

Respiratory Research

**Open Access**

Research

Peroxisome Proliferator-Activated Receptor α (PPAR α) down-regulation in cystic fibrosis lymphocytes

Veerle Reynders*¹, Stefan Loitsch¹, Constanze Steinhauer¹, Thomas Wagner¹, Dieter Steinhilber² and Joachim Bargon¹Address: ¹Dept. of Internal Medicine, Division of Pneumology, University Hospital Frankfurt, Germany and ²Institute of Pharmaceutical Chemistry, University of Frankfurt, Frankfurt am Main, GermanyEmail: Veerle Reynders* - veerlereynders@hotmail.com; Stefan Loitsch - sm_loitsch@hotmail.com; Constanze Steinhauer - consti24@web.de; Thomas Wagner - t.wagner@em.uni-frankfurt.de; Dieter Steinhilber - steinhilber@em.uni-frankfurt.de; Joachim Bargon - bargon@em.uni-frankfurt.de

* Corresponding author

Published: 30 July 2006

Received: 16 February 2006

Respiratory Research 2006, **7**:104 doi:10.1186/1465-9921-7-104

Accepted: 30 July 2006

This article is available from: <http://respiratory-research.com/content/7/1/104>

© 2006 Reynders et al; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: PPARs exhibit anti-inflammatory capacities and are potential modulators of the inflammatory response. We hypothesized that their expression and/or function may be altered in cystic fibrosis (CF), a disorder characterized by an excessive host inflammatory response.

Methods: PPAR α , β and γ mRNA levels were measured in peripheral blood cells of CF patients and healthy subjects via RT-PCR. PPAR α protein expression and subcellular localization was determined via western blot and immunofluorescence, respectively. The activity of PPAR α was analyzed by gel shift assay.

Results: In lymphocytes, the expression of PPAR α mRNA, but not of PPAR β , was reduced (-37%; $p < 0.002$) in CF patients compared with healthy persons and was therefore further analyzed. A similar reduction of PPAR α was observed at protein level (-26%; $p < 0.05$). The transcription factor was mainly expressed in the cytosol of lymphocytes, with low expression in the nucleus. Moreover, DNA binding activity of the transcription factor was 36% less in lymphocytes of patients ($p < 0.01$). For PPAR α and PPAR β mRNA expression in monocytes and neutrophils, no significant differences were observed between CF patients and healthy persons. In all cells, PPAR γ mRNA levels were below the detection limit.

Conclusion: Lymphocytes are important regulators of the inflammatory response by releasing cytokines and antibodies. The diminished lymphocytic expression and activity of PPAR α may therefore contribute to the inflammatory processes that are observed in CF.

Background

Cystic fibrosis (CF) is a common inherited disease caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), which is an epithelial chloride channel. The disorder affects multi-

ple organs and the phenotype is extremely heterogeneous. However, CF morbidity and mortality are mainly due to lung disease, which is characterized by an excessive host inflammatory response. Although CF lung disease is generally considered to be a neutrophil-mediated disorder,

recent studies suggest a potent role for lymphocytes in the pathogenesis of the disease [1,2]. In addition, inflammatory markers such as cytokines and eicosanoids are elevated, not only locally, in the airways, but also systemically, thus indicating a more generalized state of inflammation in CF [3-5].

The nuclear factor- κ B (NF- κ B) and activated protein-1 (AP-1) transcription factors are key players in the inflammatory response by inducing the expression of cytokines, chemokines, cell adhesion molecules and growth factors. The actions of NF- κ B and AP-1 can, however, be inhibited by the Peroxisome Proliferator-Activated Receptors α and γ (PPARs), which thereby exert anti-inflammatory properties [6-8]. PPARs are ligand-activated transcription factors belonging to the nuclear hormone receptor super-family. Fatty acids and eicosanoids are natural occurring PPAR ligands [9,10]; fibrates and glitazones are more specific synthetic activators for PPAR α and γ , respectively. PPARs regulate gene expression by heterodimerization with the retinoid \times receptor (RXR) and subsequent binding to specific DNA sequence elements, termed PPAR response elements (PPRE), in the promoter regions of their target genes [11]. In addition, they can repress gene transcription in a DNA-binding independent manner through inhibition of other signaling pathways by protein-protein interactions and cofactor competition [6,7,12]. At present, three distinct PPAR isoforms have been identified, called α , β and γ . PPAR α and γ agonists decrease plasma concentrations of cytokines and acute phase proteins [13-15] and induce anti-atherosclerotic effects [16,17] and are therefore able to influence the immune response. They also seem to play a role in airway inflammation. Similarly, PPAR α and γ agonists have been reported to inhibit airway inflammation in a murine model of asthma [18] and a model of airway infection [19] by inhibiting eosinophil, lymphocyte and neutrophil influx into the lung.

Moreover, CF is associated with abnormalities in fatty acid and eicosanoid metabolism. In addition to deficiencies in essential fatty acids in plasma, increased release of arachidonic acid (AA) from the cell membrane and elevated levels of pro-inflammatory eicosanoids in urine, blood and airways have been reported [3,20-24]. Even cell membrane compositions seem to be disturbed with increased levels of AA and decreased levels of docosahexaenoic acid (DHA) [25]. Fatty acids and derivatives can regulate the actions of PPARs and an imbalance may therefore cause inappropriate activation of PPARs.

In conclusion, we hypothesized that the expression of PPARs, transcription factors with anti-inflammatory capacities, is altered in CF. To check our hypothesis, we measured PPAR α , β and γ expression in peripheral blood cells, which are important mediators of the inflammatory

response through the production and release of cytokines, chemokines, and/or antibodies. We noticed differences for PPAR α levels in lymphocytes. Along the same line, an altered PPAR α activity was observed in lymphocytes, which confirmed our hypothesis.

Materials and methods

Patients

This study was approved by the Ethics Committee of the Frankfurt University Hospital. Patients with cystic fibrosis were between 22 and 43 years old and were all affected by lung disease. They had a stable condition and came for routine check-up. The clinical characteristics of our patients are represented in Table 1. An age-matched, gender-mixed healthy control group was established for all the experiments. Only healthy feeling volunteers, which had not been ill for the past weeks, and which were free from any detectable inflammation, infection or allergic disease were selected for sampling. Due to time, technical and sampling constraints, sample sizes vary between the different experiments.

Measurement of IL-8 in plasma by ELISA

A commercial ELISA kit was used to measure IL-8 concentrations in plasma (R&D Systems, Germany). The instructions of the manufacturer were followed.

Measurement of sIL-2R in plasma by ELISA

A commercial ELISA kit was applied to measure soluble IL-2 Receptor levels (R&D Systems, Germany). Prior to use, plasma was diluted 1 to 4. The instructions of the manufacturer were followed.

Isolation of peripheral lymphocytes and monocytes

To avoid circadian fluctuations of PPARs, blood samples were always taken in the morning. Mononuclear cells were isolated from whole blood by density gradient centrifugation using Lymphoprep (Axis-Shield). After washing with PBS, monocytes were separated from lymphocytes by magnetic sorting (Miltenyi Biotec, Bergisch Gladbach, Germany). Cells were incubated with saturating concentrations of anti-CD14+ monoclonal antibodies conjugated with super paramagnetic particles for 20 min. by 4°C. Subsequently, cells were resolved in PBS (containing 5 mM EDTA and 0.5% BSA) and added on top of a separation column. Unlabeled cells, *i.e.* lymphocytes, were collected through elution from the column. In order to isolate the monocytes, the separation column was detached from the strong magnet and monocytes were eluted. Purity was checked with May-Grünwald Giemsa staining and was $\geq 97\%$.

Isolation of peripheral neutrophils

Density centrifugation using Polymorphprep™ solution (Axis Shield, Heidelberg, Germany) enabled us to isolate

Table 1: Clinical characteristics of cystic fibrosis patients.

Patient	Age (years)	Gender	Genotype	P.a. ¹	CRP	FEV ₁ % pred ²	FVC % pred ²
1	33	F	dF508/R553x	+	0,9	58	91
2	25	M	dF508/dF508	+	0,5	74	90
3	30	M	dF508/dF508	+	0,7	42	72
4	34	M	dF508/?	+	0,3	59	80
5	37	M	dF508/dF508	+	0,6	52	84
6	32	F	dF508/dF508	+	2	60	75
7	26	F	dF508/dF508	+	1,32	86	87
8	28	M	dF508/?	+	0,91	31	48
9	37	M	dF508/dF508	+	< 0,3	30	43
10	32	F	dF508/dF508	+	< 0,3	76	92
11	23	M	dF508/?	+	< 0,3	103	99
12	37	M	dF508/dF508	-	< 0,3	74	101
13	34	F	dF508/dF508	+	0,94	23	59
14	39	M	dF508/dF508	-	< 0,3	60	83
15	25	M	dF508/R553x	+	1,03	85,9	79,7
16	22	F	dF508/N1303	+	0,9	53,6	64,6
17	43	M	dF508/dF508	+	< 0,3	98,8	95,5
18	23	M	dF508/dF508	+	0,8	85,3	84,5
19	39	M	dF508/G542x	+	0,4	61	79
20	40	M	dF508/?	+	0,4	64	80

¹ *Pseudomonas aeruginosa* infection² Normal: 80–120% of predicted

neutrophils from whole blood. The mononuclear and polymorphonuclear leucocytes were separated into 2 distinct bands, free from red blood cells. Neutrophils were collected, washed with PBS and checked for purity via May-Grünwald-Giemsa staining and had to be > 95%.

Reverse transcriptase – competitive multiplex PCR/real-time PCR

Total RNA from monocytes, lymphocytes and neutrophils was extracted with RNazol B™ (Wak-Chemie, Germany) and subjected to oligo(deoxythymidine)-primed first-strand cDNA synthesis using the Superscript II Preamplification System (Invitrogen, Karlsruhe, Germany). The instructions of the manufacturers were followed.

Multiplex PCR (see Loitsch et al., 1999)[26]

Construction of internal standards

The cDNA derived from monocytes and lymphocytes was amplified in the presence of a range of known concentrations of internal standards (competitors). Internal standards for the PPARs and GAPDH were constructed as wild-type fragments containing a deletion of nucleotides: PPAR α , β and γ cDNA with a 44, 41 and 106 bp deletion, respectively and GAPDH cDNA with a 55 bp deletion. The shortened fragments were obtained via PCR and the use of following antisense primers: 5'-ATC ACA GAA GAC AGC ATG GCC GTT CAG GTC CAA GTT TGC G-3' for PPAR α , 5'-CTG CCA CAA TGT CTC GAT GTA GGA TGC TGC GGG CCT TCT T-3' for PPAR β and 5'-TCA GCG GGA AGG ACT TTA TGC ACT GGA GAT CTC CGC CAA C-3' for PPAR γ . The sense primers were the same as those used for

the multiplex PCR (see next paragraph). The fragments were ligated in T-vectors (Promega) and the copy number was calculated after spectrophotometric quantification. Then, dilution series (1:3) of the internal standards were established. The internal standards share identical primer recognition sites with the wild-type target.

Competitive multiplex Polymerase Chain Reaction

Oligonucleotide primers for PCR were designed according to published sequences: PPAR α [GenBank Accession no. [Y07619](#)]: sense 5'-TGCAGATCTCAAATCTCTGG-3', antisense 5'-ATCACAGAAGACAGCATGGC-3', amplifying a 374 bp wild-type product; PPAR β [GenBank Accession no. [L07592](#)]: sense 5'-TTCCAGAAGTGCCTGGCACT-3', antisense 5'-CTGCCACAATGTCTCGATGT-3'; amplifying a 275 bp wild-type product; PPAR γ [GenBank Accession no. [D83136](#)]: sense 5'-TCTCTCCGTAATGGAAGACC-3', antisense 5'-TCTTTCCTGTCAAGATCGCC-3', amplifying a 660 bp wild-type product and, GAPDH [GenBank Accession no. [M33197](#)]: sense 5'-ATCTTCCAGGAGCGA-GATCC-3', antisense 5'-ACCACTGACACGTTGGCAGT-3', amplifying a 502 bp wild-type product.

2–10 μ l cDNA was added to a PCR master-mix, which contained all the primers mentioned above. Next, the mix was divided over a series of reaction tubes into which known concentrations of internal standards were spiked. Cycling conditions for PCR were as follows: 94°C for 3 minutes (1 cycle), followed by 40 cycles of 94°C, 58°C, 72°C, each for 45 seconds and a final extension phase at 72°C for 10 minutes (Trio-Thermoblock, Biometra).

The amplification products were separated by agarose gel electrophoresis, stained with ethidium bromide and analyzed by densitometry. Densitometric data were plotted on a log/log scale as a function of internal-standard-derived PCR products and corrected for molar equivalence.

Real-time PCR

Neutrophils exhibit low levels of mRNA in general. The classic competitive PCR was not sensitive enough and we had to establish real-time PCR. Real-time PCR was performed by using the ABI prism 7700 sequence detector (Perkin Elmer/Applied Biosystems). Primers and probes were designed using the software program Primer Express (Perkin Elmer/Applied Biosystems). For the measurement of β -actin, a published primers/probe set was applied [27]. The fluorogenic probes contained a reporter dye (FAM) covalently attached at the 5' end and a quencher dye (TAMRA) covalently attached at the 3' end. PPAR α [Genbank: [NM005036](#)]: sense 5'-CTT CAA CAT GAA CAA GGT CAA AGC-3', antisense 5'-AGC CAT ACA CAG TGT CTC CAT ATC A-3', probe 5'-CGG GTC ATC CTC TCA GGA AAG GCC-3', amplicon length 99 bp; PPAR β [Genbank: [L07592](#)]: sense 5'-GGG CAT GTC ACA CAA CGC TAT-3', antisense 5'-GCA TTG TAG ATG TGC TTG GAG AA-3', probe 5'-CTT CTC AGC CTC CGG CAT CCG A-3', amplicon length 147 bp; PPAR γ [Genbank: [D83233](#)]: sense 5'-GAA ACT TCA AGA GTA CCA AAG TGC AA-3', antisense 5'-AGG CTT ATT GTA GAG CTG AGT CTT CTC-3', probe 5'-CAA AGT GGA GCC TGC ATC TCC ACC TTA TT-3', amplicon length 87 bp; β -actin [Genbank: [D28354](#) and [X00351](#)]: sense 5'-AGC CTC GCC TTT GCC GA-3', antisense 5'-CTG GTG CCT GGG GCG-3', probe 5'-CCG CCG CCC GTC CAC ACC CGC C-3', amplicon length 174 bp.

Specific external controls were constructed for all target genes by cloning a partial cDNA fragment (the amplicon of interest obtained by classic PCR amplification) into a pCR^{2.1} vector (Invitrogen). A standard curve was generated: in each PCR run, 10-fold serial dilutions of the corresponding plasmid clone were included, with known amounts of input copy number. In order to normalize for inefficiencies in cDNA synthesis and RNA input amounts, the mRNA expression of the housekeeping gene β -actin was quantified for each sample. cDNA samples were diluted 10 times prior to PCR amplification. PCR amplifications were performed in a total volume of 25 μ l, containing 5 μ l cDNA sample, 12.5 μ l Taqman Universal PCR Master Mix (Perkin Elmer/Applied Biosystems), 200–800 nM of each primer and 200 nM detection probe (Eurogentec). Each PCR amplification was performed in triplicate, using the following conditions: 2 min. at 50°C and 10 min. at 95°C, followed by a total of 45 two-temperature cycles: 15 s at 94°C and 1 min. at 60°C for PPARs and

67°C for β -actin. PCR data were analyzed through the application of the software 'Sequence Detector 7.1' (Perkin Elmer/Applied Biosystems).

Western blot analysis for lymphocytes

Equal amounts (80 μ g) of total cell proteins were resolved by 10% SDS-PAGE and transferred to a PVDF membrane (Millipore Corporation, Bedford) at 80 V for 1 hour. Membranes were incubated with mouse PPAR α monoclonal antibodies (1:2000 dilution) (clone B11.80A, generous gift from Dr. Winegar, Glaxo Smith Kline) at 4°C overnight. Protein levels were normalized using a mouse monoclonal antibody against β -actin (1:10,000 dilution) (Sigma). Proteins were subsequently detected through the use of horseradish peroxidase-conjugated secondary antibodies and the chemiluminescence system ECL (Amersham Pharmacia Biotech, Buckinghamshire, UK). After scanning (DocuGel V-System, Scanalytics), band intensities were analyzed using the software package Zero-D-ScanTM (Scanalytics).

Immunofluorescence assay

Cytospin glass slides were prepared by centrifugation of 10⁵ lymphocytes using a cytospin centrifuge (Cytospin 4, Thermo Shandon). After cells were fixed in ice-cold methanol and blocked with a solution of 2% BSA in PBS overnight at 4°C, they were permeabilized with Perm/Wash buffer (BD Biosciences Pharmingen) and then incubated for 2 hours with monoclonal PPAR α antibody, diluted 1:10 in Perm/Wash/2%BSA buffer (clone P α B32.51 kindly provided by Dr. Winegar, Glaxo Smith Kline [28]). After washing, the second antibody (cy3 labeled goat-anti-mouse, Caltag) was added in a 1:250 dilution in Perm/Wash/2%BSA buffer for 30 min. Following washing and air-drying, the cells were embedded in Aquatex (Merck) and evaluated by immunofluorescence microscopy.

Gel shift assay

Nuclear proteins from lymphocytes were prepared as described by Dignam and coworkers [29]. A gel shift kit for PPAR α was obtained from Panomics, Inc. and the instructions of the manufacturer were followed. Equal amounts of nuclear protein extracts (10 μ g as determined by Bradford assay) were incubated for 30 min. with biotin-labeled oligonucleotide probe, which corresponds to the PPAR binding site, and then subjected to non-denaturing PAGE. Afterwards, proteins were blotted on a Pall-Biodyne^B (PALL Corporation) membrane and bands were visualized after exposure to HyperfilmTMECL (Amersham Biosciences, UK). Subsequently, equal loading was checked via Coomassie Blue staining of the membrane. Band intensities were analyzed using the software package Zero-D-ScanTM (Scanalytics).

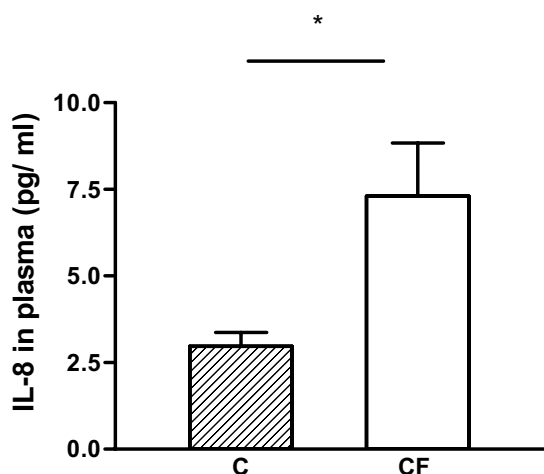


Figure 1
IL-8 levels in plasma of CF patients and healthy persons measured by ELISA. IL-8 levels are significantly higher in CF patients (n = 15) than in control persons (n = 11). Results are shown as mean \pm standard error. * Significantly different ($p < 0.03$).

Statistical analysis

Results are expressed as mean \pm SE. Statistical comparisons were made using the unpaired Student's t-test (Sigma-plot). A value of $p < 0.05$ was considered significant.

Results

Interleukin-8 levels in plasma

IL-8 in plasma was measured via ELISA to demonstrate that the patients in this study exhibit the typical elevated systemic cytokine levels [30]. As expected, IL-8 levels were significantly higher in CF patients compared with control persons (7.3 pg/ml vs 2.9 pg/ml, respectively; $p < 0.03$) (Fig. 1). We can therefore assume that the inflammation cascade is not restricted to the airways, but is also found systemically.

PPAR mRNA expression in peripheral blood cells

In order to check for differences in the expression of PPARs between CF patients and healthy persons, we started screening at mRNA level. All data were normalized to the expression levels of the housekeeping genes GAPDH or β -actin, which were equally expressed in samples of CF patients and control persons.

Monocytes and lymphocytes

Competitive multiplex PCR products were loaded on an agarose gel, electrophorised and stained with ethidium bromide (see fig. 2). Bands were scanned and analyzed with the software package Zero-D-Scan™ (Scanalytics).

PPAR α

PPAR α mRNA levels were significantly lower (-37%, $p < 0.002$) in lymphocytes of CF patients compared with control persons (Fig. 3). In monocytes, no differences were observed in the expression of PPAR α between the healthy subjects and the CF patients (Fig. 4).

PPAR β

For both lymphocytes and monocytes, no statistical differences in the mRNA expression of PPAR β were detected between CF patients and healthy persons (Fig. 3 and 4).

PPAR γ

PPAR γ mRNA was detected in a few samples of monocytes and lymphocytes, but was not quantifiable due to the extremely low expression levels.

Neutrophils

Neutrophils are considered end-cells as DNA and most, but not all, mRNA and protein synthesis, cease once the myeloid cells are mature enough to enter the blood. For that reason, mRNA levels were rather low in neutrophils and PPAR mRNA was difficult to quantify via the classic competitive multiplex PCR. We therefore developed real-time PCR, a highly sensitive and accurate method.

PPAR α

PPAR α mRNA levels were equal in neutrophils of CF patients and healthy persons (Fig. 5A)

PPAR β

Idem, PPAR β mRNA levels were similar in both groups (Fig. 5B).

PPAR γ

PPAR γ mRNA was detectable, but the low expression levels did not allow quantification.

PPAR α protein levels in peripheral blood lymphocytes measured via western blotting

mRNA analysis revealed less expression of PPAR α in lymphocytes of CF patients compared with healthy persons. On the basis of this finding we further examined the expression of the receptor at protein level via western blotting. A single band for PPAR α was observed around 60 kDA (Fig. 6A). Analysis of the band intensities demonstrated that protein levels of PPAR α are significantly lower (-26%, $p < 0.05$) in lymphocytes of CF patients compared with control subjects (Fig. 6B). β -actin was measured for normalization.

Localization of PPAR α in human peripheral blood lymphocytes

In order to identify the subcellular localization of PPAR α within peripheral blood lymphocytes, an immunofluores-

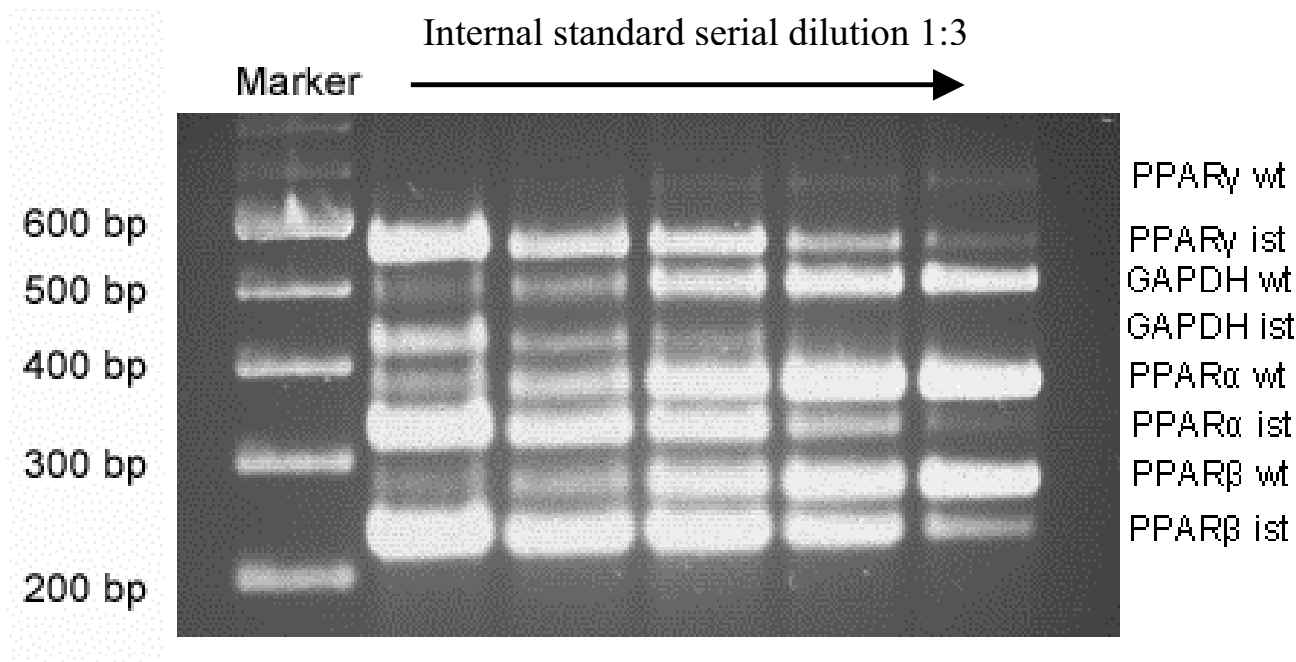


Figure 2

RT-competitive multiplex PCR for PPAR α , β , and γ and GAPDH in human peripheral blood lymphocytes. Picture of ethidium bromid stained agarose gel after electrophoresis of the amplified products. wt = wild-type amplicon, ist = internal standard amplicon.

cence assay was developed. As shown in Fig. 7A and 7B, the highest concentration of the protein is observed in the cytosol, whereas the nucleus contains only trace amounts of the transcription factor. In the context of our study, the technique was not found appropriate for quantifying PPAR α protein levels by means of measuring the fluorescence intensity. Activity was therefore measured via gel shift assay.

Activity of the PPAR α transcription factor

Since PPAR α expression is lower in lymphocytes of CF persons, it was deemed useful to check for the activity of the transcription factor, which was determined via gel shift assay (Fig. 8). To this end, a commercially available kit for PPAR α was used (Panomics). The DNA-binding element (PPRE) was not radioactive-, but biotin-labeled. Equal amounts of nuclear extracts were loaded. The measurement of band intensities showed that PPAR α DNA binding activity was 36% less in lymphocytes of CF patients, compared with control subjects ($p < 0.01$) (Fig. 8B). In order to evaluate the binding specificity, competition analysis was performed by adding 60-fold cold specific (PPRE) and unspecific oligonucleotide (see fig. 8A: lane 2 and 3, respectively). The upper band faded

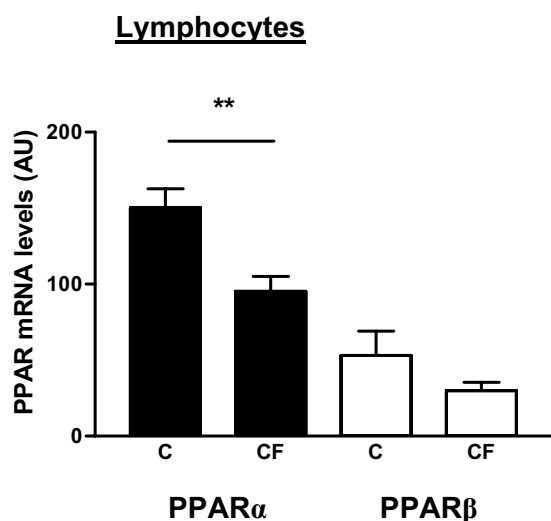
strongly by adding cold PPRE, but remained unaltered after adding cold unspecific oligonucleotide. Equal loading of nuclear extracts was verified via Coomassie Blue staining of the membrane.

sIL-2 R levels in plasma

Soluble IL-2 receptor (sIL-2R), a well-known marker for T-lymphocyte activation, was measured in plasma of stable CF patients and control persons via ELISA (Fig. 9). Normal values for sIL-2R levels in plasma are around 1020 pg/ml. Statistical analysis revealed that CF patients exhibit significantly higher levels of sIL-2R in plasma than healthy persons (CF: 1521 ± 84.15 pg/ml vs C: 970 ± 56.44 pg/ml). These data indicate that peripheral T-lymphocytes of CF patients are more activated than lymphocytes of healthy subjects.

Discussion

The mechanisms behind the disturbed immune response in CF are still largely unknown and require further research. The aim of our study was to measure the expression of the PPAR transcription factors in patients with cystic fibrosis and healthy subjects. Because of their known regulatory functions in inflammatory processes,

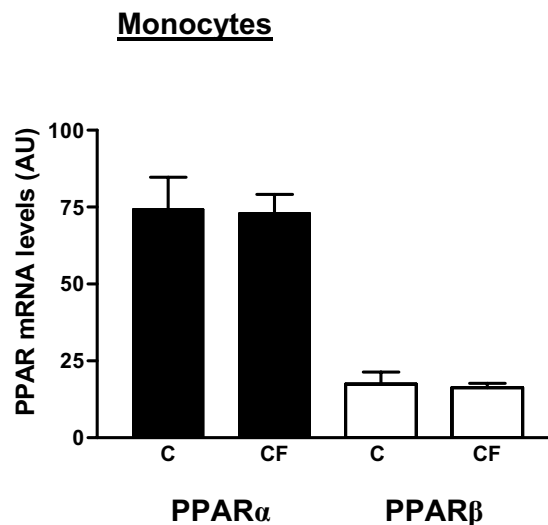
**Figure 3**

Human peripheral blood lymphocytes from CF patients (CF, n = 15; M(ale)/F(emale): 9/6) and healthy subjects (C, n = 11; M/F: 6/5) were subjected to RT-competitive multiplex PCR and densitometry in order to measure PPAR α and PPAR β mRNA expression levels. Data are normalized to GAPDH expression levels. Values are represented as means \pm standard error. PPAR α expression levels were 37% lower in CF patients compared to control persons. ** Significantly different ($p < 0.002$).

we hypothesized that their expression and/or function may be altered in cystic fibrosis, a disorder characterized by an excessive host inflammatory response.

Our study confirmed that systemic inflammation was present in our CF patients on the basis of the observed increased levels of plasma IL-8. Our data revealed that both PPAR α mRNA and protein levels were significantly lower in peripheral lymphocytes of CF patients than in healthy control persons. Immunofluorescence experiments demonstrated that just a small fraction of PPAR α resides in the nucleus, whereas the cytosol contains the larger part of the transcription factor. This was observed for both groups. Differences in activity were demonstrated via gel shift assay, i.e. a significant reduction of PPAR α DNA binding activity in lymphocytes of CF persons compared with healthy subjects. Finally, increased levels of soluble IL-2 Receptor in plasma suggest that peripheral lymphocytes are activated in cystic fibrosis.

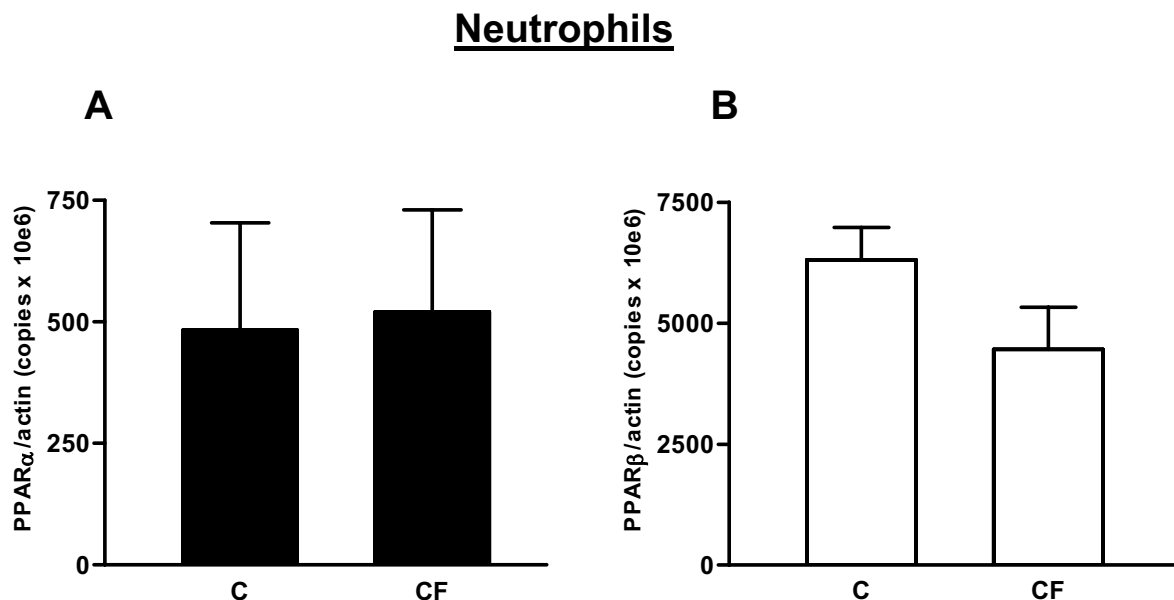
Most CF patients become chronically infected with specific bacterial pathogens, such as *Pseudomonas aeruginosa*, which cause a destructive inflammatory response in the lung. However, several studies provide evidence that

**Figure 4**

PPAR α and PPAR β mRNA expression levels were measured in human peripheral blood monocytes from CF patients (CF, n = 19; M/F: 13/6) and healthy subjects (C, n = 10; M/F: 6/4) via RT-competitive multiplex PCR and densitometry. Data are normalized to GAPDH mRNA expression levels. Values are means and standard error. Both PPAR levels were similar in the two groups.

inflammation can occur prior to infection and that CF lungs are primed for inflammation [30-32]. Nevertheless, the inflammatory processes are not restricted to the respiratory tract as shown by the elevated levels of pro-inflammatory markers in the blood circuit of CF patients [4,30,33]. Our study also demonstrated elevated levels of IL-8 in plasma of CF patients. Therefore, monocytes, lymphocytes and neutrophils were studied, as they are important mediators of the inflammatory response, i.e. through the release of cytokines, chemokines, and through the production of antibodies.

Our study revealed that PPAR α and PPAR β are abundantly expressed in freshly isolated monocytes and lymphocytes at mRNA level, whereas little or no PPAR γ was detected. Both PPAR α and PPAR β mRNA could be measured via real-time PCR in neutrophils; PPAR γ mRNA on the other hand was not quantifiable. Statistical analysis showed that PPAR α mRNA, but not PPAR β mRNA, is significantly less expressed (-37%) in lymphocytes of CF patients compared with control persons. The same difference could be detected at protein level via western blotting. The expression of PPAR α and β mRNA in monocytes and neutrophils was not significantly different in patients and healthy persons. These data are supported by several studies. First, there is evidence that PPAR α mRNA and

**Figure 5**

PPAR mRNA expression in freshly isolated human neutrophils was determined by real-time PCR for CF patients (n = 12; M/F: 7/5) and control persons (n = 12; M/F: 6/6). Data are presented as the mean PPAR mRNA level relative to the β -actin mRNA expression [(number of PPAR copies/number of β -actin copies) $\times 10^6$] and the standard error. Each measurement was performed in triple. (A) PPAR α mRNA expression. (B) PPAR β mRNA expression. No differences were seen between the two groups for both PPAR α and PPAR β mRNA levels.

protein expression are directly regulated by its own ligands [34,35]. Fatty acids and eicosanoids, which are natural PPAR activators, are found in disturbed levels in CF and may therefore cause a diminished expression of PPAR α . Second, PPAR α expression within T-lymphocytes is rapidly down-regulated following cellular activation [36]. Our present study demonstrated increased plasma soluble interleukin-2 receptor (sIL-2 R) concentrations in CF patients, which is in line with the findings of other research groups [37,38]. sIL-2 R is a generally accepted marker for T-lymphocyte activation [39]. Therefore, T-lymphocytes appear to be in some sort of activated state in CF patients, which may be responsible for the decreased PPAR α levels. The mechanism responsible for this down-regulation has not yet been elucidated. Third, the pro-inflammatory cytokines IL-6, TNF- α and IL-1 have been demonstrated to cause a reduction in the expression of PPAR α [40,41]. CF patients exhibit increased levels of IL-2, TNF- α , IL-6 and IL-8 in sputum and serum [4,5,32,37]. However, this can not be the major explanation for the decreased PPAR α levels in lymphocytes, as the expression of the transcription factor was unaltered in monocytes and neutrophils. And finally, an interesting abstract by Andersson and team reported that a CF tracheal epithelial cell line expressed less PPAR α protein than a normal tracheal epithelial cell line, which is com-

parable with our data [42]. The same research team found decreased PPAR γ levels in tissues specifically regulated by CFTR in a CF mice model [43] and their data suggest that CFTR may play a role in PPAR expression. A functional CFTR is also expressed in lymphocytes of healthy humans. Consequently, a defect CFTR in CF lymphocytes could result in altered PPAR expression. In addition, research has shown that PPAR expression may differ significantly in target organs where inflammation occurs. For example, a recent study reported that induction of PPAR α is lacking in the liver of CF mice compared to wild type animals following colitis induced bile duct injury [44].

Following our findings that PPAR α expression is down-regulated in CF lymphocytes, the question arose whether the activity of the transcription factor was also altered. Our immunofluorescence experiments revealed that for both groups, the transcription factor is primarily located in the cytosolic compartment and only a small fraction resides in the nucleus. A similar cellular distribution was reported in human macrophages [45] and in mice lymphocytes [36]. This meant that gel shift analysis had to be applied to measure possible differences in the activity of PPAR α . The gel shifts indeed showed that PPAR α DNA binding activity was 36% lower in lymphocytes of CF patients compared with control persons. A decreased

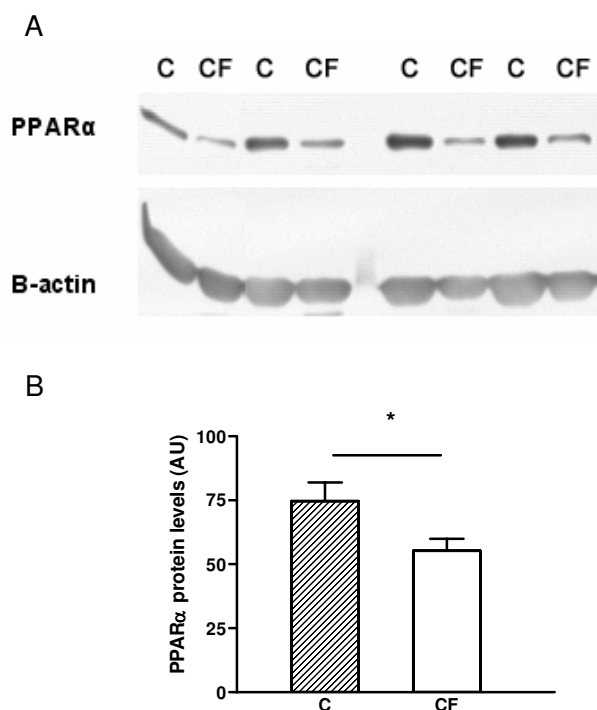


Figure 6

Western blot analysis of total protein extracts derived from human peripheral blood lymphocytes from control persons (C, n = 10; M/F: 4/6) and CF patients (CF, n = 11; M/F: 6/5). (A) A single band was detected at 60 kDa for PPAR α . β -actin protein expression was measured for normalization. (B) Analysis of band intensities revealed that PPAR α protein levels are down-regulated (-26%) in CF patients compared to healthy subjects. Densitometry data are expressed as means \pm standard error. * Significantly different ($p < 0.05$).

DNA binding activity of PPAR γ was also seen in tissues of CFTR knock-out mice. Treatment of these mice with rosiglitazone, a PPAR γ agonist, restored DNA binding [43].

PPAR α was the first isotype recognized for its *in vivo* role in inflammatory processes. Inflammation induced by leukotriene B₄, a PPAR α ligand, has been reported to be prolonged in PPAR α knock-out mice, suggesting an anti-inflammatory role for PPAR α [46]. Ligand-induced activation of PPAR α in lymphocytes antagonized DNA binding activity of NF- κ B and decreased IL-2 and TNF- α production [36,47], inhibited IFN- γ secretion but promoted IL-4 secretion and production [47,48]. These data indicate that PPAR α may have a significant influence on the lymphocytic immune response. Consequently, a decrease in PPAR α expression and function may contribute to the excessive host inflammatory response. Our data suggest that administration of ligands, such as the natural DHA or synthetic fibrates, may serve as a therapy to help reduce the inflammatory processes in CF by upregulating the

activity of PPAR α (see fig. 10). Further studies need to be carried out to test this hypothesis.

CF lung disease is well-known as a neutrophil-mediated disease. However, as pointed out by Moss, the behavior and biological function of lymphocytes is also altered in CF. Lymphocytes are important immune cells because they determine the specificity of the immune response. Quantitative analysis of inflammatory cells in CF lung tissues revealed a lymphocyte-dominated immune response in the CF bronchial wall, beneath the surface epithelium [1]. These lymphocytes may release cytokines, such as IL-17, that may attract neutrophils into the airways [49,50]. CF peripheral lymphocytes also exhibit an altered pattern in cytokine-release and production after stimulation [51-53], which could indicate an impairment of the immune response at the systemic level. Moreover, CF lymphocytes are characterized by a specific incapacity to respond to *P. aeruginosa* antigens [54]. Consequently, this defect could contribute to the inability to eradicate lung infection and inflammation due to *P. aeruginosa*. Summarized, the function of lymphocytes is altered in CF and they are therefore an interesting target to be studied.

In conclusion, our study revealed that both the expression and activity of PPAR α , a transcription factor with anti-inflammatory capacities, is down-regulated in peripheral lymphocytes of CF patients, which may render lymphocytes into cells that promote the inflammatory response and consequently lead to increased inflammation. In addition, the natural activators of PPAR α are known to be present in disturbed proportions in CF and may therefore cause an improper activation of PPAR α . We therefore hypothesize that the expression and activity of PPAR α may be up-regulated via the administration of natural or synthetic agonists which eventually may lead to a diminished immune response.

Abbreviations

AA: Arachidonic acid

AP-1: Activator protein-1

CF: Cystic fibrosis

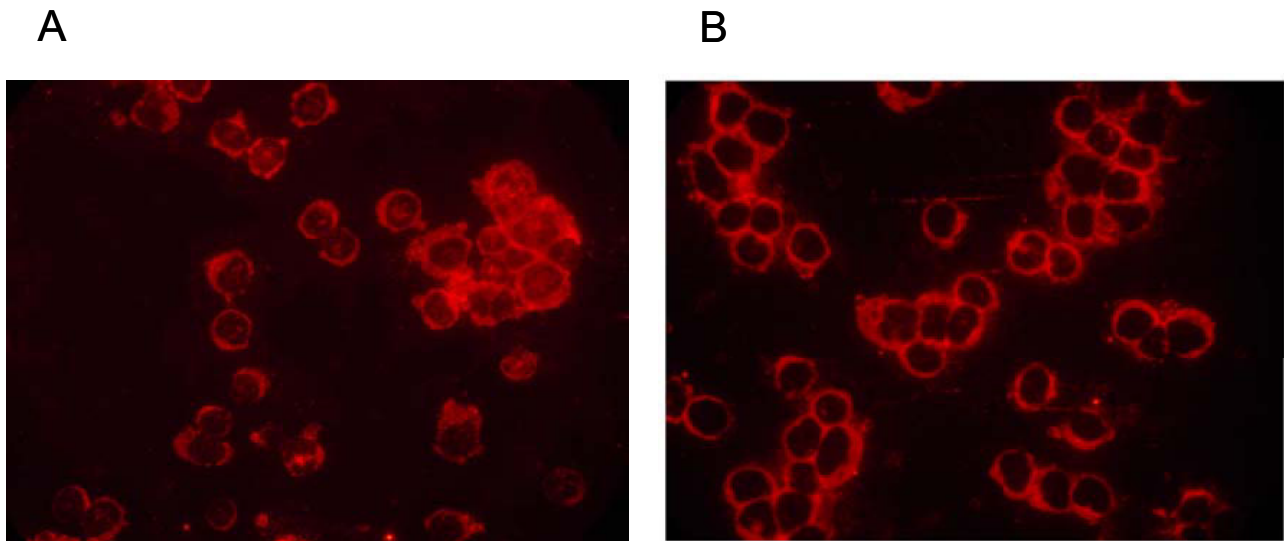
CFTR: Cystic fibrosis transmembrane conductance regulator

DHA: Docosahexaenoic acid

NF- κ B: Nuclear factor- κ B

PPAR: Peroxisome Proliferator-Activated Receptor

PPRE: PPAR response element

**Figure 7**

The subcellular localization of PPAR α protein in freshly isolated human peripheral blood lymphocytes was determined via immunofluorescence assay (C: n = 5 and CF: n = 5). Microscopy analysis revealed that the transcription factor is mainly situated in the small cytoplasmic area. (A) Representative immunofluorescence picture of lymphocytes derived from healthy control blood and (B) from a CF patient.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

VR carried out the experiments, wrote the manuscript and participated in the study design. SL designed the multiplex competitive PCR for PPARs, participated in the study design and helped evaluating the results and techniques. CS provided technical assistance. TW and DS provided the work with critical comments. JB participated in the coordination of the project and corrected the article.

Acknowledgements

The present work was supported by the Deutsche Forschungsgemeinschaft: Internationales Graduiertenkolleg 757/I.

The authors thank Glaxo-Smith Kline, especially Dr. Winegar, for the kind gift of the PPAR α antibodies. They also thank the patients and healthy volunteers for their cooperation. Comments from Dr. Hirche are gratefully acknowledged.

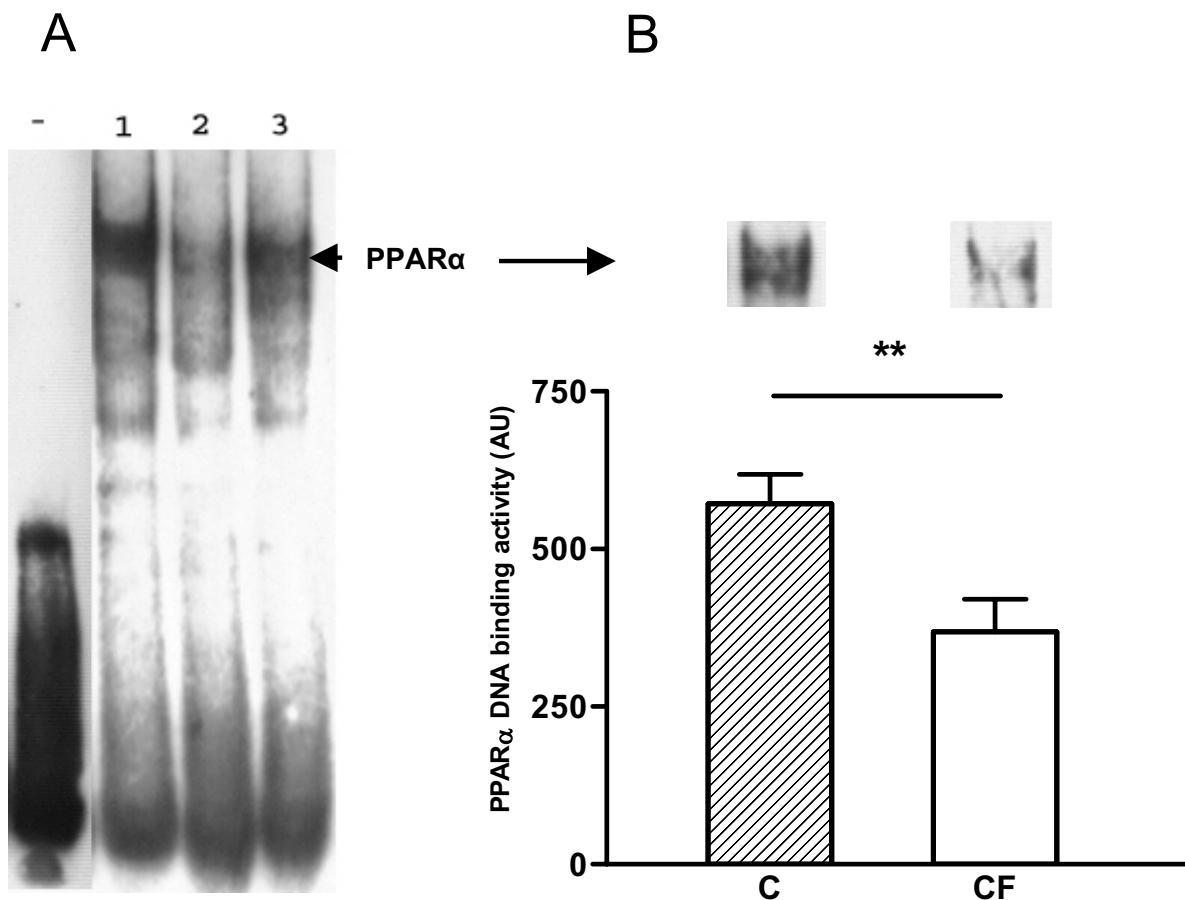


Figure 8
 Differential PPAR α binding to PPRE in peripheral lymphocytes. PPAR α DNA binding was analyzed via gel shift assay in peripheral lymphocytes of CF patients (CF, n = 11; M/F: 6/5) and healthy subjects (C, n = 11; M/F: 6/5). The DNA binding element was biotin-labeled. (A) A representative gel shift. Lane (-) represents the biotin-labeled DNA binding element, without the addition of nuclear extract. Lane 1: Lymphocytic control sample. Lane 2: Specific cold oligonucleotide binding competition assay: a 60-fold excess of cold synthetic PPRE was added. Lane 3: Unspecific cold oligonucleotide binding competition assay. A 60-fold excess of unspecific synthetic oligonucleotide was used. (B) Densitometry data derived from the gel shift assays are expressed as means and standard error. These data show that PPAR α DNA binding activity of CF patients is reduced by 36% compared to healthy persons. ** Significantly different ($p < 0.01$). On top: representative bands from a control person and a patient.

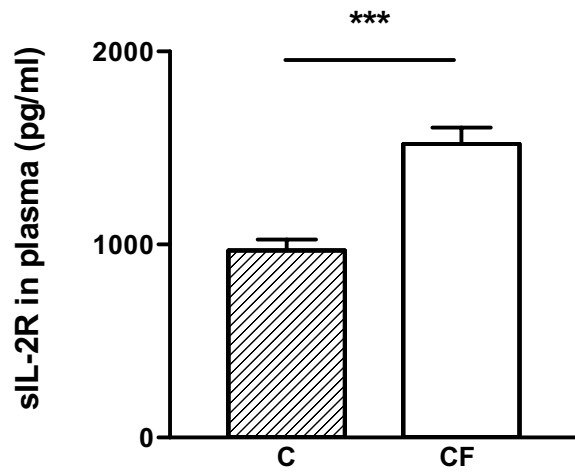


Figure 9
sIL-2R levels were measured in plasma of CF (n = 19) and control persons (n = 18). sIL-2R levels are significantly higher in CF patients than in control persons. Data are represented as mean and standard error. *** p < 0.0001.

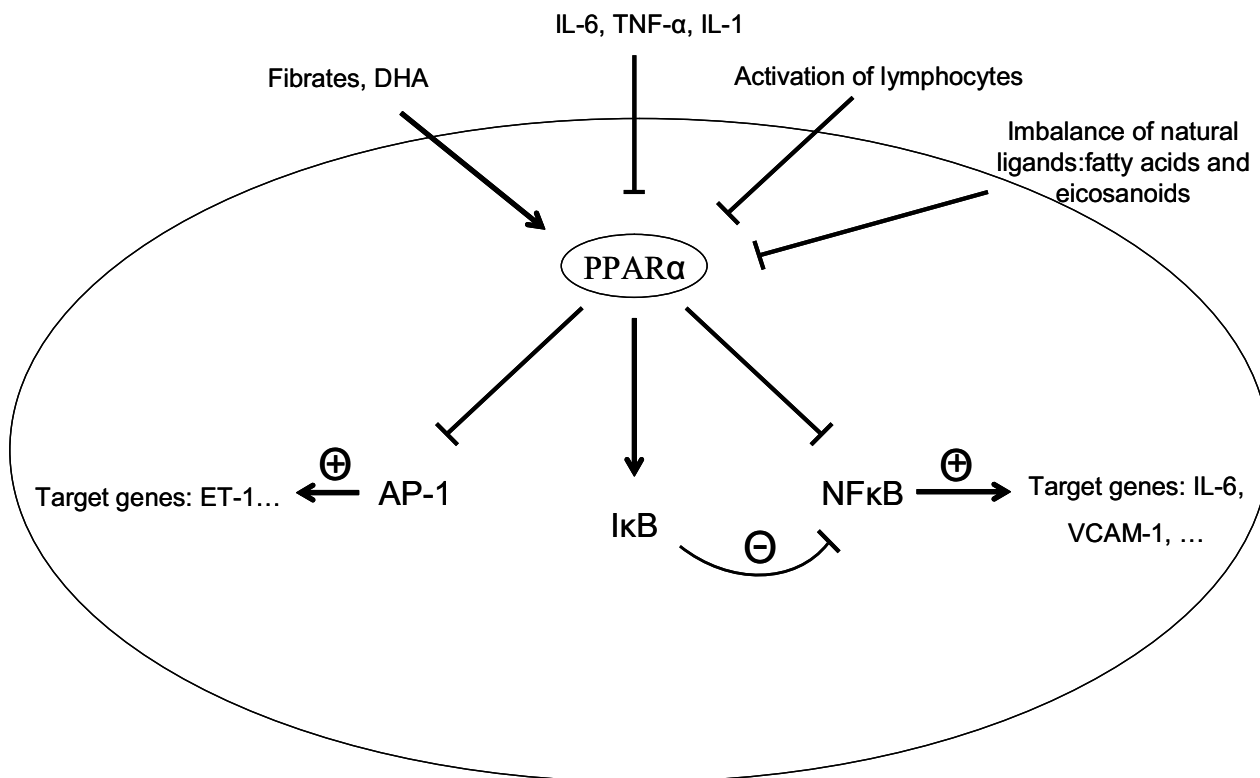


Figure 10

Summarizing picture: PPAR α in lymphocytes. PPAR α inhibits the actions of the pro-inflammatory transcription factors AP-1 and NF κ B through protein-protein interactions and by up-regulating the expression of I κ B. The nuclear hormone receptor is inhibited by specific cytokines and by lymphocyte activation. In addition, we suggest that the imbalance of natural ligands in CF leads to deficiencies in PPAR α activation. Therefore, lymphocytes may be turned into cells that promote inflammation in CF. We hypothesize that the addition of synthetic or natural ligands, such as fibrates and DHA respectively, may restore the activity and expression of PPAR α , resulting in a more balanced lymphocytic immune response.

References

- Hubeau C, Lorenzato M, Couetil JP, Hubert D, Dusser D, Puchelle E, Gaillard D: **Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa.** *Clin Exp Immunol* 2001, **124**:69-76.
- Moss RB: **Lymphocytes in cystic fibrosis lung disease: a tale of two immunities.** *Clin Exp Immunol* 2004, **135**:358-360.
- Reineck P, Artlich A, Hoerer C, Hüls G, Lindemann H: **Leukotriene im Atemkondensat von Kindern und Jugendlichen mit Asthma bronchiale und zystischer Fibrose.** *Atemw-Lungenkrkh* 2004, **3**:103-108.
- Ionescu AA, Nixon LS, Luzio S, Lewis-Jenkins V, Evans WD, Stone MD, Owens DR, Routledge PA, Shale DJ: **Pulmonary function, body composition, and protein catabolism in adults with cystic fibrosis.** *Am J Respir Crit Care Med* 2002, **165**:495-500.
- Nixon LS, Yung B, Bell SC, Elborn JS, Shale DJ: **Circulating immunoreactive interleukin-6 in cystic fibrosis.** *Am J Respir Crit Care Med* 1998, **157**:1764-1769.
- Poynter ME, Daynes RA: **Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging.** *J Biol Chem* 1998, **273**:32833-32841.
- Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, Fruchart JC, Tedgui A, Haegeman G, Staels B: **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**:32048-32054.
- Chung SW, Kang BY, Kim SH, Pak YK, Cho D, Trinchieri G, Kim TS: **Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B.** *J Biol Chem* 2000, **275**:32681-32687.
- Forman BM, Chen J, Evans RM: **Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta.** *Proc Natl Acad Sci U S A* 1997, **94**:4312-4317.
- Wahli W, Devchand PR, Ijpenberg A, Desvergne B: **Fatty acids, eicosanoids, and hypolipidemic agents regulate gene expression through direct binding to peroxisome proliferator-activated receptors.** *Adv Exp Med Biol* 1999, **447**:199-209.
- Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S: **The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene.** *EMBO J* 1992, **11**:433-439.
- Shipley JM, Waxman DJ: **Down-regulation of STAT5b transcriptional activity by ligand-activated peroxisome proliferator-activated receptor (PPAR) alpha and PPARgamma.** *Mol Pharmacol* 2003, **64**:355-364.

13. Madej A, Okopien B, Kowalski J, Zielinski M, Wysocki J, Szygula B, Kalina Z, Herman Z: **[Levels of tumor necrosis factor alpha in serum of patients with hyperlipoproteinemia IIB before and after micronized fenofibrate therapy].** *Pol Arch Med Wewn* 1998, **99**:308-313.
14. Despres JP, Lemieux I, Pascot A, Almeras N, Dumont M, Nadeau A, Bergeron J, Prud'homme D: **Gemfibrozil reduces plasma C-reactive protein levels in abdominally obese men with the atherogenic dyslipidemia of the metabolic syndrome.** *Arterioscler Thromb Vasc Biol* 2003, **23**:702-703.
15. Marx N, Froehlich J, Siam L, Ittner J, Wierse G, Schmidt A, Scharnagl H, Hombach V, Koenig W: **Antidiabetic PPAR gamma-activator rosiglitazone reduces MMP-9 serum levels in type 2 diabetic patients with coronary artery disease.** *Arterioscler Thromb Vasc Biol* 2003, **23**:283-288.
16. Staels B: **[Glitazones and atherosclerosis].** *Ann Endocrinol (Paris)* 2005, **66**:1524-1531.
17. Israelian-Konarakis Z, Reaven PD: **Peroxisome proliferator-activated receptor-alpha and atherosclerosis: from basic mechanisms to clinical implications.** *Cardiol Rev* 2005, **13**:240-246.
18. Trifilieff A, Bench A, Hanley M, Bayley D, Campbell E, Whitaker 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.
19. Birrell MA, Patel HJ, McCluskie K, Wong S, Leonard T, Yacoub MH, Belvisi MG: **PPAR-gamma agonists as therapy for diseases involving airway neutrophilia.** *Eur Respir J* 2004, **24**:18-23.
20. Roulet M, Frascarolo P, Rappaz I, Pilet M: **Essential fatty acid deficiency in well nourished young cystic fibrosis patients.** *Eur J Pediatr* 1997, **156**:952-956.
21. Farrell PM, Mischler EH, Engle MJ, Brown DJ, Lau SM: **Fatty acid abnormalities in cystic fibrosis.** *Pediatr Res* 1985, **19**:104-109.
22. Sampson AP, Spencer DA, Green CP, Piper PJ, Price JF: **Leukotrienes in the sputum and urine of cystic fibrosis children.** *Br J Clin Pharmacol* 1990, **30**:861-869.
23. Zakrzewski JT, Barnes NC, Piper PJ, Costello JF: **Detection of sputum eicosanoids in cystic fibrosis and in normal saliva by bioassay and radioimmunoassay.** *Br J Clin Pharmacol* 1987, **23**:19-27.
24. Carlstedt-Duke J, Bronnegard M, Strandvik B: **Pathological regulation of arachidonic acid release in cystic fibrosis: the putative basic defect.** *Proc Natl Acad Sci U S A* 1986, **83**:9202-9206.
25. Freedman SD, Blanco PG, Zaman MM, Shea JC, Ollero M, Hopper IK, Weed DA, Gelrud A, Regan MM, Laposata M, Alvarez JG, O'Sullivan BP: **Association of cystic fibrosis with abnormalities in fatty acid metabolism.** *N Engl J Med* 2004, **350**:560-569.
26. Loitsch SM, Kippenberger S, Daultbaev N, Wagner TO, Bargon J: **Reverse transcription-competitive multiplex PCR improves quantification of mRNA in clinical samples--application to the low abundance CFTR mRNA.** *Clin Chem* 1999, **45**:619-624.
27. Kreuzer KA, Lass U, Landt O, Nitsche A, Laser J, Ellerbrok H, Pauli G, Huhn D, Schmidt CA: **Highly sensitive and specific fluorescence reverse transcription-PCR assay for the pseudogene-free detection of beta-actin transcripts as quantitative reference.** *Clin Chem* 1999, **45**:297-300.
28. Su JL, Simmons CJ, Wisely B, Ellis B, Winegar DA: **Monitoring of PPAR alpha protein expression in human tissue by the use of PPAR alpha-specific MAb.** *Hybridoma* 1998, **17**:47-53.
29. Dignam JD, Lebovitz RM, Roeder RG: **Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei.** *Nucleic Acids Res* 1983, **11**:1475-1489.
30. Dean TP, Dai Y, Shute JK, Church MK, Warner JO: **Interleukin-8 concentrations are elevated in bronchoalveolar lavage, sputum, and sera of children with cystic fibrosis.** *Pediatr Res* 1993, **34**:159-161.
31. Balough K, McCubbin M, Weinberger M, Smits W, Ahrens R, Fick R: **The relationship between infection and inflammation in the early stages of lung disease from cystic fibrosis.** *Pediatr Pulmonol* 1995, **20**:63-70.
32. Khan TZ, Wagener JS, Bost T, Martinez J, Accurso FJ, Riches DW: **Early pulmonary inflammation in infants with cystic fibrosis.** *Am J Respir Crit Care Med* 1995, **151**:1075-1082.
33. Augarten A, Paret G, Avneri I, Akons H, Aviram M, Bentur L, Blau H, Efrati O, Szeinberg A, Barak A, Kerem E, Yahav J: **Systemic inflammatory mediators and cystic fibrosis genotype.** *Clin Exp Med* 2004, **4**:99-102.
34. Akbiyik F, Ray DM, Bozkaya H, Demirpence E: **Ligand- and species-dependent activation of PPARalpha.** *Cell Physiol Biochem* 2004, **14**:269-276.
35. Inoue I, Shino K, Noji S, Awata T, Katayama S: **Expression of peroxisome proliferator-activated receptor alpha (PPAR alpha) in primary cultures of human vascular endothelial cells.** *Biochem Biophys Res Commun* 1998, **246**:370-374.
36. 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.
37. Grealley P, Hussain MJ, Vergani D, Price JF: **Serum interleukin-1 alpha and soluble interleukin-2 receptor concentrations in cystic fibrosis.** *Arch Dis Child* 1993, **68**:785-787.
38. Dagli E, Warner JA, Besley CR, Warner JO: **Raised serum soluble interleukin-2 receptor concentrations in cystic fibrosis patients with and without evidence of lung disease.** *Arch Dis Child* 1992, **67**:479-481.
39. Waldmann TA: **The structure, function, and expression of interleukin-2 receptors on normal and malignant lymphocytes.** *Science* 1986, **232**:727-732.
40. Parmentier JH, Schohn H, Bronner M, Ferrari L, Batt AM, Dauca M, Kremers P: **Regulation of CYP4A1 and peroxisome proliferator-activated receptor alpha expression by interleukin-1beta, interleukin-6, and dexamethasone in cultured fetal rat hepatocytes.** *Biochem Pharmacol* 1997, **54**:889-898.
41. Beier K, Volkl A, Fahimi HD: **TNF-alpha downregulates the peroxisome proliferator activated receptor-alpha and the mRNAs encoding peroxisomal proteins in rat liver.** *FEBS Lett* 1997, **412**:385-387.
42. Andersson C, Ollero M, Junaidi O, Mergely M, Freedman SD: **Selective block in DHA biosynthesis and PPARalpha expression in airway epithelial cystic fibrosis cell lines.** *Pediatr Pulmonol* 2003, **Supplement 25**:236.
43. Ollero M, Junaidi O, Zaman MM, Tzamelis I, Ferrando AA, Andersson C, Blanco PG, Bialecki E, Freedman SD: **Decreased expression of peroxisome proliferator activated receptor gamma in cfr-1 mice.** *J Cell Physiol* 2004, **200**:235-244.
44. Pall H, Zaman MM, Andersson C, Freedman SD: **Decreased peroxisome proliferator activated receptor alpha is associated with bile duct injury in cystic fibrosis transmembrane conductance regulator-1 mice.** *J Pediatr Gastroenterol Nutr* 2006, **42**:275-281.
45. Chinetti G, Griglio S, Antonucci M, Torra IP, Delerive P, Majd Z, Fruchart JC, Chapman J, Najib J, Staels B: **Activation of proliferator-activated receptors alpha and gamma induces apoptosis of human monocyte-derived macrophages.** *J Biol Chem* 1998, **273**:25573-25580.
46. 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.
47. Marx N, Kehrle B, Kohlhammer K, Grub M, Koenig W, Hombach V, Libby P, Plutzky J: **PPAR activators as antiinflammatory mediators in human T lymphocytes: implications for atherosclerosis and transplantation-associated arteriosclerosis.** *Circ Res* 2002, **90**:703-710.
48. Lovett-Racke AE, Hussain RZ, Northrop S, Choy J, Rocchini A, Matthes L, Chavis JA, Diab A, Drew PD, Racke MK: **Peroxisome proliferator-activated receptor alpha agonists as therapy for autoimmune disease.** *J Immunol* 2004, **172**:5790-5798.
49. Linden A, Laan M, Anderson GP: **Neutrophils, interleukin-17A and lung disease.** *Eur Respir J* 2005, **25**:159-172.
50. McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, Steele C, Finder JD, Pilewski JM, Carrero BM, Goldman SJ, Pirhonen J, Kolls JK: **Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis.** *J Immunol* 2005, **175**:404-412.
51. Moss RB, Hsu YP, Olds L: **Cytokine dysregulation in activated cystic fibrosis (CF) peripheral lymphocytes.** *Clin Exp Immunol* 2000, **120**:518-525.
52. Moss RB, Bocian RC, Hsu YP, Dong YJ, Kemna M, Wei T, Gardner P: **Reduced IL-10 secretion by CD4+ T lymphocytes expressing**

mutant cystic fibrosis transmembrane conductance regulator (CFTR). *Clin Exp Immunol* 1996, **106**:374-388.

53. Hubeau C, Le Naour R, Abely M, Hinnrasky J, Guenounou M, Gaillard D, Puchelle E: **Dysregulation of IL-2 and IL-8 production in circulating T lymphocytes from young cystic fibrosis patients.** *Clin Exp Immunol* 2004, **135**:528-534.
54. Sorensen RU, Stern RC, Chase PA, Polmar SH: **Changes in lymphocyte reactivity to Pseudomonas aeruginosa in hospitalized patients with cystic fibrosis.** *Am Rev Respir Dis* 1981, **123**:37-41.

Publish with **BioMed Central** and every scientist can read your work free of charge

"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:
http://www.biomedcentral.com/info/publishing_adv.asp

