten B: Serum levels of phospholipid fatty acids in mothers and their babies in relation to allergic disease. Eur J Pediatr 1998, 157:298–303
50. Serhan CN, Devchand PR: Novel antiinflammatory targets for asthma. A role for PPARgamma? Am J Respir Cell Mol Biol 2001, 24:658–661
51. Lambrecht BN, Salomon B, Klatzmann D, Pauwels RA: Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. J Immunol 1998, 160:4090–4097