

Original article

PPAR γ expression profile and its cytokine driven regulation in atopic dermatitis

Background: Recent studies point to the pathophysiological role of the peroxisome proliferators-activated receptor gamma (PPAR γ) in the inflammatory immune response. We have showed that activation of PPAR γ by specific ligands attenuates the allergic immune response via monocytes and lymphocytes. The objective of this study was to analyse the PPAR γ expression and its regulation via inflammatory cytokines.

Methods: We examined the PPAR γ expression in the lesional and nonlesional skin of atopic patients by immunohistochemistry. The expression patterns of PPAR γ mRNA and its isoforms were investigated in peripheral mononuclear blood cells of atopic and nonatopic donors and in cytokine-stimulated populations by quantitative real-time RT-PCR.

Results: Our data show an increased PPAR γ expression in lesional skin from atopic dermatitis patients. The analysis of PPAR γ mRNA reveals a significantly up-regulated expression of PPAR γ 1 but not of PPAR γ 2 in monocytes and CD4⁺ T-cells from atopic dermatitis patients. Furthermore, we demonstrate that Th-cytokines, like IL-4, IL-13 and IFN γ , which regulate the biphasic atopic immune response, directly regulate the expression of PPAR γ 1.

Conclusion: Taken together, these data demonstrate that PPAR γ isoforms are differently expressed and regulated by the local cytokine-milieu. Whether the increased expression of the PPAR γ 1 receptor may be beneficial or not for a PPAR γ ligand-based treatment of atopic dermatitis, is currently under investigation.

A. Dahten¹, S. Mergemeier²,
M. Worm¹

¹Department of Dermatology and Allergy, Allergy-Center-Charité Campus Mitte Universitätsmedizin Berlin; ²Congen Biotechnology GmbH, Berlin, Germany

Key words: atopic dermatitis; cytokines; monocytes; PPAR γ ; T-cells.

Prof. Dr Margitta Worm
Department of Dermatology and Allergy
Charité – Universitätsmedizin Berlin (CCM)
Charitéplatz 1
10117 Berlin, Germany

Accepted for publication 30 April 2007

Atopic dermatitis (AD) is the cutaneous manifestation of a systemic disorder, which gives rise to asthma, food allergy and allergic rhinitis (1, 2). Interactions between the local and systemic immunological response play a crucial role in the manifestation of AD.

The peroxisome proliferator-activated receptor gamma (PPAR γ) belongs to the family of nuclear receptors, and shows pleiotrophic effects in the inflammatory processes in its capacity as a ligand dependent transcription factor (3). Functional human PPAR γ was originally cloned from the human bone marrow, and plays a role in the regulation and development of immune cells like monocytes and T-lymphocytes (4). Previously, it has been shown that PPAR γ is differently expressed in monocytes and lymphoid cells (5–7). Known are the two isoforms PPAR γ 1 and PPAR γ 2. However, the existence of alternative splice variants for PPAR γ mRNAs in humans and mice suggests cell-specific expression patterns and functions of these receptor proteins (8–10).

The PPAR γ gene gives rise to three RNA transcripts, which are identical except for their different first exons (Fig. 3A). PPAR- γ 1 and PPAR- γ 3 are translated to the

identical protein PPAR γ 1, whereas PPAR γ 2 is translated from its own unique mRNA. Several studies support the role of PPAR γ in inflammatory processes as its expression appears to be altered by (pro)-inflammatory cytokines (11,12). A different PPAR γ expression profile due to the changes of the local inflammatory milieu may result in differential functional effects of the immune response. Current studies indicate that the activation of PPAR γ can improve skin disorders through specific agonists, which belong to the group of thiazolidinediones (TZDs) (13). Furthermore, TZDs elicit immune regulatory effects in Th2-mediated allergic diseases, as it has been shown in murine asthma models (14, 15). Our own previous studies showed that the TZD ciglitazone inhibited the allergic immune response by the inhibition of IgE production but also (pro)inflammatory cytokines of the Th-response *in vitro* as also *in vivo* (16). Mechanisms involving the transcriptional regulation of Th2-cell development play an important role in the pathogenesis of AD. Furthermore, these data indicate that PPAR γ activation by specific ligands may affect the function of T-lymphocytes and monocytes in a different

manner with promising effects concerning the treatment of AD.

The aim of the present study was to analyze the expression profile of PPAR γ and its isoforms in lymphocytes and monocytes from atopic donors. Furthermore, we elucidate the impact of AD relevant inflammatory cytokines (IL-4, IL-13, IFN γ) on PPAR γ expression.

Materials and methods

Patients/controls

Peripheral venous blood samples were obtained from 12 patients, six females and six males (mean age 35 years), suffering from a moderate to severe extrinsic form of atopic dermatitis, according to the criteria of Hanifin and Raika (17) (see Table S1). The severity of AD was defined by using the SCORAD score, which evaluates the extension, intensity and subjective symptoms of AD (18, 19). All patients were polysensitized with positive skin prick tests to house dust mite, pollen and/or cat dander. The total serum IgE levels of the AD donors had a minimum of 500 kU/l (average $\geq$ 2000 kU/l). None of the included donors received a systemic immunosuppressive medication or UV therapy during the past 3 months before the blood sample was taken. Twelve age and sex matched healthy volunteers, without skin lesions and a negative history of type 1 allergies or a positive family history of atopic diseases, were chosen as control group for blood samples. Skin biopsy specimens (3 mm each) were taken from seven atopic patients with typical AD lesions (erythema, edema, crusting, excoriation and lichenification). None of these patients had other skin diseases. From each patient, a maximum of two specimens (lesional and nonlesional) were obtained. Acute AD lesions were present for $<$ 5 days and chronic lichenified AD lesions present at least for 4 weeks by the patient's history. Nonlesional skin samples were defined as noninflammatory skin, and were taken apart from inflammatory skin areas, with a minimum space of 5 cm. Local ethics committee approved the investigation and all donors gave informed consent.

Immunohistochemistry

Representative biopsy specimens were obtained from acute-, chronic- and nonlesional skin of patients with AD. Frozen tissue blocks were cut in to 5- μ m sections, adhered onto microscope slides and fixed in acetone. Sections were blocked with 10% normal goat serum in TBS for 20 min. The primary monoclonal antibodies were applied 75 min for PPAR γ (Santa Cruz, Heidelberg, Germany) and 45 min for CD68 (DAKO, Hamburg, Germany) at a concentration of 4 μ g/ml and 10 min for CD3 (clone F7.2.38). The specificity of PPAR γ staining was controlled by the usage of an appropriate blocking peptide (sc-7273 P) and isotype control. Signals were detected using the LSAB method by Chem-Mate[®] alkaline phosphatase detection Kit (Dako, Hamburg, Germany). Colorimetric detection was performed with Fast Red and BCIP/NBT (Dako). Sections were counterstained with hematoxylin.

Isolation and culture of mononuclear cells

Monocytes and lymphocytes from each donor were isolated and cultured as previously described (16). Briefly, human peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation with Histopaque-1077 (Biochrom, Berlin, Germany) from the heparinized whole blood of the AD patients and the

healthy subjects. Lymphocytes (CD4⁺, CD8⁺, CD19⁺) and monocytes (CD14⁺) were purified by positive selection enrichment with a magnetic cell sorting system (Miltenyi-Biotec, Bergisch Gladbach, Germany) according to the manufacturer's guidelines. For cytokine stimulation, freshly purified cells were plated with a density of 10⁶ cells and incubated with either 10 ng/ml IL-4 (ImmunoTools, Friesoythe, Germany), IL-13 (PeproTech, Frankfurt, Germany) or 20 ng/ml IFN γ for 24 h.

RNA extraction, real-time PCR and sequencing

Total RNA was extracted from purified mononuclear cell populations using RNeasy Mini Kit (Qiagen, Hilden, Germany) and reverse transcribed by SuperScript (Invitrogen, Karlsruhe, Germany) according to the manufacturer's guidelines. To analyze the PPAR γ mRNA isoforms (1–3), transcript specific primer pairs were generated using *Primer Premier* Software tools (PREMIER Biosoft International, Palo Alto, CA, USA). Primer pairs for PPAR γ were: cttgcagtgaggatgtctca for sense and cgccaacacctctctct for anti-sense, resulting in a 75-bp amplicon. For PPAR γ transcript variants, the following primers were used: mRNA1 (116-bp) gtggccgcagattgaaag (sense) and gaatggcatctctgtgtcaacc (anti-sense), mRNA3 (130-bp) ttctgcttaattcccttcttag (sense) and anti-sense (see mRNA1), mRNA2 (69-bp) acccatttccatgctgtatg (sense) and aaggatcgcttctgggtc (anti-sense). The expression levels of PPAR γ mRNA were quantified relative to the expression of the house-keeping gene PBGD with the following primer pairs: agatgtattcgcgtgggtacc (sense) and cctgtgtgtgacatagcaatg (anti-sense) by real-time RT-PCR using the LightCycler 1.5 (Roche Diagnostics, Mannheim, Germany). As negative control the template was replaced by sterile water. Amplification products were purified by electrophoresis in a 2.5% agarose gel stained with ethidium bromide. Using the ABI PRISM BigDye Terminator Cycl sequencing kit (Applied Biosystems, Darmstadt, Germany), PCR products were subjected to automatic sequencing by ABI Prism 310 Genetic Analyser (Applied Biosystems). The melting curve analysis was used to control the melting point specificity. Efficiency corrected relative quantification was performed by 2^{- $\Delta\Delta$ CT} method (20).

Protein isolation and Western blotting

Protein extracts of 1 $\times$ 10⁷ magnetic sorted cells were prepared with NE-PER extraction reagent (Perbio, Bonn, Germany) and a protease inhibitor cocktail (Roche, Penzberg, Germany) and quantified by standard Bradford protein assay. 50 μ g cytosolic and 20 μ g nuclear extracts were separated in 10% Tris-HCl gel. Proteins were transferred to polyvinylidene difluoride membrane (PVDF), blocked for 1 h with 5% dry milk in Tris-buffered saline-Tween (TBST) and were incubated with the primary antibody for PPAR γ sc-7196 (Santa Cruz, Heidelberg, Germany), with a dilution of 1 : 800, over night at 4°C. After washing the membrane with TBST, HRP-conjugated goat anti-rabbit IgG (Chemicon, München, Germany) was added at a concentration of 0,1 μ g/ml for 1 h at room temperature. The antigen detection was performed with the chemiluminescent detection system ECL Plus (Amersham Biosciences, Freiburg, Germany). The integrity of PPAR γ was confirmed by two-dimensional protein separation related to the specific isoelectric point in comparison with the molecular weight (Proteome Factory, Berlin, Germany).

Statistical analysis

Data were compared by nonparametric analysis using the Wilcoxon signed rank test for matched pairs and the Mann-Whitney *U*-test

(SPSS GmbH Software, Munich, Germany) or Students *t*-test (sigma plot; Systat Software GmbH, Erkrath, Germany) for independent groups. *P*-values < 0.05, were considered statistically significant.

Results

PPAR γ expression is increased in lesional skin from AD patients

The expression of PPAR γ protein was analyzed in lesional and nonlesional skin of AD patients. In nonlesional skin sections PPAR γ , was low detectable (Fig. 1A,G). The immunohistochemical (IHC) analysis from biopsies of AD-lesional skin showed a strong epidermal and dermal expression of PPAR γ protein (Fig. S1), which was accompanied by a strong dermal infiltration of mononuclear cells (Fig. 1B, C). A further analysis of the infiltrate by IHC revealed that these infiltrates consist

mainly of T-cells (Fig. 1E) and monocytes/macrophages (Fig. 1F). In acute lesions displaying clinical signs like erythema and/or crusting, the perivascular T-cell infiltrates in the dermis dominated (Fig. S1), whereas skin-sections from chronic lesions show lichenification, the number of macrophages were increased (Fig. S1). Interestingly, the expression of PPAR γ in suprabasal epidermal cells was associated with the increasing dermal infiltrates of mononuclear cells (Fig. 1A–C). For each section, the specificity of PPAR γ staining was confirmed by blocking with a specific PPAR γ peptide (Fig. 1D,J, exemplarily shown).

Increased expression of PPAR γ mRNA in CD4 $^{+}$ T-lymphocytes and CD14 $^{+}$ monocytes from atopic donors

As AD is a systemic disorder, we evaluated the expression of PPAR γ in monocytes and lymphocytes from the peripheral blood of AD patients compared with nonatopic donors (control). For this, PPAR γ mRNA expression in CD14 $^{+}$ monocytes, CD4 $^{+}$ and CD8 $^{+}$ T-cells, was quantified by real-time PCR in detail. In all investigated cell populations PPAR γ mRNA was strongly detectable. Figure 2 shows a statistically significant enhanced PPAR γ expression in monocytes (fourfold) and CD4 $^{+}$ T-lymphocytes (threefold) from atopic patients. Monocytes, CD4 $^{+}$ T-lymphocytes as well as CD19 $^{+}$ B-cells of nonatopic donors showed comparable PPAR γ mRNA expression levels. Analysis of CD8 $^{+}$ and CD19 $^{+}$ lymphocytes revealed no statistical significant differences of

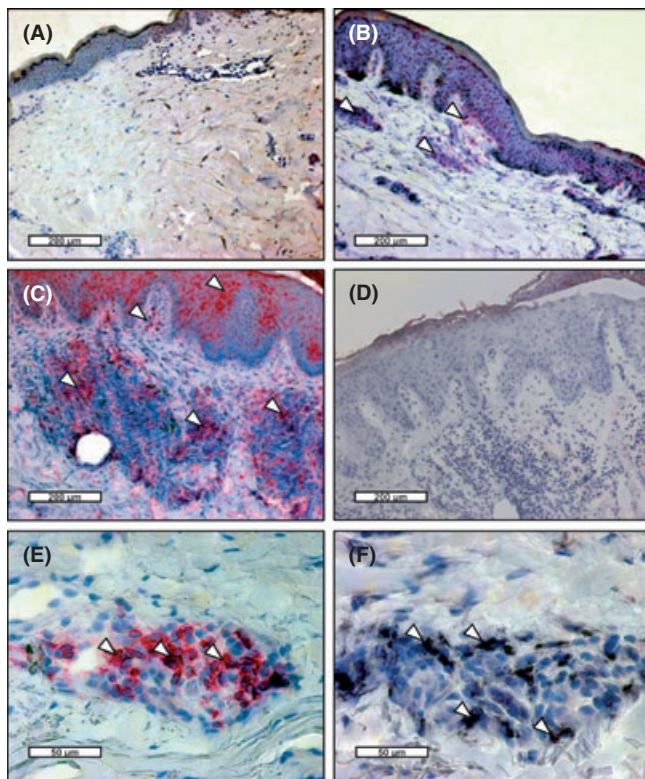


Figure 1. Peroxisome proliferator-activated receptor gamma (PPAR γ) expression in lesional and nonlesional skin of AD patients (A–D). PPAR γ (red) is low detectable in unaffected skin sections (A) and rises with increasing infiltration of mononuclear blood cells in the dermis (B, C), consisting of CD3 $^{+}$ (E, red) and/or CD68 $^{+}$ cells (F, black). PPAR γ was blocked by specific peptide as control (1 D). Results shown are representative of skin biopsy specimens from AD patients ($n = 7$) with individual skin lesions. Arrows indicate positive stained cells. Pictures were taken at 100 $\times$ magnification (scale bar = 200 μ m) and 400 $\times$ magnification (scale bar = 50 μ m).

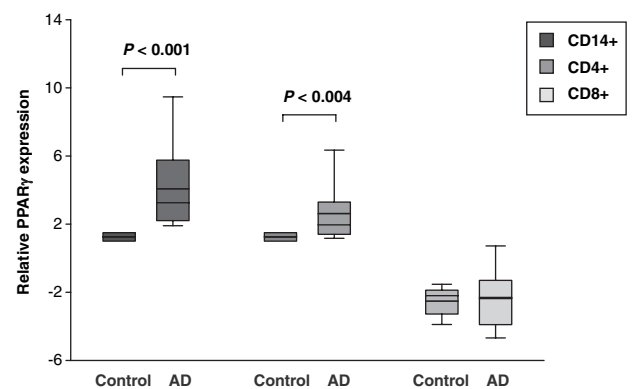


Figure 2. Expression of peroxisome proliferator-activated receptor gamma (PPAR γ) mRNA in lymphocytes and monocytes from peripheral blood. PPAR γ mRNA from atopic donors [AD] is significantly increased (approximately threefold) in CD14 $^{+}$ monocytes ($n = 12$) and CD4 $^{+}$ T-cells ($n = 10$) compared to nonatopic donors [control]. No significant differences in PPAR γ expression levels were detected in CD8 $^{+}$ T-cells. Statistical significance [*P*] is based on the Mann–Whitney *U*-test; *x*-fold PPAR γ expression was calculated by $2^{\Delta\Delta CT}$ -method normalized to PBGD, relative to data of CD4 $^{+}$ /CD14 $^{+}$ control. Data represent median (black line), mean (gray line), 5th, 25th, 75th and 95th percentiles as vertical boxes with error bars.

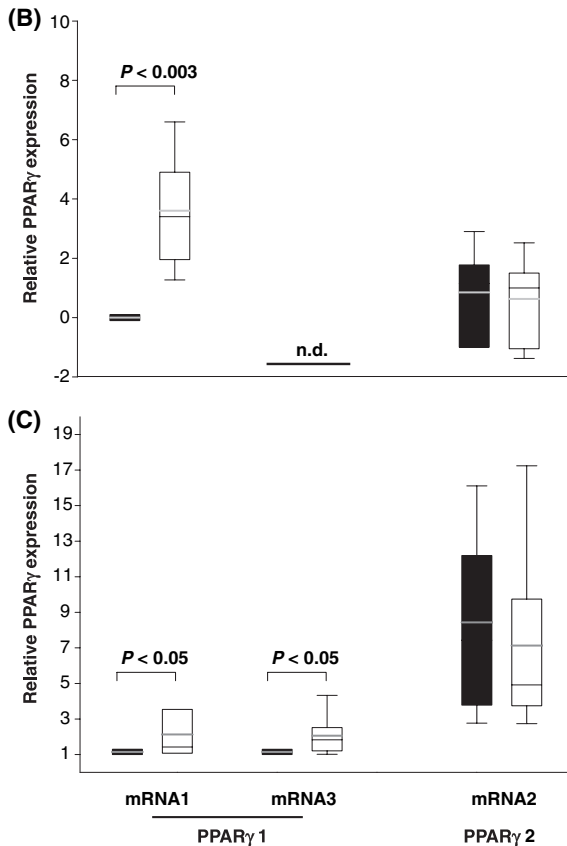
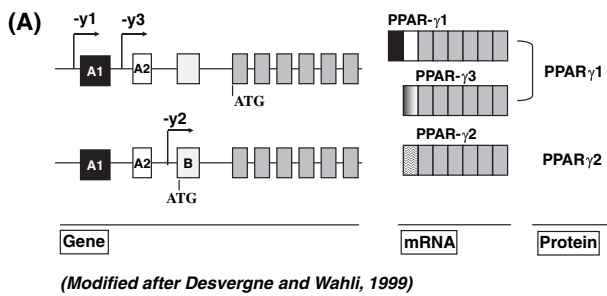


Figure 3. Expression of two peroxisome proliferator-activated receptor gamma (PPAR γ) isoforms by differential use of three promoters. (A) The mRNA1 (eight exons) and mRNA3 (seven exons) transcripts translate into the PPAR γ 1 protein, transcription from promoter γ 2 results in the PPAR γ 2 protein. Transcript variants differ in their first exons, while the last six coding exons (grey) are common to all three RNAs. (B) PPAR γ 1 expression is significantly increased in CD14⁺ monocytes and (C) in CD4⁺ T-cells from AD donors [white box] compared to nonatopic donors [full box]. Statistical significance [P] is based on the Mann–Whitney U -test; x -fold PPAR γ expression was calculated by $2^{\Delta\Delta CT}$ -method normalized to PBGD, relative to mRNA1 levels of control. Data represent median (black line), mean (grey line), 5th, 25th, 75th and 95th percentiles as vertical boxes with error bars. [n.d.] indicates data out of range (CT > 35) or non detectable relative to the total number of measured donors in CD4⁺ T-cells: 6/10 (mRNA1) and 6/10 mRNA3 of nonatopic donors and 3/10 (mRNA1) of atopic donors.

PPAR γ mRNA expression in samples derived from nonatopic and atopic donors (data not shown). Within the AD patients we did not observe a clear association between clinical severity and PPAR γ expression (see Table S1).

Enhanced PPAR γ expression is restricted to PPAR γ 1 and PPAR γ 3 mRNA

As PPAR γ can be expressed as alternative splice variants, we next studied the expression levels of the different PPAR γ mRNA isoforms in CD14⁺ and CD4⁺ cells. PPAR- γ 1 and - γ 3 encode for the gene product PPAR γ 1, whereas - γ 2 gives rise to PPAR γ 2 protein (Fig. 3A). Figure 3B shows differently expressed mRNA isoforms in monocytes. In monocytes derived from atopic donors the γ 1 isoform was significantly increased expressed (approximately threefold), whereas the γ 3 isoform was not detectable in any of the monocytes tested ($n = 8$). By contrast, γ 2 expression levels widely differed between the donors, but to a similar extent in atopic but also nonatopic donors. The more detailed analysis of PPAR γ isoform expression in CD4⁺ T-lymphocytes revealed comparable results (Fig. 3C). In contrast to monocytes, both isoforms encoding PPAR γ 1 were significantly increased compared with nonatopic donors, but only weakly expressed. However, throughout the examined cell populations (CD4⁺, CD8⁺, CD19⁺) PPAR γ 2 was the predominantly expressed isoform.

Expression profile of PPAR γ protein

To analyze whether the detected mRNA profiles were accompanied by protein expression, we determined the expression of PPAR γ 1/2 protein in lymphocytes and monocytes by Western blot. As shown in Fig. 4A, the PPAR γ protein was detectable in the cytosolic but also in the nuclear extracts of the analyzed cells. However the expression of PPAR γ protein within these compartments was heterogeneous. A cytosol-specific band at *c.* 50 kDa and a nucleus-specific band at *c.* 67 kDa were present in monocytes and in lymphocytes. In contrast, we determined a reproducible band of *c.* 75 kDa in lymphoid cell populations. As PPAR γ 2 mRNA is predominantly expressed in T- and B-lymphocytes, we suppose the existence of the PPAR γ 2 protein in these cells. The detection of the *c.* 50 kDa band may indicate a cytosol specific degradation of the PPAR γ protein. By applying two-dimension gel electrophoresis (Fig. 4B), we excluded other proteins which may have an equal molecular mass, like PPAR α (55 kDa) or PPAR δ (52 kDa) and which may be recognized by the used antibodies as well. Interestingly, the repeated protein analysis with the same cell revealed a loss of the protein at *c.* 67 kDa (data not shown).

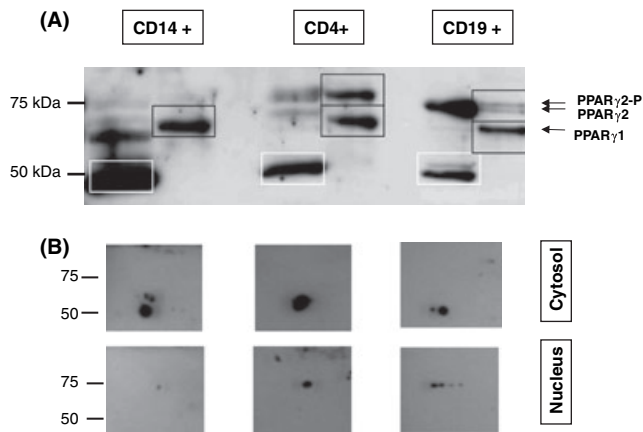


Figure 4. Cytosolic (50 μ g) and nuclear extracts (20 μ g) of CD14⁺, CD4⁺ and CD19⁺ cells were analyzed by Western blot ($n = 4$). Detection of basal PPAR γ 1/2 protein in cytosolic extracts by *c.* 50 kDa (white frame) and nuclear extracts by *c.* 67 kDa (black frame) suggest the expression of PPAR γ 1 in monocytes (CD14⁺), T- (CD4⁺) and B-lymphocytes (CD19⁺), whereas PPAR γ 2 seems to be restricted to the lymphocytes populations (A). Two-dimension gel electrophoresis spots with equal isoelectric points [horizontal₄₋₈] and molecular weight [vertical] indicate the detection of the same protein (B). Blots are representative of three similar results.

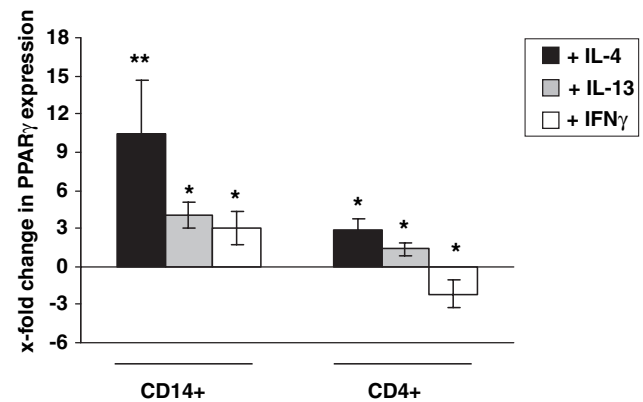


Figure 5. Peroxisome proliferator-activated receptor gamma (PPAR γ) mRNA expression is regulated by Th-cytokines. CD14⁺ monocytes and CD4⁺ T-cells were incubated with IL-4, IL-13 (10 ng/ml) and IFN γ (20 ng/ml) for 24 h and relative amounts of PPAR γ were measured in comparison with untreated cells. The values are calculated by $2^{\Delta\Delta CT}$ -method normalized to PBGD relative to the baseline cytokine expression in untreated cells. ** $P < 0.01$, * $P < 0.05$, statistical significance is based on the Wilcoxon-Test. Data are presented as mean boxes with ± 2 SD bars.

Regulation of PPAR γ expression by Th1/Th2-cytokines

As our expression profile analysis of PPAR γ indicated an increased PPAR γ expression not only in monocytes but also in CD4⁺ lymphocytes from atopic dermatitis

patients, we subsequently determined the role of cytokines, which are relevant in pathogenesis of AD. It has been recorded that allergen-specific T-cells are increased in frequency and produce IL-4 and IL-13, but little interferon γ (IFN γ). Our data show that the expression of PPAR γ in CD14⁺ monocytes was significantly not only induced by Th2 cytokines like IL-4 and IL-13 but also by Th1 cytokine IFN γ after 24 h. By contrast, IL-4 and IL-13 had a weak impact on the PPAR γ expression in CD4⁺ T-lymphocytes. Furthermore, and in contrast to monocytes, treatment with IFN γ decreased the PPAR γ expression in CD4⁺ T-cells. Similar results were obtained in CD8⁺ T-lymphocytes (data not shown). The detailed analysis of PPAR γ expression in CD14⁺ (Fig. 6A) and CD4⁺ cells (Fig. 6B) revealed that the examined cytokines significantly modulate PPAR γ 1 and PPAR γ 3 isoforms only. These isoforms exclusively result in the PPAR γ 1 protein.

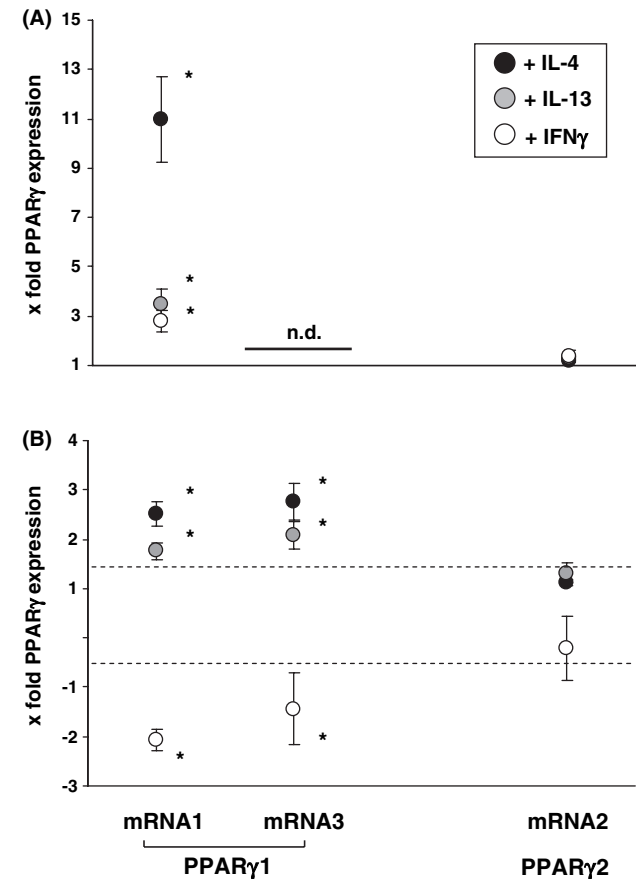


Figure 6. Cytokine-mediated modulation of the peroxisome proliferator-activated receptor gamma (PPAR γ) expression in CD14⁺ monocytes (A) and CD4⁺ T-cells (B) is restricted to the mRNA1 and the mRNA3 isoform. PPAR γ mRNA2 expression was not significantly altered by IL-4, IL-13 or IFN γ . The values are calculated by $2^{\Delta\Delta CT}$ -method normalized to PBGD relative to baseline cytokine expression in untreated cells. Data are presented as horizontal point blots with ± 2 SD bars. * $P < 0.05$, statistical significance is based on the Wilcoxon-Test.

Discussion

In the present study, we demonstrate, for the first time that the expression of PPAR γ 1 is increased in monocytes and CD4⁺ T-cells from atopic patients. Moreover, PPAR γ 1 expression is directly regulated by inflammatory Th-cytokines like IL-4, IL-13 and IFN γ .

Peroxisome proliferator-activated receptors are transcription factors, which convert environmental, nutritional and inflammatory signals by transcriptional regulation to a cellular response. They regulate gene expression in response to the binding of low molecular weight ligands (21). Recent data pointed to the physiological role of PPARs and their corresponding ligands in the modulation of the inflammatory immune responses (3, 21–24). Our own previous data showed that PPAR α and PPAR γ agonists inhibited the allergic immune response in atopic individuals via monocytes but also T-cells (16). Dallas et al. have shown that murine T-cell activation results in a down regulation of PPAR α expression, while PPAR γ expression is increased under the same conditions of T-cell activation (25). Other observations pointed to a constitutive role of PPAR γ in activated T-cells (26, 27).

Atopic dermatitis is a chronic relapsing skin disorder maintained by the interaction of migrating mononuclear blood cells and epidermal keratinocytes (28, 29). In this study we demonstrate that PPAR γ is expressed in the dermal cell infiltrate of eczematous AD skin lesions, which mainly consist of lymphocytes and monocytes. Homing of distinct proinflammatory cells such as CD4⁺ cells and monocytes/macrophages into the skin contributes to the inflammatory immune response, resulting in typical AD lesions (30). Our present study demonstrates that not only monocytes but also T-lymphocytes from atopic patients suffering from the extrinsic form of AD express significantly increased PPAR γ levels compared to nonhealthy donors. Similar results were observed in a murine asthma model (31). It has been shown that antigen-induced inflammation by systemic sensitisation, associated with IgE production promotes the PPAR γ expression in the lungs. These data and the fact that AD commonly occurs in concert with other atopic diseases, like asthma or allergic rhinitis (1, 2, 32) suggest the possibility that PPAR γ expression may be uniquely regulated in these atopic diseases. Whether sex, age or individual clinical criteria of AD, like severity or associated respiratory atopic diseases correlate with the PPAR γ expression, needs to be addressed in further studies with larger groups and clearly dissected atopic phenotypes.

Additionally, we analyzed the different PPAR γ mRNA isoforms in monocytes and lymphocytes. We observed increased PPAR γ expression levels of mRNAs, which are coding for the PPAR γ 1 protein, but PPAR γ 2 mRNA also was detectable at relevant levels. PPAR γ 1 can be

translated from its own unique mRNA or from the translation of the PPAR γ 2 mRNA at the AUG codon. Previous studies indicated that PPAR γ 2 mRNA was not detectable in mononuclear blood cells (26, 33, 34). However, the protein can be present even when the mRNA has disappeared, which may result in false negative findings because of technical errors or a high background (6).

Previous analyses showed that PPAR γ protein was expressed in the cytosol as well as in the nucleus (34, 35). In this study, we confirmed the detection of PPAR γ 1/2 protein expression by Western blot analysis. Similarly, several studies could detect a strong band at 50 kDa in whole protein extracts of CD4⁺ cells and monocytes (27, 35). But it has also been demonstrated that the PPAR γ protein is detectable as a 67 kDa band in total protein extracts from mice lymphocytes (34, 36). As several studies indicated a ligand dependent degradation of PPAR γ by ubiquitin-proteasome, we consider the observed band of 50 kDa as a fragment of the PPAR γ protein in the cytosol.

As our mRNA expression data have shown PPAR γ 2 as the predominantly expressed isoform, the existence of the PPAR γ 2 protein is also presumed in lymphoid cells, because an additional band at 75 kDa was detectable in the nuclear extracts in these cells. The observed time-dependent gradual loss of the 67 kDa protein (Fig. 4B), fortifies our assumption, because this observation was previously described for the PPAR γ 1 protein only (37).

Several studies suggest that during inflammatory processes, the PPAR γ expression is modulated differently (12, 38). The cell specific pattern of PPAR γ expression observed in atopic and nonatopic donors indicates that PPAR γ 1 expression is modulated by a typical cytokine milieu (IL-4, IL-13, IFN γ) present in the atopic immune response. The development of the AD phenotype is influenced by local and systemic cytokine expressing cells. Extrinsic AD patients exhibit a dominating Th2-immune response with an increased frequency of IL-4 as well as IL-13 producing CD4⁺ T-cells (Th2) and a decrease in IFN γ -producing T-cells (Th1). Similar observations are known for acute eczematous skin of AD patients. Chronic AD skin lesions are characterized by significantly less IL-4 and IL-13 producing cells and larger numbers of IFN γ expressing cells (39).

Previous data indicate that IL-4 can induce PPAR γ expression in peritoneal macrophages and human monocytes (11, 38). Our data confirm this observation regarding monocytes but also CD4⁺ T-cells. Similar effects could be observed for the Th2 cytokine IL-13, which has been suggested to play an important role in the acute and chronic phase of AD as well. Additional, our data show that IFN γ , which is known to be highly present in the chronic AD phenotype, can induce PPAR γ expression in monocytes/macrophages, the pre-

dominant cell type in chronic AD lesions. This indicates that IFN γ exerts diverse effects on the PPAR γ expression depending on the cell type. According to these observations, our histological analysis of acute and chronic lesional AD skin shows increased numbers of PPAR γ expressing cells. Consistent with our observation of a cytokine regulated PPAR γ 1 expression, the analysis of the nucleotide sequences of PPAR γ transcripts 1–3 by public database GENOMATIX identifies potential binding sites for the signal transducers and activators (STATs) in the promotor regions encoding for PPAR γ 1 and PPAR γ 3 only.

As the activation of PPAR γ by specific PPAR γ ligands exert anti-inflammatory effects on the allergic immune response, the observation of an increased expression of PPAR γ in monocytes and CD4 $^{+}$ T-lymphocytes of atopic donors implicates the importance of PPAR γ -ligand based treatment in atopic diseases. The induction of PPAR γ expression by cytokines, which are increased in the acute phase of AD, suggests that systemic PPAR γ -ligand treatment might be even more effective during the early phases of AD. However, the increased expression of PPAR γ protein in chronic AD skin lesions also point to a positive impact during the chronic phase by topical PPAR γ -ligand treatment. Our data may provide a basis for the development of therapeutically novel molecules involving a PPAR γ targeted immune modulation.

Acknowledgments

This study was supported by grant of Charité-Universitätsmedizin Berlin, Germany. The authors would like to thank Dr Lilla Landeck, Claudia Rasche and Dr Hae-Hyuk Lee for clinical assistance and Dr rer nat. Elke. O. Luger and Dennis Ernst for excellent laboratory assistance. We are grateful to Proteome Factory (Berlin, Germany) for technical assistance in preparing 2D gel electrophoresis.

References

1. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. *J Allergy Clin Immunol* 2003;**112**:S118–S127.
2. Leung DY, Bieber T. Atopic dermatitis. *Lancet* 2003;**361**:151–160.
3. Zingarelli B, Cook JA. Peroxisome proliferator-activated receptor-gamma is a new therapeutic target in sepsis and inflammation. *Shock* 2005;**23**:393–399.
4. Greene M, Blumberg B, McBride OW, Yi HF, Kronquist K, Kwan K. Isolation of the human PPARGgamma cDNA: expression in hematopoietic cells and chromosomal mapping. *Gene Expr* 1995;**4**:281–299.
5. Chtanova T, Kemp RA, Sutherland AP, Ronchese F, Mackay CR. Gene microarrays reveal extensive differential gene expression in both CD4(+) and CD8(+) type 1 and type 2 T cells. *J Immunol* 2001;**167**:3057–3063.
6. Greene ME, Pitts J, McCarville MA, Wang XS, Newport JA, Edelstein C et al. PPARGgamma: observations in the hematopoietic system. *Prostaglandins Other Lipid Mediat* 2000;**62**:45–73.
7. 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.

Supplementary material

The following supplementary material is available for this article:

Figure S1. PPAR γ expression in the dermis of lesional and non-lesional skin of AD patients. PPAR γ (red) is low detectable in unaffected skin sections (g) but is strongly detectable in lesional skin (h and i). PPAR γ was blocked by specific peptide as control (j). Expression of CD3 $^{+}$ T-cells (red) and CD68 $^{+}$ monocytes/macrophages (black) in acute (k and m) and chronic (l and n) lesions of AD patients. Arrows indicate positive stained cells. Pictures were taken at 100 $\times$ magnification (scale bar = 200 μ m) and 400 $\times$ magnification (scale bar = 50 μ m).

Table S1. PPAR γ mRNA expression in CD4 $^{+}$ T-cells and CD14 $^{+}$ monocytes from blood samples of AD patients relative to the PPAR γ expression levels in appropriate analyzed cells of nonatopic donors. All patients suffer from moderate to severe AD and were evaluated by the SCORAD score. Severity: 25–50 points (pt.) indicates moderate AD, > 50 points indicates severe AD. From each individual gender, age (years), SCORAD, serum IgE and PPAR γ expression (x-fold to nonhealthy) are shown as single values and means.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1398-9995.2007.01444.x> (This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

8. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R et al. The organization, promoter analysis, and expression of the human PPAR γ gene. *J Biol Chem* 1997;**272**:18779–18789.
9. Fajas L, Fruchart JC, Auwerx J. PPAR γ 3 mRNA: a distinct PPAR γ mRNA subtype transcribed from an independent promoter. *FEBS Lett* 1998;**438**:55–60.
10. Sundvold H, Lien S. Identification of a novel peroxisome proliferator-activated receptor (PPAR) gamma promoter in man and transactivation by the nuclear receptor ROR α 1. *Biochem Biophys Res Commun* 2001;**287**:383–390.
11. Huang JT, Welch MR, Ricote M, Binder CJ, Willson TM, Kelly C et al. IL-4 dependent production of PPAR-gamma ligands in macrophages by 12/15-lipoxygenase. *Nature* 1999;**400**:378–382.
12. Tanaka T, Itoh H, Doi K, Fukunaga Y, Hosoda K, Shintani M et al. Down regulation of peroxisome proliferator-activated receptorgamma expression by inflammatory cytokines and its reversal by thiazolidinediones. *Diabetologia* 1999;**42**:702–710.
13. Ellis CN, Varani J, Fisher GJ, Zeigler ME, Pershadsingh HA, Benson SC et al. Troglitazone improves psoriasis and normalizes models of proliferative skin disease: ligands for peroxisome proliferator-activated receptor-gamma inhibit keratinocyte proliferation. *Arch Dermatol* 2000;**136**:609–616.
14. 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.
15. Mueller C, Weaver V, Vanden Heuvel JP, August A, Cantorna MT. Peroxisome proliferator-activated receptor gamma ligands attenuate immunological symptoms of experimental allergic asthma. *Arch Biochem Biophys* 2003;**418**:186–196.
16. Ruhl R, Dahten A, Schweigert FJ, Herz U, Worm M. Inhibition of IgE-production by peroxisome proliferator-activated receptor ligands. *J Invest Dermatol* 2003;**121**:757–764.
17. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venerol* 1980;(Suppl. 92):44–47.
18. Kunz B, Oranje AP, Labreze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997;**195**:10–19.
19. Oranje AP, Stalder JF, Taieb A, Tasset C, de Longueville M. Scoring of atopic dermatitis by SCORAD using a training atlas by investigators from different disciplines. ETAC Study Group. Early Treatment of the Atopic Child. *Pediatr Allergy Immunol* 1997;**8**:28–34.
20. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;**25**:402–408.
21. Debril MB, Renaud JP, Fajas L, Auwerx J. The pleiotropic functions of peroxisome proliferator-activated receptor gamma. *J Mol Med* 2001;**79**:30–47.
22. Lee KS, Park SJ, Hwang PH, Yi HK, Song CH, Chai OH et al. PPAR-gamma modulates allergic inflammation through up-regulation of PTEN. *FASEB J* 2005;**19**:1033–1035.
23. Daynes RA, Jones DC. Emerging roles of PPARs in inflammation and immunity. *Nat Rev Immunol* 2002;**2**:748–759.
24. Clark RB. The role of PPARs in inflammation and immunity. *J Leukoc Biol* 2002;**71**:388–400.
25. Jones DC, Ding X, Daynes RA. Nuclear receptor peroxisome proliferator-activated receptor alpha (PPARalpha) is expressed in resting murine lymphocytes. The PPARalpha in T and B lymphocytes is both transactivation and transrepression competent. *J Biol Chem* 2002;**277**:6838–6845.
26. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L, Padula SJ. The nuclear receptor PPAR γ and immunoregulation: PPAR γ mediates inhibition of helper T cell responses. *American Association of Immunologists* 2000;**164**:1364–1317.
27. Wang P, Anderson PO, Chen S, Paulsson KM, Sjogren HO, Li S. Inhibition of the transcription factors AP-1 and NF-kappaB in CD4 T cells by peroxisome proliferator-activated receptor gamma ligands. *Int Immunopharmacol* 2001;**1**:803–812.
28. Akdis CA, Blaser K, Akdis M. Apoptosis in tissue inflammation and allergic disease. *Curr Opin Immunol* 2004;**16**:717–723.
29. Leung DY, Boguniewicz M, Howell MD, Nomura I, Hamid QA. New insights into atopic dermatitis. *J Clin Invest* 2004;**113**:651–657.
30. Abramovits W. Atopic dermatitis. *J Am Acad Dermatol* 2005;**53**:S86–S93.
31. Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. *J Allergy Clin Immunol* 2004;**113**:882–888.
32. Boguniewicz M, Schmid-Grendelmeier P, Leung DY. Atopic dermatitis. *J Allergy Clin Immunol* 2006;**118**:40–43.
33. Desvergne B, Wahli W. PPARs: nuclear control of metabolism. *Endocr Rev* 1999;**20**:649–688.
34. Harris SG, Phipps RP. The nuclear receptor PPAR gamma is expressed by mouse T lymphocytes and PPAR gamma agonists induce apoptosis. *Eur J Immunol* 2001;**31**:1098–1105.
35. Hornung D, Waite LL, Ricke EA, Bentzien F, Wallwiener D, Taylor RN. Nuclear peroxisome proliferator-activated receptors alpha and gamma have opposing effects on monocyte chemotaxis in endometriosis. *J Clin Endocrinol Metab* 2001;**86**:3108–3114.
36. Padilla J, Leung E, Phipps RP. Human B lymphocytes and B lymphomas express PPAR-gamma and are killed by PPAR-gamma agonists. *Clin Immunol* 2002;**103**:22–33.
37. Floyd ZE, Stephens JM. Interferon-gamma-mediated activation and ubiquitin-proteasome-dependent degradation of PPARgamma in adipocytes. *J Biol Chem* 2002;**277**:4062–4068.
38. Ricote M, Welch JS, Glass CK. Regulation of macrophage gene expression by the peroxisome proliferator-activated receptor-gamma. *Horm Res* 2000;**54**:275–280.
39. Hamid Q, Boguniewicz M, Leung DY. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest* 1994;**94**:870–876.