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Modulation of T Lymphocyte Function by the Pregnan X Receptor

Sandrine Dubrac,* Andreas Elentner,* Susanne Ebner,* † Jutta Horejs-Hoeck, ‡ and Matthias Schmuth*

The pregnane X receptor (PXR) is a ligand-activated transcription factor regulating genes central to drug and hormone metabolism in the liver. Previous reports indicated that PXR is expressed in PBMC, but the role of PXR in immune cells remains unknown. In this paper, we report increased PXR expression in mouse and human T lymphocytes upon immune activation. Furthermore, pharmacologic activation of PXR inhibits T lymphocyte proliferation and anergizes T lymphocytes by decreasing the expression of CD25 and IFN-γ and decreasing phosphorylated NF-κB and MEK1/2. Although these effects are preceded by an increase of suppressor of cytokine signaling 1, a master switch for IFN-γ expression, in a PXR-dependent manner, T-bet expression remains unchanged. Conversely, PXR-deficient mice exhibit an exaggerated T lymphocyte proliferation and increased CD25 expression. Furthermore, PXR-deficient lymphocytes produce more IFN-γ and less of the anti-inflammatory cytokine IL-10. In summary, these results reveal a novel immune-regulatory role of PXR in T lymphocytes and identify suppressor of cytokine signaling 1 as an early signal in PXR-mediated T lymphocyte suppression. The Journal of Immunology, 2010, 184: 000–000.

T he pregnane X receptor (PXR) is a member of the nuclear receptor superfamily that includes the steroid, retinoid, and orphan receptors. PXR is activated by a broad range of compounds, including steroids, bile acids, and a wide variety of drugs including antibiotics, rifampicin, pregnenolone 16α carbonitrile (PCN), and RU-486 (1). PXR mediates the detoxification of endogenous and exogenous compounds and prevents toxic accumulation of metabolites within cells (2). DNA polymorphisms within the PXR gene have been linked to interindividual differences in drug responsiveness (3, 4).

PXR acts as a transcription factor. Once activated, PXR heterodimerizes with the retinoid X receptor (RXR), binds to regulatory DNA sequences in the nucleus, and modulates transcription of genes involved in the oxidation, conjugation, and export of compounds from cells. These include the cytochrome P-450 enzymes and the multiple drug resistance gene (MDR1/ABCB1), among many others (1, 2, 5). Notably, cytochrome P-450 enzymes are responsible for the metabolism of >50% of current prescription drugs (1).

PXR is primarily expressed in the gastrointestinal tract and liver. However, recent reports showed that PXR is expressed in immune cells, that is, PBMC, including CD4+, CD8+ T lymphocytes, CD19+ B lymphocytes, and CD14+ monocytes in humans (6, 7). Several studies showed that rifampicin, a human PXR activator, suppresses both humoral and cellular immunity and identified rifampicin as a powerful immunosuppressive drug (8–12). Subsequently, rifampicin was successfully used as an effective antipsoriatic drug (13–15). As drug metabolism and the immune system are intertwined, recent work shed initial light on the molecular aspects of this interaction. A reciprocal repression between PXR and NF-κB was shown (16, 17). In the colon, PXR-mediated repression of NF-κB target genes appears to be a critical mechanism by which PXR activation decreases inflammation (17). Whereas PXR activation inhibits NF-κB activation, conversely, p65 NF-κB disrupts the binding between PXR and RXR α to DNA and inhibits the transactivation of target genes (18, 19). In the present work, we set out to delineate the molecular mechanism involved in the control of T lymphocyte function by PXR.

Materials and Methods

Reagents and Abs

LPS, PCN, RU-486, rifampicin, PMA, and BrdU were purchased from Sigma-Aldrich (St Louis, MO); oligodeoxynucleotide containing embedded unmethylated CpG motifs (CpG) from Coleypharma (Wellesley, MA); recombinant mouse-TSPL from R&D Systems (Minneapolis, MN); and peptideoligocyn (PGN) from InvivoGen (San Jose, CA). Directly labeled primary mAb specific for mouse CD4 (clone GK 1.5), CD25 (clone PC61), IL-10-PE (clone JES5-16E3), and IFN-γ-FITC (clone XMGl2.1) were purchased from BD Pharmingen (San Diego, CA). Alexa Fluor 488 conjugate anti-BrdU was purchased from Invitrogen (Carlsbad, CA). Anti-phospho-p65 NF-κB (ser 529), phospho-SAPK/JNK (Thr 183/Tyr185), phospho-MEK1/2 (ser 217/221), phospho-p38 MAPK (Thr180/Tyr182), and cleaved caspase 3 were purchased from Cell Signaling (Danvers, MA); anti-mouse T-bet and anti-human PXR (clone H4417) from Perseus Proteomics (Tokyo, Japan); anti-mouse suppressor of cytokine signaling 1 (SOCS-1) from Imgenex (San Diego, CA); anti-human SOCS-1 from GeneTex (Irvine, CA); anti-β-actin from Sigma-Aldrich; biotinylated anti-rabbit Abs and streptavidin-APC from Amersham (GE Healthcare, U.K.); and annexin V from Bender Medsystems (Vienna, Austria). mAb specific for human CD14 (clone mC5-3), CD16 (clone 3G8), CD56 (clone N-CAM), CD19 (clone Leu-12), CD235a (clone GA-R2), CD123 (clone 9F5), CD36 (clone CB38), and HLA-DR (clone L243) were purchased from BD Biosciences (San Jose, CA) and CD45 RO (clone UCHL1) from Dako (Glostrup, Denmark).

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Abbreviations used in this paper: PCN, pregnenolone 16α carbonitrile; PGN, peptidoglycan; PXR, pregnane X receptor; RXR, retinoid X receptor; SOCS-1, suppressor of cytokine signaling 1.

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Animals
Mice of the inbred strains C57BL/6 and BALB/c were purchased from Charles River Laboratories (Sulzfeld, Germany), maintained on a standard chow diet, and used at 2–3 mo of age. PXR<sup>−/−</sup> mice were bred on a C57BL/6 background as described previously (20). All animal experiments were carried out according to governmental guidelines.

Preparation of cells and cell culture
T cell isolation. T lymphocytes were isolated from mouse lymph nodes and spleens as described earlier and further purified by negative selection (21). Purity was 98 ± 1% as determined by FACS analysis. Human PBMC were obtained from donors after informed consent in accordance with the Declaration of Helsinki. naïve CD3<sup>+</sup> T lymphocytes were isolated using a panning technique, as described previously (22). The purity of isolated CD3<sup>+</sup> cell population was >90%, as determined by FACS analysis.
Jurkat cells. Jurkat T leukemia cells (clone E6-1) derived from a human acute T cell leukemia were obtained from the American Type Culture Collection (Manassas, VA).

Epidermal cell isolation. Epidermal cells were isolated by trypsinization, as described earlier (21).

FACS analysis
Specimens were analyzed on a FACScalibur (BD Biosciences). Nonviable cells were excluded by 7-aminotactinoxin D (Sigma-Aldrich) uptake when fixation of cells was not required for analysis. After fixation and permeabilization with a cell permeabilization kit (Fix&Perm, An der Grub Bio-Research, Kaumberg, Austria) for 15 min at room temperature, the expression of phosphoproteins and T-bet was assessed on spleen cells by incubation with the first-step Ab, followed by staining with biotinylated Abs (listed above) and then with streptavidin-APC. T lymphocytes were counterstained with an anti–CD3-FITC mAb.

Cytokine detection
In vivo production of cytokines by T lymphocytes was determined as described earlier (21). Alternatively, cells were isolated from spleens by homogenization and stimulated for 48 h. Cells were cultured for 4.5 h with 1 μg/ml Brefeldin A (BD Pharmingen) to prevent cytokine secretion. Then, cells were fixed, permeabilized, and stained with mAb, as described above. Highly purified T lymphocytes were cultured, supernatants were collected and pooled, and cytokine concentrations were determined by multianalyte profiler ELISArray Kit from SuperArray Bioscience Corporation (Frederick, MD).

In vivo proliferation
In vivo proliferation of T lymphocytes was determined by BrdU incorporation. Mouse ear skin was topically treated with 20 μl 1% 2,4,6-trinitro-1-chlorobenzene picryl chloride (Kodak Eastman, Rochester, NY) at day 0. At day 5, mice were injected i.p. with 0.8 mg BrdU. At day 7, cells were isolated from lymph nodes by collagenase P digestion, as described earlier (21), and stained according to the manufacturer’s instruction.

Immunocytochemical analysis
Cytospins of highly purified human T lymphocytes were fixed in acetone for 5 min. Immunostaining was performed with anti-human PXR mAb or isotype control for 1 h at 37°C, using biotinylated sheep anti-mouse Ig (Amersham Biosciences, Piscataway, NJ) in 1% BSA followed by a streptavidin Alexa-Fluor 594 conjugate (Molecular Probes, Eugene, OR). The staining was visualized with a 40× objective using an Olympus BX60 epifluorescence microscope (Olympus, Melville, NY).

Western blot analysis
T lymphocytes were lysed in radioimmunoprecipitation assay lysis buffer in the presence of phosphatase and protease inhibitors (Pierce, Rockford, IL). Electrophoresis for SOCS-1 was performed under reducing conditions, using DT. Endogenous proteins were detected with primary Abs, as listed above, and with Alexa A680-conjugated anti-rabbit secondary Ab (Invitrogen, Carlsbad, CA). Blots were then scanned with a LI-COR Biosciences (Lincoln, NE) analyzer.

RNA quantification
Total RNA was extracted using TRizol (Life Technologies, Carlsbad, CA), and random primed cDNA was prepared (Superscript II RNase H-reverse transcriptase; Life Technologies, Vienna, Austria). Quantitative PCR analysis was performed by real-time PCR (ABI PRISM 7700 sequence detector; Applied Biosystems, Vienna, Austria). Sequences for probes and primers (synthesized by Microsynth, Balgach, Switzerland) specific for mouse TATA binding protein, PXR, CYP3A11, SULT2B1, IFN-γ, and SOCS-1 mRNA molecules and for human ribosomal protein 18S, TATA binding protein, SOCS-1, and PXR mRNA molecules were selected using Primer Express software (Applied Biosystems, Foster City, CA).

Luciferase assay
A pLUC vector containing a 1433-bp DNA fragment of the human SOCS-1 promoter was used as previously described (23). Jurkat cells were cotransfected with 2 μg pLUC-HuSOCS-1-promoter and 0.2 μg pLUC-GL4 (Promega, Madison, WI) complexed with PlasmidFect reagent (1:4) (Bioline, Taunton, MA), according to the manufacturer’s directions. After overnight incubation, 10 μg rifampicin or vehicle was added to the medium for 24 additional hours. Luciferase activity was measured using the Dual-Glo Luciferase Assay System from Promega, according to the manufacturer’s directions.

MLRs
Epidermal dendritic cell-containing interphases (21) were cocultured with allogeneic PXR<sup>−/−</sup> and PXR<sup>+/+</sup> T lymphocytes (3 × 10<sup>5</sup> per well) at various ratios for 4 d, and proliferation was measured by [3H]thymidine incorporation for the last 16 h (activity, 4 μCi/ml = 148,000 Bq/ml; New England Nuclear/PerkinElmer, Boston, MA).

Statistical analysis
Results are expressed as mean ± SEM. Data were analyzed using a paired or an unpaired Student t test as appropriate.

Results
Increased PXR expression in activated T lymphocytes
Recent reports indicated PXR expression in human CD4<sup>+</sup>, CD8<sup>+</sup> T lymphocytes, CD19<sup>+</sup> B lymphocytes, and CD14<sup>+</sup> monocytes (6, 7), but not in bone marrow-derived mouse macrophages (24). Because the functional significance of PXR expression in these cells remained unknown, we stimulated mouse T lymphocytes with various inflammatory stimuli known to activate T lymphocytes and assessed PXR expression. We used anti-CD3/CD28, which cross-links TCR or PMA, activating the protein kinase C pathway. In addition, we have combined PMA or anti-CD3/CD28 with TLR ligands, such as LPS, CpG, and PGN. LPS and CpG are TLR4 and TLR9 ligands, respectively, inducing a Th1 response in T cells. In contrast, PGN is a TLR2 ligand and induces a Th2 response. We used these different stimulation mixtures to establish whether PXR upregulation is specific to one type of stimulation or, in contrast, if PXR upregulation is a general event that occurs in all activated T cells independent of the nature of the stimulus. Real-time PCR analysis revealed a significant increase in PXR mRNA transcript upon T lymphocyte activation (Fig. 1A, Supplemental Fig. 1). Moreover, the combination of PMA or anti-CD3/CD28 with LPS and/or CpG (Th1) revealed additional effects on PXR expression that were not present with PGN (Th2) (Fig. 1A, Supplemental Fig. 1A). We also observed a parallel upregulation of cyp3a11, a known PXR downstream gene (1) (Fig. 1B). A similar PXR induction was found in human PMA-activated T lymphocytes derived from PBMC (Fig. 1C) and in Jurkat cells following different inflammatory stimuli (Fig. 1D). Time course expression of PXR shows that PXR expression is upregulated beginning 18 h postactivation of mouse T lymphocytes or 30 h postactivation of Jurkat cells (Supplemental Fig. 1B, 1C). Immunohistochemistry showed that in unstimulated resting and PMA-stimulated human T lymphocytes, PXR mainly localizes to the cytoplasmic and perinuclear compartment (Fig. 1E).

PXR activation inhibits T cell proliferation, CD25 and IFN-γ expression in vitro
We next determined the effects of PXR activation on T cell function. We first asked whether the immunogenic potential of T lymphocytes is modulated by PXR in vitro by assessing the ability of mouse
T lymphocytes to proliferate upon treatment with the mouse PXR activators PCN and RU-486. We found that, in a PXR-dependent manner, PXR activation dramatically decreased the ability of T lymphocytes to proliferate (Fig. 2A, 2B) without affecting their viability (Supplemental Fig. 3). These results indicate that PXR ligands modulate the immunogenicity of T lymphocytes in a PXR-dependent manner.

**FIGURE 1.** PXR expression is increased in activated T lymphocytes. A and B, Quantitative PCR analyses were performed to measure the expression of PXR (A) or CYP3A11 (B), using real-time PCR with the TaqMan PCR master mix from Applied Biosystems. Random-primed cDNA was prepared from total RNA isolated from highly purified unstimulated or stimulated mouse T lymphocytes. Activation stimuli included the following: anti-mouse CD3/CD28 (2 μg/ml), anti-mouse CD3/CD28 (2 μg/ml) + CpG (5 μg/ml), PMA (100 ng/ml), PMA (50 ng/ml) + LPS (50 ng/ml) + CpG (5 μg/ml), or PMA (50 ng/ml) + PGN (1 μg/ml). RNA levels were measured after 48 h; n = 3. Data were analyzed using a Student t test. p < 0.05; **p < 0.005, showing differences between activated and unstimulated mouse T lymphocytes. C, Quantitative PCR analyses were performed on random-primed cDNA prepared from total RNA isolated from highly purified or PMA-stimulated human T lymphocytes (100 ng/ml PMA, for 24 h); n = 4. Data were analyzed using a paired t test. p < 0.05. D, Quantitative PCR analyses were performed on random-primed cDNA prepared from total RNA isolated from Jurkat cells incubated for 48 h prior to RNA isolation in the presence of anti-human CD3/CD28 (2 μg/ml) or PMA (100 ng/ml). Data were analyzed using a Student t test. ***p < 0.001, showing differences between activated and unstimulated Jurkat cells. E, Immunohistochemistry showing the expression of PXR in PMA-stimulated and unstimulated (resting) human CD3+ T lymphocytes. Cytospins were prepared and stained for PXR expression, as described in Materials and Methods. The staining was visualized with a ×40 objective. Negative controls were stained with isotype control. Nuclei were visualized by DAPI staining.

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dependent manner and independent of apoptotic effects. To further delineate the immune-regulatory role of PXR, we treated spleen-derived mouse T lymphocytes with various inflammatory stimuli in combination with the PXR activators PCN or RU-486, or vehicle (1). PXR activation resulted in decreased expression of the cell surface activation marker CD25 (Fig. 2C, Supplemental Fig. 2),
whereas it did not alter the viability of activated spleen cells (Supplemental Fig. 3). 7-Aminoactinomycin D+ cells were excluded from these analyses, ensuring that the effects of PXR activity on T lymphocyte function did not depend on cell viability. Furthermore, a lack of effect in PXR activator-treated PXR−/− cells showed that the decrease was mediated by PXR (Fig. 2C). Similar results were observed in a human T cell line; that is, rifampicin and RU-486 reduced the expression of CD25 in Jurkat T cells (Fig. 2D). Finally, we tested whether PXR activation modulated cytokine production in activated splenocytes. PCN and RU-486 treatment decreased T lymphocyte-derived IFN-γ in a PXR-dependent manner (Fig. 2E, 2F). Notably, PXR−/− T lymphocytes produced more IFN-γ than did wild types after in vitro stimulation with anti-CD3/CD28 (Fig. 2E). A multi-analyte cytokine profiling ELISA assay revealed that production of IL-10 and TNF-α by T lymphocytes treated with PCN (10 μM) or DMSO was very low and did not exhibit significant changes except a 4-fold decrease in IFN-γ in PCN-treated T lymphocytes, as compared with vehicle controls.

**FIGURE 3.** PXR deficiency targets T lymphocyte function. A, Purified T lymphocytes isolated from PXR−/− and littermate control mice were cocultured with graded doses of allogenic epidermal dendritic cells for 72 h and then pulsed with [3H]-thymidine for 16 h, as described in Materials and Methods; n = 3. Data were analyzed using a Student t test; *p < 0.05. CPM values for T cells in the absence of epidermal dendritic cells were consistently below 2000. B, In vivo proliferation of CD4+ T lymphocytes in PXR−/− and littermate control mice measured by BrdU incorporation; n = 4. Data were analyzed using a Student t test. C, Flow cytometry analysis showing the production of IFN-γ by CD4+ T lymphocytes in the skin draining lymph nodes of PXR−/− and littermate control mice 4 d after skin sensitization with 2,4,6-trinitro-1-chlorobenzene picryl chloride; n = 4; representative plots are shown. Numbers in quadrants show the percentage of CD4+ IFN-γ+ cells. D, Production of IL-10 by T lymphocytes stimulated with 2 μg/ml anti-mouse CD3/CD28 for 48 h, isolated from PXR−/− or littermate control mice and analyzed by flow cytometry, as described in Materials and Methods; n = 3. Data were analyzed with a Student t test. E, Expression of CD25 on PXR−/− or littermate control T lymphocyte cell surface isolated from spleens and stimulated with anti-mouse CD3/CD28 for 24 h was analyzed by flow cytometry, as described in Materials and Methods; n = 3. The black line designates PXR−/− cells, the gray line PXR+/+ cells, and the dotted gray line the isotype control.
ROLE OF PXR IN T LYMPHOCYTES

PXR deficiency activates T lymphocytes

We next investigated whether T lymphocyte function is altered in PXR-deficient cells. We found that PXR−/− T lymphocyte proliferation was significantly increased when compared with littermate controls in vitro (Fig. 3A) and in vivo (Fig. 3B). To determine, whether PXR deficiency resulted in altered cytokine production in activated T lymphocytes, we performed intracellular cytokine staining, which demonstrated that PXR−/− T lymphocytes produced more IFN-γ (Fig. 3C) and less IL-10 (Fig. 3D). In addition, spleen-derived PXR−/− T lymphocytes mildly activated by 50 ng/ml PMA exhibited a higher percentage of CD25+ cells (PXR+/+, 21.9% ± 3.9%, n = 3; PXR−/−, 45.5% ± 1.2%, n = 3, p = 0.004). We also observed increased CD25 expression upon anti-CD3/CD28 stimulation (Fig. 3E). The altered T lymphocyte phenotype in PXR-deficient animals was not observed under baseline conditions. Increased T lymphocyte activation only became apparent upon stimulation. Additional immune parameters, including the size, cellularity, and architecture of spleen and lymph nodes, were not altered in PXR-deficient mice at baseline (data not shown). These results demonstrate that PXR deficiency leads to a hyperresponsiveness of T lymphocytes to inflammatory stimuli.

PXR activation inhibits MEK1/2 and NF-κB signaling

Activation of NF-κB and MAPKs produces important signals for T lymphocyte proliferation (25–28). Therefore, we next assessed changes in phospho-p65 NF-κB, phospho-SAPK/JNK, phospho-MEK1/2, and phospho-p38 MAPK expression in activated T lymphocytes treated with the PXR activator PCN or vehicle for MEK1/2, and phospho-p38 MAPK expression in activated T lymphocytes treated with the PXR activator PCN or vehicle for various time periods. Our results show that the level of phosphorylated p65 NF-κB and MEK1/2 in activated T lymphocytes was decreased by PCN treatment after 24 h of cell culture (Fig. 4B, Supplemental Fig. 5A) in a PXR-dependent manner (Fig. 4B), but not at earlier time points (15–60 min, Fig. 4A). Similar results were found in Jurkat cells (Supplemental Fig. 5B). In contrast, the level of phosphorylated p38 MAPK and JNK/SAPK in activated T lymphocytes was not significantly altered by PCN treatment at any time point (data not shown). Recently, it was reported that LXR activation decreases T lymphocyte proliferation by inducing SULT2B1, which would decrease cholesterol availability, a limiting factor for T lymphocyte proliferation (29). Therefore, we assessed the expression of this enzyme in our experimental conditions. PXR activation did not affect the expression of SULT2B1 after 18 and 24 h of cell culture, nor that of cleaved caspase 3 after 48 h, showing that PXR activation does not alter cholesterol availability or level of the proapoptotic protein cleaved caspase 3 in proliferating T lymphocytes (data not shown). In conclusion, PXR activation alters T lymphocyte proliferation, presumably owing to inhibition of MEK1/2 and the p65-NF-κB pathways.

PXR regulates SOCS-1 but not T-bet

Because T-bet and SOCS-1 are master switches of IFN-γ production by T lymphocytes (30–32), we next determined whether PXR activation alters T-bet and SOCS-1 expression. T lymphocytes were stimulated for various time periods in the presence of PCN or vehicle. Whereas T-bet was not altered by PXR activation (Fig. 5A), SOCS-1 was increased in PCN-treated T lymphocytes (Fig. 5B) in a PXR-dependent manner (Fig. 5C), suggesting a regulation of SOCS-1 expression by PXR. Concomitant actinomycin D treatment blocked the PCN-induced increase in SOCS-1, indicating transcriptional regulation (Fig. 5C). Moreover, we assessed the amount of SOCS-1 transcripts in activated T lymphocytes by quantitative PCR following treatment with vehicle or PCN. We found that PCN increased the amount of SOCS-1 mRNA (∼3.6 times) after 1 h (Fig. 5D). Furthermore, in Jurkat cells, SOCS-1 expression culminated 2–3 h after CD3/CD28 stimulation (Supplemental Fig. 6A) and PXR activation maintained high levels of SOCS-1 (Supplemental Fig. 6B). Again, to ensure that PXR was required, this experiment was also done in PXR−/− T lymphocytes. In the absence of PXR, the ability of PCN to induce SOCS-1 was lost (Fig. 5D). Because these data strongly supported that PCN-induced SOCS-1 expression was directly mediated by PXR, we transfected Jurkat cells with pLuc

FIGURE 4. PXR activation inhibits MEK1/2 and NF-κB signaling. T lymphocytes isolated from PXR−/− or littermate control mouse spleens were stimulated in the presence of vehicle or 10 μM PCN. Amount of phosphoproteins in T lymphocytes was determined by flow cytometry, and T lymphocytes were counterstained with an anti-mouse CD3 mAb. A. Time course expression of phosphorylated p65 NF-κB and MEK1/2. Two independent experiments were done. Results are expressed as percentage of controls 15 min poststimulation. B, Level of phosphorylated p65 NF-κB and MEK1/2 24 h poststimulation; n = 3–8. Data were analyzed using a Student t test.
containing the human SOCS-1 promoter sequence. We cultivated cells in the presence of vehicle or the human PXR activator rifampicin for 24 h, and we observed a significant increase in relative luciferase activity following rifampicin treatment when compared with vehicle (Fig. 5E). Together, these results identify SOCS-1 as an early signal in PXR-mediated suppression of T lymphocyte function.

Discussion
By regulating the transcription of a variety of genes, PXR is a powerful integrator of endogenous and exogenous metabolic and xenobiotic cues. The reports of PXR expression in T lymphocytes (6, 7) and our initial observation of a marked increase following immune activation (Fig. 1) prompted us to further explore its role in the adaptive immune response. The combination of PXR agonists with PXR−/− animals allowed us to dissect the role of PXR in T lymphocytes in detail.

We demonstrate that pharmacologic activation of PXR inhibits T lymphocyte function. PXR agonists inhibit the expression of CD25, a T lymphocyte activation marker, as well as synthesis and production of the Th1 cytokine IFN-γ by T lymphocytes in a PXR-dependent manner. Moreover, pharmacologic PXR activation dramatically reduces the ability of T lymphocytes to proliferate in response to a strong immune stimulation. Combined with prior publications reporting immunosuppressive effects of rifampicin (8–12, 33, 34), there is now accumulating evidence that PXR activation suppresses T lymphocyte function. Notably, the possible involvement of the glucocorticoid receptor in mediating the
immunosuppressive effects of PXR activators (35) was previously dismissed (36).

Because PXR is mostly known for regulating drug metabolism, our results raise the question of how PXR exerts its anti-inflammatory effects. Recent reports demonstrated a mutual repression between PXR and NF-κB (16). Indeed, in hepatocytes PXR activation decreased the transcriptional activity of NF-κB, and reciprocally NF-κB activation inhibited both PXR activity and CYP3A4 gene expression (16). Moreover, inhibition of NF-κB activity by IκBαM was able to rescue repressed PXR activity and to enhance PXR-mediated CYP3A4 expression (16). In our work, we additionally show that PCN decreases the amount of active NF-κB and MEK1/2 in T lymphocytes and that this requires the presence of PXR because PCN does not decrease levels of active NF-κB and MEK1/2 in PXR−/− T lymphocytes. It is possible that the anergy of T lymphocytes induced by PXR activation originates from the inhibition of both NF-κB and MEK1/2. We observed these effects at late (24 h) rather than early (15–60 min) time points after T lymphocyte activation. Similarly, Zhou et al. (16) recently showed in HepG2 cells that PXR activation inhibits the activity of NF-κB 24 h after PXR activation. These results suggest that the anti-inflammatory / antiproliferative effects of PXR activation are mediated by well-established inflammatory signaling pathways.

T-bet and SOCS-1 are master switches of IFN-γ production by T lymphocytes (30–32). Although PPARα, RXR, or the glucocorticoid receptor has been shown to modulate T-bet expression (37–39), in this paper we show that PXR activation did not have any effect on T-bet expression in mouse T lymphocytes (Fig. 5A). Instead, PXR activation enhances the expression of SOCS-1, which may serve as one potential mechanism of PXR-mediated T cell immunosuppression. PXR activation increased SOCS-1 rapidly in a PXR-dependent manner, (Fig. 5B, 5C). A luciferase assay demonstrates transactivation of the SOCS-1 promoter; however, a direct regulation of SOCS-1 by PXR remains to be demonstrated. Interestingly, SOCS-1 has previously been shown to bind to p65 NF-κB and to decrease its expression, presumably by promoting p65 NF-κB degradation (40). Therefore, by inducing SOCS-1, PXR may be ideally positioned at the crossroad between metabolism and the immune system.

Previously, Siest et al. reported a correlation between PXR and cytochrome P-450 enzymes that are involved in leukotriene metabolism (7, 19, 41, 42). Accordingly, PXR regulates the expression and production of leukotrienes in activated lymphocytes (41, 42). Albeit we found in the current study that the expression of CYP3A11 parallels the expression of PXR in stimulated T lymphocytes, a direct link between cytochrome P-450 enzymes and the inflammatory process is difficult to establish, and we cannot exclude that these are collateral effects of PXR upregulation.

We also report increased proliferation of PXR-deficient T lymphocytes both in vitro and in vivo (Fig. 3A, 3B). Furthermore, we show that PXR-deficient lymphocytes produce more IFN-γ and less of the anti-inflammatory cytokine IL-10. Together with a previous report showing that PXR−/− mice develop inflammation of the small intestine (16), our data strongly indicate that a lack of PXR results in exaggerated inflammation. Thus, PXR may counterbalance ongoing immune responses.

In conclusion, we show immunosuppressive effects of PXR activation. Increased PXR expression in T lymphocytes following activation with an inflammatory stimulus indicates a role of PXR in counterbalancing ongoing immune responses. Therefore, exogenous compounds could be leveraged to target PXR in inflammatory disease. Indeed, rifampicin, a well-known activator of the human form of PXR, was shown to ameliorate psoriasis, a Th1-mediated skin disease (13–15). The present study, by demonstrating a downregulation of IFN-γ production and a decrease in NF-κB and MEK1/2 preceded by increased SOCS-1 in activated T lymphocytes upon PXR activation, can explain, at least in part, such beneficial use of rifampicin in psoriasis.

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Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Fig. 1: A. PXR expression is increased in activated T-lymphocytes: Quantitative PCR analyses were performed to measure the expression of PXR using real-time PCR with the TaqMan PCR master mix from Applied Biosystems. Random-primed cDNA was prepared from total RNA isolated from highly purified unstimulated or stimulated mouse T-lymphocytes. Activation stimuli included: IL-2 (35U/ml), mouse TSLP (50 ng/ml), PMA (50 ng/ml), PMA (50 ng/ml) + LPS (50 ng/ml), and PMA (50 ng/ml) + CpG (5μg/ml). RNA levels were measured after 48h, n=3. Data were analyzed using a Student-test (activated vs unstimulated mouse T-lymphocytes). B, C. Time course expression of PXR in activated Jurkat cells (B) or mouse T-lymphocytes (C). PXR mRNA was quantified by real-time PCR analysis with the TaqMan PCR master mix from Applied Biosystems. Jurkat cells were stimulated with anti-CD3/CD28 while mouse T-lymphocytes were stimulated with anti-CD3/CD28 or PMA for various time periods, n=3-6. Data were analysed by a one-way ANOVA test followed by a Newman-Keuls post-hoc test.
Supplemental Fig. 2: PXR activation inhibits T cell activation in vitro: Expression of CD25 on wild type or PXR^{+/+} mouse T-lymphocytes after activation with anti-mouse CD3/CD28 for 48h in the presence of 10 μM PCN or RU-486 or vehicle was analysed by flow cytometry, n=3. FACS plots show representative results expressed as mean fluorescence intensity (MFI).
Supplemental Fig. 3: Treatments do not affect cell viability: Flow cytometry analysis showing the percentage of 7AAD⁺CD4⁺ T-lymphocytes after activation in the presence of 10 μM PCN or RU-486 or vehicle, n=4. FACS plots show representative results.
Supplemental Fig. 4: PXR activation reduced the production of IFN-γ by T-lymphocytes: Production of IFN-γ determined by ELISA on pooled supernatant from PXR<sup>+/+</sup> T-lymphocytes activated with anti-mouse CD3/CD28 for 48h in the presence of vehicle (DMSO) or 10 μM PCN.
**Supplemental Fig. 5: PXR activation inhibits MEK1/2 and NF-κB signalling:** Level of phosphorylated p65-NF-κB and MEK1/2 in stimulated purified T-lymphocytes treated with vehicle or 10 μM PCN (A) or in stimulated Jurkat cells (B) treated with vehicle or 10 μM rifampicin (RIF) and analyzed by Western blotting as described in Materials and Methods.
Supplemental Fig 6: PXR activation maintains high level of SOCS-1: A. Time course expression of SOCS-1 in stimulated Jurkat cells. SOCS-1 mRNA was quantified by real-time PCR analysis using the TaqMan PCR master mix from Applied Biosystems. Jurkat cells were stimulated with anti-CD3/CD28 for various time periods. Experiments were done in triplicates. B. Effects of PXR activation on SOCS-1 expression. SOCS-1 mRNA was quantified by real-time PCR analysis with the TaqMan PCR master mix from Applied Biosystems. Jurkat cells were stimulated with anti-CD3/CD28 in the presence of rifampicin or vehicle for various time periods. Experiments were done in triplicates. Level of SOCS-1 in stimulated Jurkat cells treated with vehicle or rifampicin (RIF) for 6h and analyzed by Western blotting as described in Materials and Methods.