

Peroxisome proliferator-activated receptor α regulates skin inflammation and humoral response in atopic dermatitis

Delphine Staumont-Sallé, MD,^{a,b,c,d,*} Georges Abboud, BSc,^{a,b,c,*} Céline Brénuchon, MD,^{a,b,c} Akira Kanda, MD, PhD,^{a,b,c} Thomas Roumier, PhD,^{a,b,c} Céline Lavogiez, MD,^{a,b,c,d} Sébastien Fleury,^{a,b,c} Patrick Rémy,^{a,b,c} Jean-Paul Papin,^{a,b,c} Justine Bertrand-Michel,^{e,f} François Tercé, PhD,^{e,f,g} Bart Staels, PhD,^{b,c,h} Emmanuel Delaporte, MD,^{c,d} Monique Capron, PhD,^{a,b,c} and David Dombrowicz, PhD^{a,b,c} *Lille and Toulouse, France*

Background: The peroxisome proliferator-activated receptors (PPARs) α , β/δ , and γ are ligand-activated transcription factors belonging to the nuclear receptor superfamily. In addition to their regulatory role on lipid and glucose metabolism, they exert anti-inflammatory properties. In skin both PPAR- α and PPAR- β/δ regulate keratinocyte proliferation/differentiation and contribute to wound healing. The 3 PPAR isoforms are expressed by several cell types recruited into the dermis during inflammation.

Objective: We have investigated the role of PPAR- α in the regulation of atopic dermatitis (AD), a common skin inflammatory disease.

Methods: We chose a mouse model of inflammatory dermatosis with immunologic features of AD and used epicutaneous sensitization with ovalbumin in the absence of adjuvant, which mimics the human pathology.

Results: On antigen sensitization, PPAR- α -deficient mice display increased epidermal thickening, dermal recruitment of inflammatory cells, lung inflammation, airway hyperresponsiveness, and IgE and IgG2a production compared with their wild-type counterparts. Increased inflammation was correlated to an enhancement of T_H2 and, to a greater extent, T_H1 responses and to increased skin expression of nuclear factor κB . Interestingly, PPAR- α expression was decreased in eczematous skin from patients with AD compared with skin from nonatopic donors, suggesting that defective PPAR- α expression might contribute to the pathology. Topical application of WY14643, a specific PPAR- α agonist,

significantly decreased antigen-induced skin inflammation in the AD model.

Conclusion: PPAR- α acts as a negative regulator of skin inflammation in AD. (J Allergy Clin Immunol ■■■■;■■■: ■■■-■■■.)

Key words: Peroxisome proliferator-activated receptor α , atopic dermatitis, allergy, inflammation, human, mouse

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. The PPAR family consists of 3 members, PPAR- α , PPAR- β/δ and PPAR- γ ,^{1,2} which are activated by various fatty acid metabolites produced during inflammatory responses. Furthermore, PPARs also inhibit the activity of other transcription factors, which play a key role in proinflammatory signaling pathways, such as nuclear factor κB (NF- κB),³ through ligand-dependent transrepression.⁴ PPARs function as sensors and regulators of inflammatory reactions through their ability to be activated by locally generated eicosanoids and act in a gene- and signal-specific manner.⁵ Indeed, PPARs are expressed by several cell types within the immune system, such as T lymphocytes,^{6,7} B lymphocytes,⁸ monocytes-macrophages,⁹ dendritic cells,¹⁰ mast cells,¹¹ and eosinophils.¹² *In vivo*, PPAR- α -deficient mice display prolonged leukotriene (LT) B_4 -induced ear swelling,¹³ absence of regulation of spontaneous production of proinflammatory cytokines on aging,¹⁴ exacerbated response of aortic explants to proinflammatory stimuli, increased allergic asthma and IgE production,¹² and exacerbated experimental autoimmune encephalomyelitis.⁶ The 3 PPAR isoforms have been identified in rodent and human epidermis and dermis during fetal development. Their expression decreases rapidly after birth to become undetectable in the interfollicular epidermis in adults, although it persists in the hair follicles.¹⁵ Several recent studies have demonstrated an important role for PPAR- α in the molecular mechanisms involved in skin repair^{15,16} and in epidermal differentiation contributing to permeability barrier formation.¹⁷ The combination of the properties of PPAR- α in immune responses and epidermal differentiation led us to investigate whether PPAR- α might exert a beneficial role in atopic dermatitis (AD), a skin inflammatory disease characterized, like psoriasis, by disturbed epidermal homeostasis. AD is a common, chronic, pruritic inflammatory skin disease that often begins in infancy and frequently occurs in subjects with a personal or family history of atopic disease. Examination of skin lesions in AD reveals increased epidermal thickness (acanthosis) and an inflammatory cell infiltrate in the

From ^aInserm U547, Lille; ^bInstitut Pasteur de Lille; ^cUniversité Lille 2; ^dthe Department of Dermatology, Claude Huriez Hospital, Lille; ^eIFR30, Plateau technique de Lipidologie; ^fUniversité Toulouse III, Toulouse; ^gInserm U363, Toulouse; and ^hInserm U545, Lille.

*These authors contributed equally to this work.

Supported by grants from Inserm, Université Lille 2 and Institut Pasteur de Lille and by a fellowship (to D.S.) from Inserm.

Disclosure of potential conflict of interest: D. Dombrowicz has received research support from Inserm, Université Lille 2, Institut Pasteur de Lille, ANR, FRM, the European Union, and Region Nord-Pas de Calais. M. Capron has received research support from Inserm, Université Lille 2, and Institut Pasteur de Lille. The rest of the authors have declared that they have no conflict of interest.

Received for publication July 15, 2007; revised December 20, 2007; accepted for publication December 20, 2007.

Reprint requests: David Dombrowicz, PhD, Inserm U547, Institut Pasteur de Lille 1, rue Prof Calmette BP245, 59019 Lille Cedex, France. E-mail: david.dombrowicz@pasteur-lille.fr.

0091-6749/\$34.00

© 2008 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2007.12.1165

Abbreviations used

AD:	Atopic dermatitis
AHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
NF- κ B:	Nuclear factor κ B
OVA:	Ovalbumin
Penh:	Enhanced pause
PPAR:	Peroxisome proliferator-activated receptor
TEWL:	Transepidermal water loss
TSLP:	Thymic stromal lymphopoietin
WT:	Wild-type

dermis, consisting of activated memory CD4⁺ T cells, macrophages, mast cells, and eosinophils. In acute AD lesions there is a preferential recruitment of T_H2 cells, whereas in the chronic lesions a T_H1 profile is predominant, with an accumulation of IFN- γ -producing T_H1 cells. Most patients with AD have increased levels of serum IgE, with specific IgE directed against environmental allergens or microbial proteins (*Staphylococcus aureus*).¹⁸ Moreover, a high proportion of individuals with AD have allergic asthma, rhinitis, or both later in life.

METHODS**Patients**

Skin biopsy specimens were taken after obtaining informed consent in involved areas from 8 patients (5 male and 3 female patients; average age, 25.2 years) with severe AD, according to the criteria defined by Hanifin and Rajka,¹⁹ and from 6 male nonatopic healthy individuals (average age, 30.7 years) characterized by the absence of personal or familial history of atopic disease and normal IgE serum levels. Patients received neither any topical treatment with immunosuppressive effects for at least 15 days before the collection of skin samples nor systemic treatment for at least 1 month before biopsy. Only male control subjects were selected to avoid "inaesthetic" scare in female individuals. Specimens were immediately frozen in dry ice to isolate total RNA.

Induction of inflammatory dermatosis with immunologic features of AD and ovalbumin-induced airway hyperresponsiveness

AD was induced by epicutaneous sensitization with ovalbumin (OVA), as previously described.²⁰ Two paper-disc inserts of a Finn Chamber (Promedica, La Chaussée Saint-Victor, France) soaked with 25 μ L of 0.2% OVA solution in PBS or with vehicle were applied on abdominal skin 24 hours after shaving. Patches were secured to the skin with a bio-occlusive dressing (Visulin; Hartmann, Châtenoi, France), itself protected with an elastic bandage (Optiplaste; Smith & Nephew, Vibraye, France). Patches were left on for three 1-week periods (with patch renewal at midweek), with a 2-week interval between applications. For BALB/cJ mice, abdomens were topically treated immediately before patch application with 25 μ L of the 50 μ mol/L PPAR- α agonist WY14,643 (stock 10⁻² in dimethyl sulfoxide; Alexis Biochemicals, Coger, France) in acetone/olive oil (4:1 vol/vol), whereas the corresponding untreated mice were receiving an equivalent volume of dimethyl sulfoxide. For PPAR- α ^{-/-} and 129S1/SVImJ mice, at the time of the last patch removal (day 49), animals were challenged for 20 minutes by means of aerosol nebulization with OVA (1% in PBS) by using an ultrasonic nebulizer (Systam, Villeneuve sur Lot, France), and serum was collected. On the next day, airway hyperresponsiveness (AHR) to increasing concentrations of methacholine was measured by means of whole-body plethysmography (Emka Technologies, Paris, France); results were expressed as enhanced pause (Penh) values.²¹ Animals were killed by means of cervical dislocation. Lungs were used for bronchoalveolar lavage (BAL). BAL specimens were analyzed on cytospin preparations

after RAL 555 staining. Skin samples were collected for histologic analyses and real-time PCR after RNA extraction.

Analysis of inflammatory dermatosis with immunologic features of AD and OVA-induced AHR

For more information, see the [Methods](#) section in the Online Repository at www.jacionline.org.

Statistical analysis

Statistical significance was determined with the Statview software by using the Student test, except for plethysmographic data, for which ANOVA for repeated measures was used, and Q-PCR analysis, for which the Mann-Whitney test was applied. Results were expressed as means $\pm$ SEMs. A *P* value of less than .05 was considered significant.

RESULTS**PPAR- α -deficient mice exhibit exacerbated skin inflammatory response in a model of AD**

We first assessed the effect of PPAR- α deficiency on skin inflammatory responses by comparing cutaneous responses obtained in wild-type (WT) 129S1/SVImJ and PPAR- α ^{-/-} mice in a previously described model of inflammatory dermatosis with immunologic features of AD based on antigen sensitization by means of repeated epicutaneous OVA applications. Neither strain exhibited an inflammatory phenotype in the absence of OVA sensitization. Compared with OVA-sensitized WT mice, OVA-sensitized PPAR- α ^{-/-} mice displayed an exacerbated skin inflammatory response, which was characterized by more pronounced hyperkeratosis, spongiosis, and dermal cellular infiltrates (Fig 1, A), and a 1.8-fold (45%) increase in epidermal thickening (Fig 1, B). Likewise, PPAR- α deficiency increased the number of mast cells and CD4⁺ T cells into the dermis. By contrast, eosinophil infiltration in OVA-sensitized PPAR- α ^{-/-} mice was not statistically different from the recruitment observed in their WT counterparts (Fig 1, C). These results demonstrate that the absence of PPAR- α exacerbates skin lesions observed in AD.

Exacerbated inflammation in OVA-sensitized PPAR- α -deficient mice is not due to a barrier defect

Because, in addition to immune dysregulation, alterations in the epidermal barrier might contribute to AD development, we analyzed structural parameters of the epidermis. On Nile red staining, the neutral lipid layer appeared similarly discontinuous in both PBS- and OVA-sensitized WT and PPAR- α ^{-/-} mice (see Fig E1, A, in the Online Repository at www.jacionline.org). Likewise, transepidermal water loss (TEWL) was comparable in the 4 experimental groups and significantly higher than in skin from corresponding naive WT and PPAR- α ^{-/-} animals (see Fig E1, B). Finally, except for free fatty acids and ceramide 2, which were increased by 22% and 16% in PBS-treated PPAR- α ^{-/-} mice, skin lipid composition was also similar for both genotypes after the complete sensitization process (see Fig E1, C). Taken together, these data suggest that the exacerbated responses of PPAR- α ^{-/-} animals on OVA sensitization, compared with those in WT animals, are due to a lack of immunoregulation rather than to major structural differences between the 2 genotypes.

PPAR- α deficiency increases expression of both T_H2 - and T_H1 -related molecules in OVA-sensitized mouse skin

We next examined, by means of real-time PCR, the expression of mRNAs for T_H2 - and T_H1 -associated molecules in the skin of unsensitized and OVA-sensitized WT and PPAR- $\alpha^{-/-}$ mice. OVA sensitization increased mRNA expression of both T_H2 - and T_H1 -associated molecules in both genotypes (Fig 1, D). In skin from OVA-sensitized PPAR- $\alpha^{-/-}$ mice compared with skin from OVA-sensitized WT animals, we detected a more pronounced increase in several T_H2 -associated molecules (IL-4, 2.3-fold; IL-10, 2-fold; IL-31, 4.1-fold; IL-33, 4-fold; CCL5, 3-fold; CCL11, 2.4-fold; CCL24, 2.3-fold; and CCR3, 2-fold) that contribute to eosinophil chemotaxis and CCL22 (or macrophage-derived chemokine), a chemoattractant for T_H2 lymphocytes into the inflammatory skin (3.1-fold). IL-5 mRNA expression was increased after OVA epicutaneous sensitization to the same extent in PPAR- $\alpha^{-/-}$ and WT animals. By contrast, after OVA sensitization, expression of 2 other molecules associated with skin inflammation, IL-18 and thymic stromal lymphopoietin (TSLP), which activates bone marrow-derived dendritic cells to produce T_H2 -attracting chemokines, as well as expression of immunoregulatory TGF- β , remained unchanged in both WT and PPAR- $\alpha^{-/-}$ mice (data not shown). Expression of T_H1 cytokines and chemokines was also markedly increased in the skin of OVA-sensitized PPAR- $\alpha^{-/-}$ mice compared with that seen in OVA-treated WT mice: IFN- γ (2.3-fold); IL-12 (4.1-fold), a cytokine that contributes to the switch of an early T_H2 response to a late T_H1 response; CXC chemokine ligand (CXCL) 10 and its receptor CXCR3 (4.4- and 12.7-fold, respectively); and CCR5 (5.7-fold).

Skin expression of NF- κ B and inflammatory cytokines is increased in OVA-sensitized PPAR- α -deficient mice

In addition to immunoregulatory cytokines, we also found that expression of the proinflammatory cytokines IL-1 β and IL-6 was also increased (2.6- and 4.5-fold, respectively) in the skin of OVA-sensitized PPAR- $\alpha^{-/-}$ mice compared with that seen in the skin of OVA-treated WT mice. NF- κ B is both essential to the development of inflammatory responses and a key target of PPAR- α regulation²²; we thus analyzed skin expression of NF- κ B 1 and 2. We found that NF- κ B 1 and 2 expression in unsensitized animals was not different in WT and PPAR- $\alpha^{-/-}$ mice. By contrast, OVA-sensitized PPAR- $\alpha^{-/-}$ animals exhibited a significantly increased expression of both NF- κ B 1 and 2 in lesional skin compared with their WT counterparts, suggesting that PPAR- α could negatively regulate the skin inflammatory responses by inhibiting the NF- κ B proinflammatory pathway (Fig 1, E).

PPAR- α deficiency leads to increased lung inflammation in a model of AD

In atopic individuals the same allergen can induce both skin and airway allergic inflammation. This model of inflammatory dermatosis with immunologic features of AD closely mimics this pathologic situation. Indeed, after epicutaneous sensitization, a single antigen nebulization leads to airway inflammation and hyperreactivity (AHR). Although PPAR- $\alpha^{-/-}$ mice did not exhibit an inflammatory phenotype in the skin in the absence of epicutaneous sensitization with OVA, they had a significantly higher

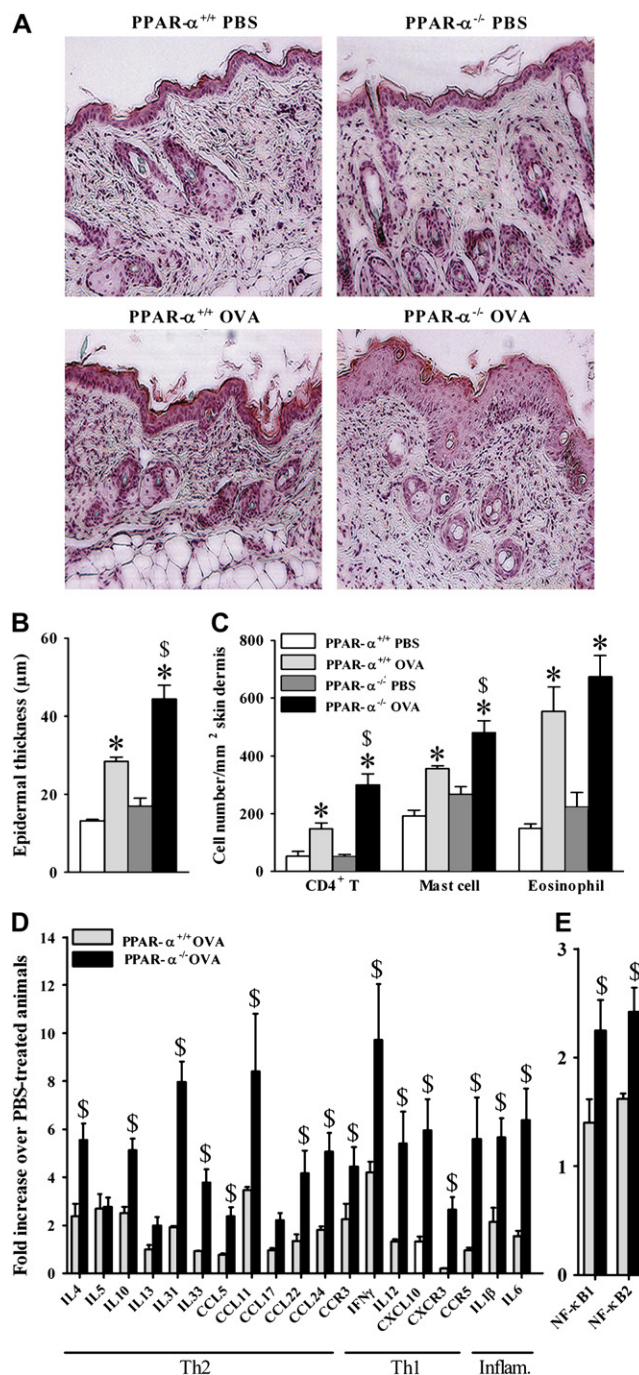


FIG 1. Increased skin inflammation in PPAR- $\alpha^{-/-}$ mice in a model of AD. **A**, Hematoxylin and eosin staining of skin sections from OVA-sensitized mice or PBS-treated PPAR- $\alpha^{-/-}$ or WT mice (original magnification $\times 100$). **B**, Epidermal thickness. **C**, Eosinophil, mast cell, and T-lymphocyte numbers in dermis at the site of sensitization and treatment (n = 6–10 animals per group). Data are expressed as means $\pm$ SEMs. *Statistically different from PBS-treated mice ($P < .05$). §, Statistically different from WT mice ($P < .05$). **D**, Skin cytokine, chemokine, and receptor expression. **E**, Skin NF- κ B 1 and 2 expression. Primers used are listed in Table E1 in the online Repository at www.jacionline.org. Results for OVA-sensitized animals are expressed as a fold increase compared with the identically responding PBS-treated animals (n = 3–6 animals per group). §, Statistically different from OVA-sensitized WT mice ($P < .05$).

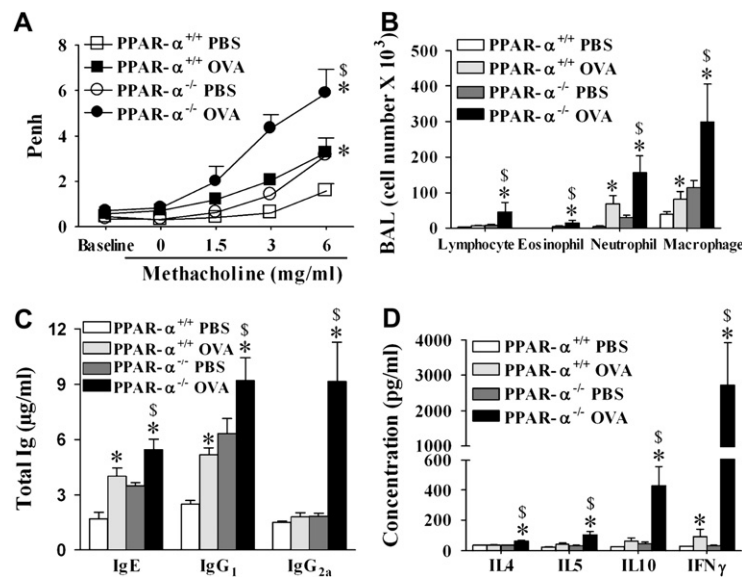


FIG 2. Increased lung inflammation and humoral response in PPAR-α^{-/-} mice in a model of AD. **A**, Measurement of AHR to increasing methacholine concentrations by means of whole-body plethysmography. **B**, Total number of macrophages, neutrophils, lymphocytes, and eosinophils in BAL fluid at the time of death (n = 3-6 animals per group). **C**, Total IgE, IgG1, and IgG2a serum concentrations. **D**, OVA-induced cytokine production by inguinal lymph nodes *in vitro* (n = 6-10 animals per group). *Statistically different from PBS-treated mice ($P < .05$). \$Statistically different from WT mice ($P < .05$).

AHR to methacholine, with a 2-fold higher response (for 6 mg/mL methacholine) compared with that of their WT counterparts. Furthermore, OVA-sensitized PPAR-α^{-/-} mice displayed higher Penh values in response to methacholine compared with WT mice, with a 1.8-fold higher Penh value for 3 and 6 mg/mL methacholine (Fig 2, A). After airway challenge, overall inflammatory infiltrate in the BAL specimens of PBS-sensitized PPAR-α^{-/-} animals was 3.3-fold higher compared with that of WT animals (data not shown). This infiltrate was predominantly composed of neutrophils and macrophages. OVA-sensitized PPAR-α^{-/-} mice displayed a total cell recruitment increased by 54% compared with that seen in OVA-sensitized WT mice (data not shown). Neutrophils, macrophages, lymphocytes, and eosinophils were 2.2-, 3.6-, 7.4-, and 8.3-fold more abundant, respectively (Fig 2, B).

PPAR-α deficiency increases both T_H2 and T_H1 humoral and cellular responses in a mouse model of AD

Serum levels of IgE and IgG1, both associated to a T_H2 response, were increased in OVA-sensitized PPAR-α^{-/-} mice compared with those in OVA-sensitized WT animals, with a 1.5- and 2.3-fold increase for total IgE and OVA-specific IgE levels, respectively, and a 1.9- and 2.1-fold increase for total and OVA-specific IgG1 levels, respectively. IgG2a antibodies, associated to a T_H1 response, were also increased in OVA-sensitized PPAR-α^{-/-} mice compared with levels seen in their WT counterparts, with a 4.4- and a 7.2-fold higher response for total and OVA-specific IgG2a, respectively (Fig 2, C, and see Fig E2, A, in the Online Repository at www.jacionline.org). Even if both T_H2 and T_H1 humoral responses were exacerbated, increase of the T_H1 response was more pronounced in PPAR-α^{-/-} mice on antigen sensitization. Indeed, the increase of total and OVA-specific

IgG2a levels in PPAR-α^{-/-} animals was about 3-fold higher than the increase of total and OVA-specific T_H2 antibodies. Furthermore, only PPAR-α^{-/-} animals displayed an increased humoral T_H1 response after allergen sensitization (Figs 2, C, and E2, A). Finally, the level of T_H2 antibodies was significantly higher in control PPAR-α^{-/-} mice than in control WT animals (Figs 2, C, and E2, A). Likewise, a similarly exacerbated cellular response (T_H2 and, to an even greater extent, T_H1 cytokine production) was observed on *in vitro* OVA restimulation of inguinal lymph node cells (Fig 2, D) from PPAR-α^{-/-} animals compared with corresponding cells from WT mice. Splenocyte restimulation only led to an exacerbated T_H1 (but not T_H2) response (Fig E2, B).

PPAR-α expression is significantly decreased in the lesional skin of patients with AD

We aimed at correlating the data observed in PPAR-α-deficient mice with the status of individuals with AD. PPAR-α and PPAR-β/δ mRNA expression was studied in the lesional skin of patients with atopic eczema and compared with that seen in the skin of nonatopic healthy age-matched individuals. Interestingly, we observed a markedly decreased expression of PPAR-α in eczematous skin of patients with AD (approximately 50% lower than in control individuals, Fig 3), whereas PPAR-β/δ mRNA expression was not significantly different in skin samples from atopic and nonatopic individuals (Fig 3).

PPAR-α agonist WY14643 reduces skin inflammatory response in a model of AD

We investigated whether PPAR-α could represent a therapeutic target in AD by using a treatment with a PPAR-α agonist: WY14643. For this purpose, WY14643 was topically applied during the sensitization phases. BALB/c background was

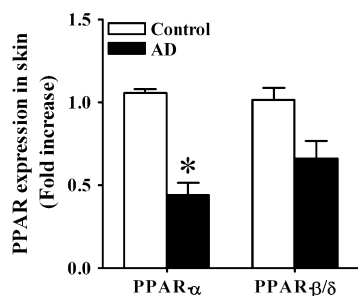


FIG 3. Decreased expression of PPAR- α in lesional skin from patients with AD. RNA was extracted from skin biopsy specimens of nontreated patients with AD ($n = 8$) and nonatopic healthy individuals ($n = 6$). Real-time PCR analysis of PPAR- α and PPAR- β/δ expression was conducted by using the primers shown in Table E1. *, Statistically different from control individuals ($P < .05$).

chosen because OVA sensitization induces a more pronounced pathology than on the 129 SV background. In vehicle-treated OVA-sensitized mice histopathologic analysis revealed typical lesions of eczema characterized by hyperkeratosis with desquamation and spongiosis, which were absent in unsensitized mice (Fig 4, A). Compared with unsensitized mice, epidermal thickness at the inflammatory site was increased 1.5-fold in vehicle-treated sensitized mice. Topical treatment with WY14643 did not affect the skin's histologic aspect in unsensitized mice. In WY14643-treated OVA-sensitized mice histologic lesions of eczema were significantly less pronounced (49% inhibition) than in vehicle-treated OVA-sensitized mice (Fig 4, B). Histologic analysis also revealed that this effect was accompanied by reduced dermal CD4⁺ T cells and mast cell and eosinophil infiltration in WY14643-treated mice (95%, 68%, and 32% reduction, respectively; Fig 4, C). In this experimental model WY14643 treatment did not affect the neutral lipid layer (Fig E1, A) and TEWL (Fig E1, B), but in OVA-sensitized animals it did modify skin lipid composition, with decreases in free fatty acids and ceramide 2, 3, and 6 content (by 27%, 34%, 34%, and 41%, respectively; Fig E1, C). Furthermore, topical WY14643 treatment also reduced lung inflammation, in particular eosinophil and neutrophil recruitment, induced on OVA sensitization (Fig E3). These data show that topical treatment with WY14643 exerts a significant inhibitory effect on skin and airway inflammatory symptoms induced by OVA sensitization.

WY14643 reduces skin expression of proinflammatory, T_H1, and T_H2 cytokines and chemokines and lung cellular inflammation

WY14643 treatment also suppressed local T_H2 and T_H1 cytokine expression, as well as that from inflammation-associated cytokines (Fig 4, D) and transcription factors (Fig 4, E). Inhibition was most effective for IL-1 β (93%), IL-31 (87%), and IL-4 (80%) and weaker for IFN- γ (57%), CCL11 (47%), and NF- κ B 1 and 2 (38% and 36%, respectively). No inhibition was observed for IL-5 expression.

Topical WY14643 treatment does not affect humoral and cellular responses

By contrast, WY14643 affected neither the humoral response (OVA-specific IgE or IgG1 production) induced on epicutaneous

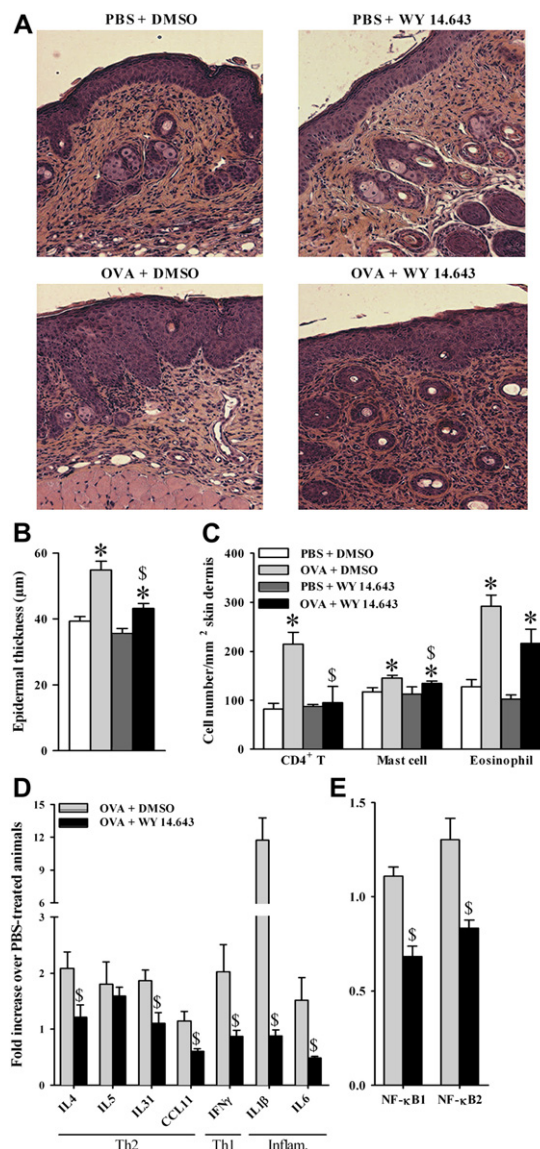


FIG 4. Improvement of skin lesions in a model of AD after topical treatment with the PPAR- α agonist WY14643. **A**, Hematoxylin and eosin staining of skin sections from OVA-sensitized or PBS-treated mice treated with dimethyl sulfoxide (DMSO) or WY14643 (original magnification $\times 200$). **B**, Epidermal thickness. **C**, CD4 T-cell, eosinophil, and mast cell numbers in dermis at the site of sensitization and treatment. **D**, Skin cytokine, chemokine, and receptor expression. Primers used are listed in Table E1. **E**, Skin NF- κ B 1 and 2 expression ($n = 6$ -10 animals per group). *Statistically different from PBS-treated mice ($P < .05$). \$, Statistically different from DMSO-treated mice ($P < .05$).

sensitization (Fig E2, E) nor the cellular response from inguinal lymph node cells or splenocytes *in vitro* (except for a slight decrease in IL-5 production after OVA restimulation of splenocytes; Fig E2, C and D). Thus the systemic effects of topical WY14643 appear to be limited.

DISCUSSION

In this article we demonstrate for the first time the anti-inflammatory role of PPAR- α in a murine model of inflammatory dermatosis with immunologic features of AD. In this model

development of the pathology was dependent on the immune response induced on epicutaneous OVA sensitization but independent of barrier alterations induced by repeated patch changes/removal. Absence of PPAR- α led to exacerbated skin inflammation, with increased epidermal thickness; dermal recruitment of CD4⁺ T cells, mast cells, and eosinophils; and inflammatory cytokine production, as well as increased AHR in response to methacholine provocation and cellular infiltration in lung tissue on antigen airway challenge. These exacerbated functional and cellular responses were associated with both skin, cellular, and humoral T_H1 and T_H2 responses in OVA-sensitized PPAR- α ^{-/-} animals, as found in human AD, where chronic lesions are characterized by a switch from a T_H2 toward a T_H1 cytokine profile.¹⁸ Comparative study of T_H1 and T_H2 immunoglobulin isotypes suggested that naive PPAR- α ^{-/-} mice were biased toward a T_H2 polarization but developed a particularly strong T_H1 response on allergen sensitization. PPAR- α might modulate T_H1/T_H2 balance through its ability to negatively regulate T-bet transcription factor, which is essential for T_H1 polarization²³ and its T_H2 counterpart, GATA-3.¹² However, we did not find a significant difference in skin eosinophil recruitment and IL-5 mRNA expression between OVA-sensitized PPAR- α ^{-/-} animals and their WT counterparts. By contrast, exacerbation of lung inflammation observed in OVA-sensitized PPAR- α ^{-/-} mice is associated to an effect of PPAR- α on bronchial recruitment of eosinophils, as previously described in a classical asthma model.^{12,24} These differences between skin and lung compartments might be due to the fact that eosinophils can be recruited at an early stage of skin pathology, and differences might disappear in the chronic stage at the time of death, whereas BAL eosinophilia was observed only 24 hours after a single airway challenge. Likewise, in our model OVA sensitization did not induce skin expression of TSLP (data not shown), which is in contrast to another model of genetically induced AD triggered by a rapid and strong enhancement of expression of TSLP in retinoid X receptor-ablated keratinocytes.²⁵ *In vitro* studies have shown that human TSLP activates dendritic cells, which induce T_H2 differentiation, as well as CD4⁺ T-cell proliferation.²⁶ Again, in an AD model TSLP might have exerted its role at an earlier phase of the disease. Next, we investigated the mechanisms by which PPAR- α can exert its anti-inflammatory properties in this model of inflammatory dermatosis with immunologic features of AD. We found that the exacerbated skin inflammatory response observed in PPAR- α -deficient animals was not dependent on a lack of Foxp3 expression by CD4⁺CD25⁺ regulatory T cells (data not shown) or on the production of immunoregulatory cytokines, such as TGF- β (data not shown), but might be linked to an inhibition of the proinflammatory NF- κ B pathway by PPAR- α . Indeed, we detected increased mRNA expression for NF- κ B 1 and 2 transcription factors in the skin of OVA-sensitized PPAR- α -deficient animals compared with that seen in their WT counterparts. It has been demonstrated that PPAR- α agonists can exert their anti-inflammatory effects by antagonizing the NF- κ B pathway, leading to an attenuated transcription of genes encoding inflammatory cytokines.²⁷ PPAR- α ligands act at several levels along the signaling cascade upstream of NF- κ B, such as on I κ B, or through transrepression.^{3,28} We next investigated whether a lack of PPAR- α expression could contribute to the skin inflammatory status in patients with AD and found a 50% decrease in PPAR- α expression in eczematous skin of patients with AD compared with that seen in control

individuals, whereas mRNA expression of the β/δ isoform was not significantly different from that seen in nonatopic patients. To our knowledge, only one recent publication provided indirect information concerning the expression level of PPAR- α in human AD skin. Indeed, Plager et al²⁹ used microarrays to identify cutaneous gene transcription changes associated with early AD inflammation and found a differential expression for genes associated with PPAR- α -regulated transcription. Nevertheless, PPAR- α expression has never been studied in AD skin lesions, regardless of the disease stage. Finally, we confirmed the potent beneficial effect of PPAR- α by demonstrating that topical application with the PPAR- α agonist WY14643 during sensitization phases significantly decreased the skin inflammatory response, with reduced eczematous lesions and dermal eosinophil and mast cell recruitment. Effects of PPAR- α activators on immune response in mice have been described by Cunard et al,³⁰ who showed that oral administration of WY14643 alters the humoral (total IgG and IgG1) and cytokine responses to a defined antigen and also causes profound decrease in splenocyte numbers in both immunized and nonimmunized animals. Nevertheless, the beneficial role of PPAR- α in skin disease might not be only due to its anti-inflammatory properties but also to its role in skin barrier function. In our model involving multiple patch applications and removal, PPAR- α affected neither histologic distribution of neutral lipids nor TEWL. However, it induced minor alterations in skin lipid composition. Nevertheless, recent studies have demonstrated the important role of PPAR- α in skin repair^{15,31} and in epidermal differentiation contributing to barrier formation.¹⁷ Michalik and colleagues^{15,31} observed that skin injury stimulates PPAR- α re-expression in keratinocytes during wound healing. Furthermore, PPAR- α ^{-/-} mice have a transient delay in initial wound healing. Kömüves et al¹⁷ showed that clofibrate, a PPAR- α ligand added to fetal rat skin explants, stimulates epidermal differentiation by activating stratum corneum development and leads to an acceleration of barrier formation. As in our work, Kömüves et al noticed that the skin and in particular the epidermis of PPAR- α ^{-/-} animals appeared normal. They only mentioned a thinner stratum granulosum, with granular cells having fewer keratohyalin granules and focal parakeratosis in the stratum corneum. Conversely, Yang et al³² reported that the skin phenotype of transgenic mice constitutively overexpressing PPAR- α in the epidermis was characterized by epidermal thinning and sparse fur, contributing to lethality within 2 days after birth. PPAR- α might transcriptionally regulate epidermal development by interacting with various other transcription factors, such as lymphoid enhancer-binding factor-1 and T-cell factor-3, which have been investigated for their role in the epidermal stem cell niche³³ and with several proteins implicated in the coordination of the keratinocyte cell cycle, such as p21 (Waf1/Cip1), which is increased in cultured keratinocytes from 1-day-old mice overexpressing PPAR- α .³²

In conclusion, the present study provides *in vivo* evidence for the role of PPAR- α in controlling skin and systemic inflammatory responses in patients with AD. These results are pointing in the direction of PPAR- α being an interesting therapeutic target in skin diseases like AD characterized by inflammatory infiltrates and barrier dysfunction. In our study epicutaneously OVA-sensitized PPAR- α ^{-/-} mice represent a new murine model that faithfully mimics human AD by reproducing a natural pathway of skin and lung sensitization against an allergen and by exhibiting both T_H1 and T_H2 immune response.

We thank Josette Fontaine and Bruno Derudas for their technical assistance, Dr Hervé Cotten for his contribution to histologic work, and the members of the Department of Dermatology in Lille for help with patient recruitment and skin biopsies.

Clinical implications: PPAR- α agonists could have potential therapeutic use in AD.

REFERENCES

1. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, et al. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. *Pharmacol Rev* 2006;58:726-41.
2. Glass CK, Ogawa S. Combinatorial roles of nuclear receptors in inflammation and immunity. *Nat Rev Immunol* 2006;6:44-55.
3. Lefebvre P, Chinetti G, Fruchart JC, Staels B. Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J Clin Invest* 2006;116:571-80.
4. Ricote M, Glass CK. PPARs and molecular mechanisms of transrepression. *Biochim Biophys Acta* 2007;1771:926-35.
5. Moraes LA, Piqueras L, Bishop-Bailey D. Peroxisome proliferator-activated receptors and inflammation. *Pharmacol Ther* 2006;110:371-85.
6. Dunn SE, Ousman SS, Sobel RA, Zuniga L, Baranzini SE, Youssef S, et al. Peroxisome proliferator-activated receptor (PPAR)alpha expression in T cells mediates gender differences in development of T cell-mediated autoimmunity. *J Exp Med* 2007;204:321-30.
7. Hontecillas R, Bassaganya-Riera J. Peroxisome proliferator-activated receptor gamma is required for regulatory CD4+ T cell-mediated protection against colitis. *J Immunol* 2007;178:2940-9.
8. Schlezinger JJ, Jensen BA, Mann KK, Ryu HY, Sherr DH. Peroxisome proliferator-activated receptor gamma-mediated NF-kappa B activation and apoptosis in pre-B cells. *J Immunol* 2002;169:6831-41.
9. Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors: new targets for the pharmacological modulation of macrophage gene expression and function. *Curr Opin Lipidol* 2003;14:459-68.
10. 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;170:5295-301.
11. Maeyama K, Emi M, Tachibana M. Nuclear receptors as targets for drug development: peroxisome proliferator-activated receptor gamma in mast cells: its roles in proliferation and differentiation. *J Pharmacol Sci* 2005;97:190-4.
12. Woerly G, Honda K, Loyens M, Papin JP, Auwerx J, Staels B, et al. Peroxisome proliferator-activated receptors alpha and gamma down-regulate allergic inflammation and eosinophil activation. *J Exp Med* 2003;198:411-21.
13. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W. The PPARalpha-leukotriene B4 pathway to inflammation control. *Nature* 1996;384:39-43.
14. Spencer NF, Poynter ME, Im SY, Daynes RA. Constitutive activation of NF-kappa B in an animal model of aging. *Int Immunol* 1997;9:1581-8.
15. Michalik L, Desvergne B, Tan NS, Basu-Modak S, Escher P, Rieusset J, et al. Impaired skin wound healing in peroxisome proliferator-activated receptor (PPAR)alpha and PPARbeta mutant mice. *J Cell Biol* 2001;154:799-814.
16. Michalik L, Wahli W. Peroxisome proliferator-activated receptors (PPARs) in skin health, repair and disease. *Biochim Biophys Acta* 2007;1771:991-8.
17. Komuves LG, Hanley K, Lefebvre AM, Man MQ, Ng DC, Bikle DD, et al. Stimulation of PPARalpha promotes epidermal keratinocyte differentiation in vivo. *J Invest Dermatol* 2000;115:353-60.
18. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;113:651-7.
19. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venerol* 1980;92(suppl):44-7.
20. Spergel JM, Mizoguchi E, Brewer JP, Martin TR, Bhan AK, Geha RS. Epicutaneous sensitization with protein antigen induces localized allergic dermatitis and hyperresponsiveness to methacholine after single exposure to aerosolized antigen in mice. *J Clin Invest* 1998;101:1614-22.
21. Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG, et al. Non-invasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997;156:766-75.
22. Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappaB and AP-1. *J Biol Chem* 1999;274:32048-54.
23. Tripp RA, Dakhama A, Jones LP, Barskey A, Gelfand EW, Anderson LJ. The G glycoprotein of respiratory syncytial virus depresses respiratory rates through the CX3C motif and substance P. *J Virol* 2003;77:6580-4.
24. Trifileff 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;139:163-71.
25. Li M, Messaddeq N, Teletin M, Pasquali JL, Metzger D, Chambon P. Retinoid X receptor ablation in adult mouse keratinocytes generates an atopic dermatitis triggered by thymic stromal lymphopoietin. *Proc Natl Acad Sci U S A* 2005;102:14795-800.
26. Watanabe N, Hanabuchi S, Soumelis V, Yuan W, Ho S, de Waal Malefyt R, et al. Human thymic stromal lymphopoietin promotes dendritic cell-mediated CD4+ T cell homeostatic expansion. *Nat Immunol* 2004;5:426-34.
27. Vanden Berghe W, Vermeulen L, Delerive P, De Bosscher K, Staels B, Haegeman G. A paradigm for gene regulation: inflammation, NF-kappaB and PPAR. *Adv Exp Med Biol* 2003;544:181-96.
28. Bailey ST, Ghosh S. "PPAR"ting ways with inflammation. *Nat Immunol* 2005;6:966-7.
29. Plager DA, Leontovich AA, Henke SA, Davis MD, McEvoy MT, Sciallis GF 2nd, et al. Early cutaneous gene transcription changes in adult atopic dermatitis and potential clinical implications. *Exp Dermatol* 2007;16:28-36.
30. Cunard R, Ricote M, DiCampli D, Archer DC, Kahn DA, Glass CK, et al. Regulation of cytokine expression by ligands of peroxisome proliferator activated receptors. *J Immunol* 2002;168:2795-802.
31. Tan NS, Michalik L, Noy N, Yasmin R, Pacot C, Heim M, et al. Critical roles of PPAR beta/delta in keratinocyte response to inflammation. *Genes Dev* 2001;15:3263-77.
32. Yang Q, Yamada A, Kimura S, Peters JM, Gonzalez FJ. Alterations in skin and stratified epithelia by constitutively activated PPARalpha. *J Invest Dermatol* 2006;126:374-85.
33. Nguyen H, Rendl M, Fuchs E. Tcf3 governs stem cell features and represses cell fate determination in skin. *Cell* 2006;127:171-83.

METHODS

Animals

BALB/cJ and 129S1/SVImJ mice were purchased from Charles River Laboratories (L'Arbresle, France). PPAR- α -deficient mice were obtained from Dr F. J. Gonzalez (National Institutes of Health, Bethesda, Md).^{E1} Eight- to 12-week-old female mice were used for all experiments. Animals were bred within the specific pathogen-free facility from the Institut Pasteur de Lille. Experiments were performed after approval by the Ethics Committee for Animal Experimentation from the Nord-Pas-de-Calais Region.

TEWL

TEWL was measured with a Tewameter TM300 (Courage and Khazaka, Köln, Germany) on the abdominal skin of naive animals and at the treated abdominal sites at the time of and 24 hours after the last patch removal, according to the manufacturer's instructions.

Skin lipid analysis

Ceramides, cholesterol, and fatty acids from skin were analyzed by means of high-performance thin layer chromatography. Skin samples were crushed twice in 1.5 mL of methanol/5 mmol/L ethyleneglycol-bis-(β -aminoethyl-ether)-N,N,N',N'-tetraacetic acid (2:1 vol/vol) by using a Fast Prep (MP Biochemicals, Illkirch, France). Aliquots were removed and evaporated, and the dry pellets were dissolved in 0.5 mL of NaOH (0.1 mol/L) overnight. Proteins were measured with the Bio-Rad Protein Assay (Bio-Rad, Marnes-la-Coquette, France). Lipid extraction was performed with a dounce after addition of 1 mL of chloroform and further completed in chloroform/methanol/water (2.5:2.5:2.1, vol/vol/vol), according to the method of Bligh and Dyer.^{E2} The lipid (chloroform) phase was evaporated to dryness, dissolved in 20 μ L of chloroform/methanol (1:1, vol/vol), and applied on a TLC Silica Gel 60 plate (Merck, Fontenay-sous-Bois, France). Chromatograms were developed twice in chloroform/methanol/acetic acid (190:9:1, vol/vol/vol) to 17 cm and then in hexane/diethyl-ether/formic acid (55:45:1, vol/vol/vol) to the top of the plate. After drying, the chromatogram was sprayed with 10% (wt/vol) cupric sulphate hydrate in 8% (wt/vol) phosphoric acid and charred by heating at 110°C for 24 hours. A digitized image was captured, and quantification (ImageJ 1.38x) of lipid fractions was based on known quantities of the following standards analyzed on the same plate: cholesterol and nonadecanoic acid (Sigma, Saint-Quentin Fallavier, France), ceramide 2 (nonhydroxy ceramide), ceramide 3 (phytosphinganine), and ceramide 5 (hydroxy ceramide; Matreya-LLC, Marne la Vallée, France).

Histology and immunohistochemistry

Tissue biopsy specimens were fixed in 4% buffered formalin and embedded in paraffin for general histology, fixed in a formaldehyde-free zinc fixative (ImmunohistoFix, Intertiles, Brussels, Belgium), and embedded in ImmunohistoWax (Intertiles) for immunohistochemistry, as previously described.^{E3} Serial paraffin-embedded sections of 5- μ m thickness were stained with hematoxylin and eosin and acidic toluidine blue for measurement of epidermal thickness and eosinophil and mast cells counts. Epidermal thickness was determined at 250-fold magnification with an ocular micrometer; an average of 10 measures was calculated for each sample. Eosinophils and mast cells were enumerated by using an eyepiece equipped with a calibrated grid by examining 20 random fields at 1000-fold magnification; results were expressed as cells per square millimeter. For immunohistochemical analysis, 5- μ m sections were dewaxed in acetone for 5 minutes and immunostained with anti-CD4 mAb (BD Biosciences PharMingen, Le Pont de Claix, France), as previously described.^{E3}

Nile red staining

Frozen sections (10 μ m) were stained for neutral lipids with Nile red. Briefly, staining solution was freshly prepared from stock solution of Nile red (500 μ g/mL; Sigma, Saint-Quentin Fallavier, France) in acetone 1:100 dilution in 75% glycerol, followed by brisk vortexing. After degasing by

means of vacuum to remove bubbles, a drop of solution was added onto the sections and immediately covered with a coverslip. Sections were examined after 5 minutes by means of fluorescence microscopy (450- to 500-nm band pass excitation filter). Neutral lipids stained yellow-gold.

Measurement of immunoglobulin concentrations

Total IgE concentrations were measured in mouse serum by means of ELISA with 2 monoclonal anti-IgE antibodies (BD PharMingen), as previously described.^{E4} Total IgG1 and IgG2a levels were measured in serum with the immunoassay kit SBA Clonotyping System-HRP (Southern Biotech, Birmingham, Ala). Serum anti-OVA IgE levels were measured by means of ELISA with anti-IgE (BD PharMingen) as the capture antibody and biotinylated OVA and horseradish peroxidase-conjugated streptavidin (Amersham Biosciences, Orsay, France) for detection. Serum anti-OVA IgG1 and IgG2a levels were measured by means of ELISA with OVA-coated plates and horseradish peroxidase-conjugated anti-mouse IgG1 and IgG2a (Southern Biotech). Two-fold serial dilutions were prepared for each serum (starting dilution 1:25 for IgE, 1:5000 for IgG1, and 1:250 for IgG2a titrations). Antibody titers were calculated as the dilution corresponding to twice the mean absorbance value obtained for nonsensitized mouse sera.

Quantitative RT-PCR

RNA extraction from skin biopsy specimens was performed as previously described by Chirgwin et al.^{E5} Reverse transcription was performed with 1 μ g of RNA by using Superscript reverse transcriptase (Invitrogen, Cergy Pontoise, France), according to the manufacturer's instructions. cDNAs were amplified by means of PCR in triplicate assays for 40 cycles in SYBR Green PCR Master Mix (Molecular Probes, Europe BV, Leiden, The Netherlands) on an ABI PRISM 7000 sequence detection system (Applied Biosystems, Courtaboeuf, France). Oligonucleotide primers specific for mouse cytokines, chemokines, and their receptors and nuclear transcription factors are listed in Table E1. Primers were used for amplification in triplicate assays. PCR amplification of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase was performed to control for sample loading and to allow normalization between samples. Because mRNA levels in the tissues from PBS-sensitized PPAR- α ^{-/-} animals were identical to those measured in PBS-sensitized WT mice, results for OVA-sensitized animals were expressed as the mean fold induction compared with the mean expression level in PBS-treated mice.

In vitro cytokine production

Spleen and inguinal lymph node cells were isolated at day 50 and restimulated in 96-well plates in triplicate with 10 μ g/mL OVA or PBS. Culture supernatants were harvested 4 days later. T_H1 (IFN- γ) and T_H2 (IL-4, IL-5, and IL-10) cytokine production was quantified with a Cytometric Bead Array kit and FCAP Array software (BD PharMingen), according to the manufacturer's instruction.

REFERENCES

- E1. Lee SS, Pineau T, Drago J, Lee EJ, Owens JW, Kroetz DL, et al. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. *Mol Cell Biol* 1995;15:3012-22.
- E2. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911-7.
- E3. Angeli V, Staumont D, Charbonnier A-S, Hammad H, Gosset P, Pichavant M, et al. Activation of the D prostanoid receptor 1 regulates immune and skin allergic responses. *J Immunol* 2004;172:3822-9.
- E4. Dombrowicz D, Flamand V, Miyajima I, Ravetch JV, Galli SJ, Kinet JP. Absence of Fc epsilonRI alpha chain results in upregulation of Fc gammaRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between Fc epsilonRI and Fc gammaRIII for limiting amounts of FcR beta and gamma chains. *J Clin Invest* 1997;99:915-25.
- E5. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979;18:5294-9.

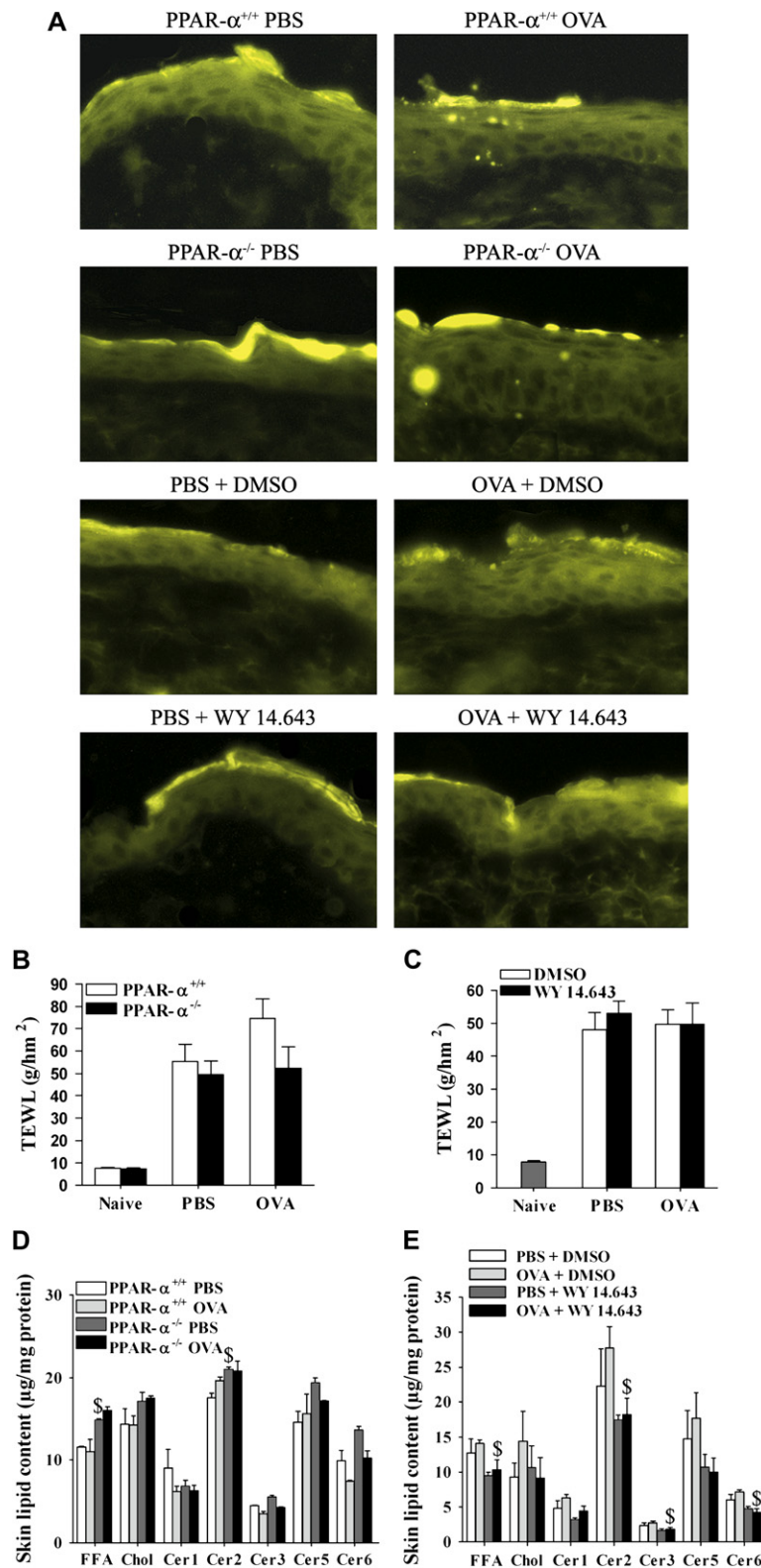


FIG E1. Analysis of skin barrier function in WT and PPAR- $\alpha^{-/-}$ mice and PPAR- α agonist-treated WT animals in a model of AD. **A**, Nile red staining of skin neutral lipids (200-fold magnification). **B** and **C**, TEWL. **D** and **E**, Skin lipid content ($n = 4$ animals per group). \$, Statistically different from dimethyl sulfoxide (DMSO)-treated mice ($P < .05$).

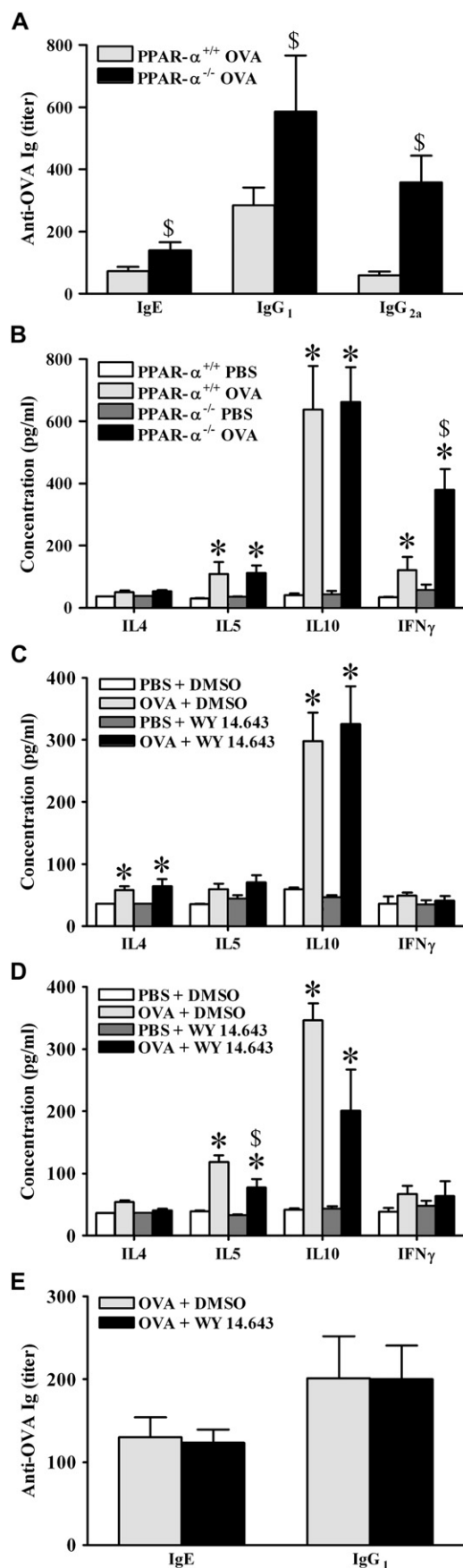


FIG E2. Humoral and cellular response in WT and PPAR- $\alpha^{-/-}$ mice and PPAR- α agonist-treated WT animals in a model of AD. **A**, OVA-specific IgE, IgG₁, and IgG_{2a} concentrations in WT and PPAR- $\alpha^{-/-}$ mice. **B-D**, Cytokine production by splenocytes (Fig E2, B and C) and inguinal lymph nodes (Fig E2, D) from PPAR- $\alpha^{-/-}$ and WT mice (Fig E2, B) and WY14643- or dimethyl sulfoxide (DMSO)-treated WT mice (Fig E2, C and D) after *in vitro* OVA restimulation ($n = 5-6$ animals per group). **E**, OVA-specific IgE and IgG₁ concentrations in WY14643- or DMSO-treated mice. OVA-specific IgG_{2a} levels remained undetectable. *Statistically different from PBS-treated mice ($P < .05$). \$, Statistically different from control WT mice ($P < .05$).

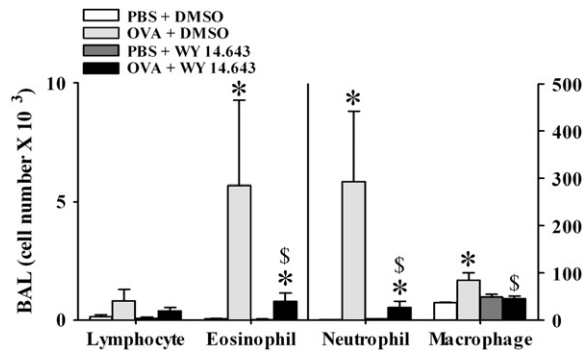


FIG E3. Decreased lung inflammation in a model of AD after topical treatment with the PPAR- α agonist WY14643. The total number of macrophages, neutrophils, lymphocytes, and eosinophils in BAL fluid at the time of death is shown ($n = 3-6$ animals per group). *DMSO*, Dimethyl sulfoxide.

TABLE E1. Primers used for real-time PCR

Gene	Primer	Size	Sequence
Mouse CCL5/RANTES	Forward	21	5' CCT GCT GCT TTG CCT ACC TCT 3'
	Reverse	20	5' ACT TGG CGG TTC CTT CGA GT 3'
Mouse CCL11/eotaxin 1	Forward	20	5' GCT TTC AGG GTG CAT CTG TT 3'
	Reverse	20	5' CAC GGT CAC TTC CTT CAC CT 3'
Mouse CCL17/TARC	Forward	20	5' GCT CGA GCC ACC AAT GTA GG 3'
	Reverse	19	5' ACA CGA TGG CAT CCC TGG A 3'
Mouse CCL22/MDC	Forward	20	5' TGG TGC CAA TGT GGA AGA CA 3'
	Reverse	21	5' GGC AGG ATT TTG AGG TCC AGA 3'
Mouse CCL24/eotaxin 2	Forward	21	5' GAC CAT CCC CTC ATC TTG CTG 3'
	Reverse	21	5' GGT GAT GAA GAT GAC CCC TGC 3'
Mouse CCL27/cTACK	Forward	20	5' CAT GGA ACT GCA GGA GGC TG 3'
	Reverse	20	5' TTG GCG TTC TAA CCA CCG AG 3'
Mouse CCR3	Forward	20	5' TGA CCC CAG CTC TTT GAT TC 3'
	Reverse	24	5' CTG GAC TCA TAA AGG ACT TAG CAA 3'
Mouse CCR4	Forward	20	5' CGC CAT CCA GGC TAC AGA AA 3'
	Reverse	19	5' ACC CCG GCA TGT TCT GAA G 3'
Mouse CCR5	Forward	22	5' GCA AAA AGC TGA AGA GCG TGA C 3'
	Reverse	20	5' TGC AGC ATA GTG AGC CCA GA 3'
Mouse CXCL10/IP-10	Forward	19	5' ACC CAA GTG CTG CCG TCA T 3'
	Reverse	20	5' CAT TCT CAC TGG CCC GTC AT 3'
Mouse CXCR3	Forward	20	5' TGA GCA GCA CGG ACA CCT TC 3'
	Reverse	20	5' AAC CCA CTG GAC AGC AGC AT 3'
Mouse Foxp3	Forward	21	5' CCC AGG AAA GAC AGC AAC CTT 3'
	Reverse	18	5' TTC TCA CAA GGC CAC TTG 3'
Mouse GAPDH	Forward	20	5' TGC CCA GAA CAT CAT CCC TG 3'
	Reverse	20	5' TCA GAT CCA CGA CGG ACA CA 3'
Mouse IFN- γ	Forward	20	5' ACC CTG TCG TAT GCT GGG AA 3'
	Reverse	21	5' GTT GGT GCA GGA ATC AGT CCA 3'
Mouse IL-1 β	Forward	20	5' AGG TGC TCA TGT CCT CAT CC 3'
	Reverse	20	5' CAG GCA GGC AGT ATC ACT CA 3'
Mouse IL-6	Forward	20	5' CCA CGG CCT TCC CTA CTT CA 3'
	Reverse	22	5' CCA CGA TTT CCC AGA GAA CAT G 3'
Mouse IL-4	Forward	20	5' GCA TGG AGT TTT CCC ATG TT 3'
	Reverse	20	5' AGA TGG ATG TGC CAA ACG TC 3'
Mouse IL-5	Forward	22	5' CTC ACC GAG CTC TGT TGA CAA G 3'
	Reverse	22	5' GAA CTC TTG CAG GTA ATC CAG G 3'
Mouse IL-10	Forward	21	5' TTC ATG GCC TTG TAG ACA CCT 3'
	Reverse	20	5' TGA ATT CCC TGG GTG AGA AG 3'
Mouse IL-12p35/IL-12a	Forward	20	5' GAA GCA GGA TGC AGA GCT TC 3'
	Reverse	20	5' CTA GAC AAG GGC ATG CTG GT 3'
Mouse IL-13	Forward	20	5' GGA ATC CAG GGC TAC ACA GA 3'
	Reverse	20	5' AGG AGC TGA GCA ACA TCA CA 3'
Mouse IL-17	Forward	22	5' AAG TCT TTA ACT CCC TTG GCG C 3'
	Reverse	19	5' AGG GTC TTC ATT GCG GTG G 3'
Mouse IL-18	Forward	21	5' GGA ATC AGA CAA CTT TGG CCG 3'
	Reverse	22	5' CCT CGA ACA CAG GCT GTC TTT T 3'
Mouse IL-31	Forward	21	5' GAC GAA TCA ATA CAG CTG CCG 3'
	Reverse	20	5' GGT TAA TGC TTC CCG GTC CA 3'
Mouse IL-33	Forward	19	5' ATC AGG CGA CGG TGT GGA T 3'
	Reverse	21	5' GAC GTC ACC CCT TTG AAG CTC 3'
Mouse NF- κ B 1	Forward	21	5' ATG TGT GTG AAG GCC CAT CAC 3'
	Reverse	19	5' CAG GCC CCA CAT AGT TGC A 3'
Mouse NF- κ B 2	Forward	20	5' GTT GAT GAC GCC GAG GTA CT 3'
	Reverse	20	5' ACA GGA CGA GAA CGG AGA CA 3'
Mouse TGF- β	Forward	21	5' TAC TCT GGA GAC GGT TTG CCA 3'
	Reverse	20	5' CAT GAA GAA AGT CTC GCC CG 3'
Mouse TSLP	Forward	19	5' TAT CCC TGG CTG CCC TTC A 3'
	Reverse	21	5' TGT GCC ATT TCC TGA GTA CCG 3'
Mouse PPAR- α	Forward	20	5' ATG CCA GTA CTG CCG TTT TC 3'
	Reverse	20	5' CCG AAT CTT TCA GGT CGT GT 3'
Mouse PPAR- β/δ	Forward	21	5' CTC TTC ATC GCG GCC ATC ATT 3'

(Continued)

TABLE E1. (*Continued*)

Gene	Primer	Size	Sequence
Human PPAR- α	Reverse	21	5' TCT GCC ATC TTC TGC AGC AGC 3'
	Forward	20	5' GGT GGA CAC GGA AAG CCC AC 3'
Human PPAR- β/δ	Reverse	20	5' GGA CCA CAG GAT AAG TCA CC 3'
	Forward	20	5' GGG ACC ACA GCA TGC ACT TC 3'
	Reverse	20	5' TGC AGT TGG TCC AGC AGT GA 3'

TARC, Thymus and activation-regulated chemokine; *MDC*, macrophage-derived chemokine; *cTACK*, cutaneous T cell-attracting chemokine; *IP-10*, IFN- γ -inducible protein-10; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.