Regulation of dectin-1 mediated dendritic cell activation by peroxisome proliferator-activated receptor-gamma ligand troglitazone

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Running title: Regulation of Dectin-1 activated DCs by PPAR-gamma
Abstract

Dectin-1 is the major receptor for fungal β-glucans. Activation of Dectin-1 leads to the upregulation of surface molecules on dendritic cells (DCs) and cytokine secretion. Furthermore, Dectin-1 is important for recruitment of leukocytes and production of inflammatory mediators. PPAR-gamma and its ligands, cyclopentenone prostaglandins or thiazolidinediones, have modulatory effects on B cell, T cell and DC function.

In our study we analyzed the effects of troglitazone (TGZ), a high affinity synthetic PPAR-gamma ligand, on the Dectin-1 mediated activation of monocyte-derived human DCs.

Dectin-1 mediated activation of DCs was inhibited by TGZ characterized by downregulation of costimulatory molecules and reduced secretion of cytokines and chemokines involved in T-lymphocyte activation. Furthermore, TGZ inhibited the T cell stimulatory capacity of DCs. These effects were not due to a diminished expression of Dectin-1 or a reduced phosphorylation of Syk. They were mediated by the inhibition of downstream signaling molecules such as mitogen-activated protein kinases and NF-κB. Furthermore, curdlan-mediated accumulation of CARD9 in the cytosol was inhibited by TGZ.

Our data demonstrate that the PPAR-gamma ligand TGZ inhibits Dectin-1 mediated activation by interfering with CARD9, MAP kinase and NF-κB signaling pathways. This confirms their important role as negative feedback regulators of potentially harmful inflammatory responses.
Introduction

Dendritic cells (DCs) form a heterogeneous population of antigen presenting cells linking the innate and the adaptive immune system. Immature DCs mostly reside in the body's tissues and in part travel through the blood and lymph stream. In their immature state, DCs take up particles and large amounts of extracellular fluids by receptor-mediated endocytosis, phagocytosis and macropinocytosis. When they encounter foreign antigens, DCs undergo a variety of phenotypic and functional changes and differentiate into mature cells. They upregulate the expression of MHC class I and class II molecules as well as the co-stimulatory molecules CD80, CD83, CD86 and CD40 and adhesion molecules like CD54, CD102 and CD209. They secrete a variety of cytokines as IL-6, IL-12, IL-1β and TNF-α and chemokines like MIP-3α and RANTES to generate an optimal environment for the following immune response and to attract lymphocytes.

A variety of antigenic structures recognized by DCs are pathogen-associated molecular patterns (PAMPs) which are shared by groups of pathogens. Well known examples are lipopolysaccharides (LPS), which are characteristic for bacteria, or double-stranded RNA, which are typical for certain viruses. PAMPs are recognized by pattern recognition receptors that are activated after binding of their ligand structures. The Dectin-1 receptor belongs to the group of pattern recognition receptors and is mainly expressed on DCs and macrophages. The receptor is a member of the C-type lectin receptor family and able to recognize carbohydrates with its extracellular carbohydrate recognition domain. Several isoforms of human Dectin-1 (hDectin-1) with different functions exist due to alternative splicing. Interestingly,
only the isoforms hDectin-1a and -1b can bind zymosan, a β-glucan-rich particle from the cell wall of *Saccharomyces cerevisiae*\(^6\). Isoform hDectin-1b was found to be the main form expressed in human monocyte-derived DCs (mDCs), while isoform hDectin-1a is mainly expressed in myeloid and plasmacytoid DCs\(^5\). Other structural features of hDectin-1 are its transmembrane region and a cytoplasmic domain, which entails an immunoreceptor tyrosine-activation motif (ITAM)-like motif. Through the ITAM-like motif, hDectin-1 initiates signaling cascades via activation of Syk and MAP kinases\(^7\),\(^7\),\(^8\). hDectin-1 generates proinflammatory cytokine responses together with Toll-like receptor (TLR) 2\(^9\),\(^10\). However, the TLR2-dependent and the Syk-dependent pathways can operate independently from each other\(^11\). Furthermore, hDectin-1 signaling induces the expression of the caspase recruitment domain (CARD)-containing adaptor protein Card9 and therefore activates NF-κB in DCs\(^12\)-\(^14\). Card9 is a key adaptor for non TLR pattern recognition receptor (PRR) signal transduction and links hDectin-1/Syk activation with NF-κB activation. Card9-deficient DCs were reported to show defective activation of NF-κB and an impaired cytokine response upon stimulation with zymosan\(^12\). As NF-κB controls the expression of genes encoding inflammatory cytokines, chemokines and cell surface adhesion molecules, hDectin-1 and its ligands transduce signals via the described pathways to raise an immune response.

Peroxisome proliferator activated receptor gamma (PPAR-γ) is a nuclear hormone receptor and lipid-activated transcription factor. It is highly expressed in adipose tissue, but also in several immune cells like DCs and macrophages\(^15\)-\(^17\). Natural ligands of PPAR-γ are polyunsaturated fatty acids, prostaglandin derivats like 15-deoxy-\(\Delta^{12-14}\)-Prostaglandin-J\(_2\) (15d-PGJ\(_2\)) and linoleic acid metabolites\(^18\),\(^19\). Synthetic ligands are amongst others thiazolidinediones like troglitazone (TGZ)\(^20\).
The activation of PPAR-γ by its natural or synthetic ligands affects DC differentiation and function 17, 21, 22. In mDCs, PPAR-γ activation inhibits differentiation processes, shown by the impaired expression of CD1a 22. Furthermore, PPAR-γ agonists inhibit the maturation process: The expression of the co-stimulatory molecules CD83 and CD40 is decreased 17, 22. The chemokine receptor CCR7, which is important for DC migration to draining lymph nodes, is down-regulated 22. The secretion of IL-12 and RANTES is inhibited 17. Finally, PPAR-γ activation impairs mDC stimulation via TLR 2, 3, 4 and 7 ligands 23.

As the natural ligand of PPAR-γ, 15d-PGJ₂, is produced during the late phase of inflammation and due to the obvious anti-inflammatory effects of PPAR-γ activation, its role in the restriction of immune responses and induction of tolerance has been investigated 24-26.

In this work, we analyzed the effects of PPAR-γ activation on the hDectin-1-mediated stimulation of mDCs.

Material and methods

Generation of monocyte-derived dendritic cells (mDCs)

mDCs were generated from adhering monocytes as described previously 27. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll/Paque (Biochrom, Berlin, Germany) density gradient centrifugation from buffy coats of healthy voluntary donors from the blood bank of the University of Bonn. PBMCs were seeded into 75 cm² cell culture flasks (BD, Heidelberg, Germany) in X-VIVO medium (Lonza, Cologne, Germany) at a density of 10⁸ cells/10 ml/flask. After 1.5 h
incubation at 37°C, 5% CO₂, non-adherent cells were removed by extensive washing. Adhering monocytes were cultured in RP10 medium (RPMI 1640 containing GlutaMAX, supplemented with 10% heat-inactivated fetal calf serum and 100 units/ml penicillin/streptomycin). Differentiation into DCs was induced by addition of 100 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF, sargramostim, Berlex, Richmond, VA) and 20 ng/ml Interleukin-4 (IL-4, R&D Systems, Wiesbaden, Germany) every second day. Cells were harvested on day seven after start of culture.

Depending on the experiment, mDCs were treated with one or a combination of the following substances: troglitazone (TGZ, 5 µM, Biomol, Hamburg, Germany) every second day; curdlan (100 µg/ml, Wako, Neuss, Germany) on day 6 of culture. Equal amounts of DMSO were added to non-treated cells as a control to exclude solvent effects.

**Immunostaining and flow cytometry analysis**

The phenotype of generated mDCs was analyzed by flow cytometry. Cells were stained with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated mouse monoclonal antibodies against CD1a, CD40, CD54, CD80, CD83, HLA-DR and mouse IgG (all purchased from Beckman Coulter, Krefeld, Germany) and Dectin-1, DC-LAMP and CCR7 (all purchased from R&D Systems, Wiesbaden, Germany). For intracellular staining, cells were fixed with a 2% formaldehyde solution and permeabilized with 0.1% Triton X-100 followed by staining with antibodies. All flow cytometry analysis was performed on a Cytomics FC 500 (Beckman Coulter) using CXP Analysis software (Beckman Coulter).
Preparation of whole-cell lysates

Whole-cell lysates were prepared as described previously \textsuperscript{23}. mDCs were incubated in lysis buffer containing 1% Igepal CA-630, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 2 mM ethylene diamine tetra acetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 µg/ml aprotinin and 1 mM sodium orthovanadate for 20 to 30 minutes. Lysates were centrifuged for 15 minutes at 20,000g and the protein-containing supernatant was harvested. The protein concentration was measured using a bicinchoninic acid (BCA) assay (PIERCE, Perbio Science, Bonn, Germany).

Preparation of nuclear extracts

Nuclear extracts were prepared as described \textsuperscript{28}. In brief, \(10^6\) mDCs were incubated in 400 µl Buffer A containing 10 mM HEPES pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT and 0.5 mM PMSF. Cellular membranes were destroyed by addition of 10% Igepal CA-630 and vigorous vortexing. Nuclei were pelleted by centrifugation and resuspended in buffer C containing 20 mM HEPES pH 7.9, 0.4 M sodium chloride, 1 mM EDTA, 1 mM EGTA, 1 mM DTT and 0.5 mM PMSF. Nuclear proteins were recovered by centrifugation for 5 minutes at 20,000g.

PAGE and Western blotting

For analysis of cytosolic proteins, approximately 20 µg of whole-cell lysates were separated on a 10% SDS-polyacrylamide gel by electrophoresis and transferred onto a nitrocellulose membrane (Whatman, Germany). The blot was probed with
antibodies recognizing hDectin-1 (rabbit polyclonal), CARD9 (T-17, goat polyclonal, Santa Cruz Biotechnology Inc., Heidelberg, Germany), phospho-ERK1/2 (Thr202/Tyr204), rabbit polyclonal), ERK1/2 (137F5, rabbit monoclonal), phospho-p38 (Tyr180/Tyr182, rabbit polyclonal), p38 (rabbit polyclonal, all purchased from Cell Signaling Technology Inc., Frabkfurt, Germany), phospho-Syk (pY348, mouse monoclonal) and Syk (mouse monoclonal, both from BD Biosciences Pharmingen, Heidelberg, Germany). To ensure that equal amounts of protein had been loaded into each lane of the SDS gel, antibodies against GAPDH (10B8, mouse monoclonal) and Aktin (I-19, goat polyclonal, Santa Cruz Biotechnology) were used as controls.

To analyze nuclear proteins, 20 µl of nuclear extracts were separated on a 10% SDS polyacrylamide gel and blotted on a nitrocellulose membrane. Ponceau S staining was performed to ensure equal loading of the gel. The blot was probed with antibodies against RelB (C-19, rabbit polyclonal) and phospho-NFATc1 (Ser257, rabbit polyclonal, both purchased from Santa Cruz Biotechnology).

For all performed Western blots, suitable secondary antibodies conjugated with horseradish peroxidase were used. Protein bands were visualized using the enhanced chemiluminescence (ECL) staining system (GE Healthcare, München, Germany).

**Determination of cytokine production**

Secretion of cytokines and chemokines in mDC culture supernatants was measured using commercially available ELISAs and following the manufacturer’s instructions. The concentrations of IL-6, IL-1β and TNF-α were analyzed with ELISA kits from Immunotech, Beckman Coulter. The concentrations of MIP-3α and RANTES were
analyzed with ELISAs from R&D Systems. The read-out was performed using a Synergy2 (BioTek, Bad Friedrichshall, Germany).

**Mixed lymphocyte reaction**

PBMCs from allogeneic blood donors were seeded into flat-bottom 96-well microtiter plates at $10^5$ cells/well (Greiner Bi-One GmbH, Frickenhausen, Germany). $10^5$, $10^4$ or $10^3$ mDCs were added as stimulator cells and incubated for 5 days at 37°C, 5% CO$_2$. The assay was performed in 4-fold replicates as described previously 29. Cells were pulsed with $^3$H-thymidine (Hartmann Analytic, Braunschweig, Germany) and incubated for another 16 hours at 37°C, 5% CO$_2$. Cells were harvested using a Filter Mate Harvester (Perkin Elmer, Waltham, USA) and uptake of $^3$H-thymidine was measured by a Microbeta TriLux (Perkin Elmer).

**Results**

**PPAR-$\gamma$-activation inhibits Dectin-mediated alteration of cell surface molecules**

Activation of Dectin-1 with zymosan or the more specific ligand curdlan was demonstrated to activate human dendritic cells (DCs) characterized by upregulation of costimulatory molecules and CD83.

To determine the effect of PPAR-$\gamma$-activation on the expression of cell surface molecules, we incubated DCs during their differentiation from peripheral blood monocytes with or without TGZ, a highly specific PPAR-$\gamma$ ligand, and activated them with curdlan. On day 7 of culture mDCs generated in the presence of GM-CSF and IL-4 showed a strong expression of CD1a (Figure 1a) and a light expression of CD83 (Figure 1a), a phenotype characteristic for immature mDCs 25. Treatment with
curdlan for 16 h (stimulation on day 6 of culture) resulted in up-regulation of surface molecules associated with the mature DC phenotype as CD83, CD54, CD40, CCR7, HLA-DR and DC-LAMP (Figure 1a). TGZ-treatment during the generation of mDCs inhibited as expected the expression of CD1a and costimulatory molecules. When curdlan was added to TGZ-treated mDCs, the Dectin-1 mediated upregulation of CD83 and costimulatory molecules was dramatically inhibited (Figure 1a). To ensure that the inhibitory effect of TGZ was not due to a reduced expression of Dectin-1, we performed Western blot analysis and found no relevant differences in the protein expression of Dectin-1 in used cell populations (Figure 1b). We used Western blotting for analysis of Dectin-1 expression as activation of mDCs with beta-glucans leads to the internalization of Dectin-1.

**PPAR-γ-activation inhibits Dectin-mediated secretion of cytokines and chemokines**

Upon activation, DCs secrete a variety of cytokines and chemokines to attract and stimulate T lymphocytes. Treatment of mDCs with curdlan induced secretion of IL-6, TNF-α, IL-1β, MIP-3α and RANTES in comparison to DMSO treated cells. Additional treatment with TGZ strongly decreased the secretion of all cytokines and chemokines to background levels or only slightly above (Figure 2).

**PPAR-γ-activation impairs the ability of Dectin-1-activated mDCs to stimulate lymphocyte proliferation**

One characteristic feature of activated DCs is the induction of lymphocyte proliferation. We next analyzed the influence of PPAR-γ-activation on the ability of Dectin-1-activated DCs to stimulate T cells in MLR. Curdlan activated mDCs induced a strong proliferative response in allogeneic T cells as compared to immature DC.
This effect was inhibited by additional treatment with TGZ, where lymphocyte proliferation was comparable to immature mDCs (Figure 3).

**Effects of PPAR-γ-activation are mediated through MAPK signaling pathways but independent from Syk**

Dectin-1 has an ITAM-like motif in its cytoplasmic tail, which mediates the downstream signaling by phosphorylation of Syk and activation of MAP kinase pathways after stimulation with the Dectin-1 ligand curdlan. In the next set of experiments, we addressed the role of tyrosine kinase Syk in TGZ-induced alteration of Dectin-1 signaling. Stimulation of mDCs with curdlan resulted in an increase in Syk phosphorylation that was not affected by TGZ (Figure 4a). However, we found that treatment of mDCs with curdlan induced phosphorylation and thus activation of MAPKs p38 and ERK1/2 that was inhibited by addition of TGZ (Figure 4b).

**PPAR-γ-activation impairs nuclear localization of NF-κB through inhibition of Card9 expression**

We next analyzed the effects of TGZ on the nuclear expression of transcription factors involved in Dectin-1 mediated activation of DC by Western blotting. The nuclear localization of NF-κB family member RelB was strongly increased upon treatment of mDCs with curdlan. Additional treatment of cells with TGZ inhibited the activation and nuclear localization of these NF-κB members (Figure 5a). To further characterize the signaling processes leading to NF-κB translocation, we determined the protein expression of Card9 in nuclear extracts. Card9 is a known transducer of Dectin-1 signaling triggering NF-κB activation. As expected,
stimulation of mDCs with curdlan induced the expression of Card9. When mDCs were treated additionally with TGZ, the Card9 expression was dramatically inhibited in curdlan stimulated cells (Figure 5b). This was not due to an increased proteasomal degradation of the protein as addition of bortezomib, a specific proteasome inhibitor, to the cell cultures had no effect on Card9 expression in TGZ treated cells (data not shown). In addition we could not find a relevant down regulation of Card9 transcripts in these cells as analyzed by PCR (data not shown).

We further analyzed the activation of the transcription factor NFAT that was demonstrated to be involved in Dectin-1 induced DC stimulation. Treatment with curdlan resulted in enhanced phosphorylation of NFAT in mDCs that was not affected by TGZ (Figure 5c).

**Discussion**

Innate immune cells sense invading pathogens through pattern recognition receptors, which recognize highly conserved molecular structures. Besides Toll-like receptors and NOD proteins, dendritic cells express Dectin-1, a member of the C-type lectin family that represents the main receptor for fungal and bacterial β-glucans. Characterized ligands for hDectin-1 are zymosan, a β-glucan rich particlal from the cell wall of *Saccharomyces cerevisiae*, and curdlan, a linear β-1,3-glucan polymer from *Alcaligenes faecalis*. As zymosan also stimulates TLR2 besides hDectin-1, it is difficult to differentiate between the effects that are induced via hDectin-1 or the TLR2/MyD88 signaling pathways. The polysaccharide curdlan is a more specific hDectin-1 agonist and mediates its effects independently from TLR2. We therefore used curdlan in our experiments to exclude TLR stimulation and to solely analyze hDectin-1 signaling.
The transcription factor peroxisome-proliferator activated receptor gamma gets activated by a variety of natural and synthetic ligands. Amongst its natural ligands is the cyclopentenone prostaglandin 15d-PGJ$_2$, which gets produced during the late phase of inflammation. The inhibitory effects of 15d-PGJ$_2$ or the more specific synthetic ligands such as TGZ on the activation of DCs regarding antigen presentation, migration or cytokine secretion have been shown previously. Considering its inhibitory properties on antigen presenting cells and the production of cyclopentenone prostaglandins by COX-2 during the late phase of inflammation, an assumed function of PPAR-$\gamma$ in the immune system is the termination of initiated immune responses. While the initiation of immune responses is essential for defending the organism against pathogens, its termination is pivotal for the body's integrity. Infinite inflammatory processes might lead to autoimmune diseases and cause severe damage. PPAR-$\gamma$ activation might thus play an important role as a negative feedback mechanism in the regulation of immune responses.

In our work, we analyzed the modulation of Dectin-1 mediated DC activation by PPAR-$\gamma$ ligand. While treatment of mDCs with the hDectin-1 ligand curdlan caused the upregulation of CD83 and maturation-associated molecules, their expression was inhibited by the addition of TGZ. In line with these results, the secretion of pro-inflammatory cytokines and chemokines that were shown to be important for T cell activation and attraction such as IL-12 or MIP-3$\alpha$ and RANTES was inhibited by TGZ.

Finally, the inhibitory effects of the PPAR-$\gamma$ activation on the hDectin-induced maturation of mDCs were confirmed in the MLR assays. The proliferation of T lymphocytes, after stimulation with hDectin-1-activated mDCs, was reduced when TGZ was administered to mDCs, making an immune response towards a fungal
pathogen impossible. Taken together, these results demonstrate that TGZ interferes
with the Dectin-1 induced activation of antigen presenting cells and can therefore
prevent the generation of an effective immune response in curdlan-treated mDCs,
simulating a fungal infection.

In the next set of experiments we analyzed the underlying mechanisms involved in
the inhibitory effects of PPAR-γ. We showed that the seen effects are not caused by
the induction of apoptosis by TGZ. We also determined the activation and expression
of several members of intracellular signaling cascades. It is well known, that the
activation of hDectin-1 by fungal PAMPs leads to the phosphorylation of Syk. In the
next steps, the Card9/Bcl10/Malt-1 (CBM) complex is recruited and leads to the
polyubiquitination of IKK, followed by the phosphorylation and degradation of IκB and
finally the nuclear translocation of the transcription factor NF-κB. Additionally,
Syk mediates the activation of MAP kinase cascades and leads to the
phosphorylation of p38 and ERK1/2, which activate several transcription factors as a
consequence.

We found, that the phosphorylation of Syk is induced upon hDectin-1 stimulation with
curdlan and that Syk activation is not altered when PPAR-γ is activated by TGZ. The
beginning of the signaling pathway is obviously not influenced by PPAR-γ activation.
The phosphorylation of p38 and ERK1/2 MAP kinases, however, is inhibited when
TGZ-treated mDCs are stimulated with curdlan. This indicates that the signaling
cascade is inhibited between Syk and p38 or ERK1/2.

Looking at the nuclear translocation of the NF-κB member RelB, we found that the
nuclear localization of the transcription factor was inhibited in curdlan-stimulated,
TGZ-treated mDCs. The signaling cascade seemed to be restrained at some point
between Syk and NF-κB. As the activation of NF-κB depends on the CBM complex, we analyzed the expression of Card9 and found that Card9 expression is induced in mDCs stimulated with curdlan, but inhibited when cells are co-stimulated with TGZ. These findings provide an explanation for the impaired nuclear translocation of NF-κB in TGZ- and curdlan-treated cells. In addition, the data show that upon activation of hDectin-1, the signaling cascade Syk/CBM/NF-κB is not only activated by phosphorylation and ubiquitination processes, but additionally strengthened by the upregulated Card9 expression. The increased expression of the signal mediator Card9 might help to amplify the incoming signal.

It has been shown, that the stimulation of hDectin-1 leads to the activation of the transcription factor NFAT in DCs. Similar to NFAT lymphocyte signaling, the signaling cascade is mediated via Syk, which then activates phospholipase C-γ (PLC-γ). PLC-γ catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to inositol 1,4,5-trisphosphate (IP₃) and 1,2-diacylglycerol (DAG). IP₃ leads to intracellular calcium (Ca²⁺) release and activation of calcineurin. The phosphatase calcineurin dephosphorylates NFAT, allowing its nuclear translocation. While NFAT has been found to be constitutively expressed in the nucleus of myeloid cells, it gets induced after hDectin-1 stimulation. We found, that mDC treatment with curdlan strongly increased the nuclear translocation of NFAT, which stayed unchanged when cells were co-treated with TGZ. The results point out that the signaling pathway Syk/PLC-γ/IP₃/Ca²⁺/Calcineurin/NFAT is not inhibited by activation of PPAR-γ.

It is still unclear, how PPAR-γ activation mediates all its effects on the DC activation by fungal pathogens. PPAR-γ activation mainly decreases antigen presentation via HLA-molecules and CD1a, inhibits cytokine secretion and induction of lymphocyte
proliferation. In this work we show that activation of PPAR-γ leads to the inhibition of hDectin-1-induced activation of NF-κB and MAP kinase signaling cascades by reducing Card9 expression, while NFAT signaling remains unaffected.

Acknowledgments

We thank N. Gallala for her technical assistance.

Authorship Contribution

G.K. performed research and wrote the paper, A.B. designed experiments, performed research and wrote the paper, S.A.E. Held performed research, S.D. performed research, A.H. analyzed data, P.B. designed the research and wrote the paper. G.K. and A.B. contributed equally to this work.

Disclosure of Conflicts of Interest

The authors have no conflict of interest to declare.

References


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**Figure Legends**

**Figure 1: PPAR-γ activation inhibits the hDectin-1-induced maturation in mDCs independently from hDectin-1 expression.** mDCs were generated from adhering monocytes in the presence of PPAR-γ ligand TGZ or DMSO as control. Cells were stimulated with curdlan on day 6 of culture. (A) Expression of surface maturation markers was analyzed on day 7 by flow cytometry. The figure shows percentages of positive cells as well as MFI values in square brackets. (B) The protein expression of hDectin-1 was analyzed on day 7 either by Western blotting. The data shown are representative of at least 3 independent experiments.

**Figure 2: PPAR-γ activation inhibits the hDectin-1-induced secretion of inflammatory cytokines and chemokines.** mDCs were generated in the presence of PPAR-γ ligand TGZ or DMSO as a control and were stimulated with curdlan on day 6 of culture. Cytokine and chemokine secretion in culture supernatants was
determined 24 hours later using ELISAs. The data shown are representative of at least 3 independent experiments.

**Figure 3:** PPAR-γ activation reduces the hDectin-1-induced proliferation of lymphocytes. DMSO- or TGZ-treated mDCs were stimulated with curdlan for 24 h before using the cells as stimulators in a MLR assay. Incorporation of ³H-thymidine representing a measurement for lymphocyte proliferation was analyzed. The data shown are representative of at least 3 independent experiments. The error bars represent the deviation of the quadruplicates.

**Figure 4:** Activation of MAP kinases in response to hDectin-1 stimulation is impaired upon PPAR-γ activation. DMSO- or TGZ-treated DCs were stimulated with curdlan for 4-24 h and whole cell lysates were prepared. The phosphorylation and thus activation of Syk (A) as well as p38 and ERK (B) were analyzed by Western blotting. The data shown are representative of at least 3 independent experiments.

**Figure 5:** Inhibitory effects of PPAR-γ activation are mediated via the Card9/NF-κB signaling pathway. Nuclear extracts and whole cell lysates were prepared from mDCs treated with TGZ or DMSO and stimulated with curdlan. The nuclear translocation of NF-κB member RelB was inhibited after PPAR-γ activation (A). The expression of Card9 was reduced after TGZ-treatment and correlated with the RelB translocation (B). The transcription factor NFAT was not affected by PPAR-γ activation (C). The data shown are representative of at least 3 independent experiments.
Figure 2

Graphs showing the concentration of various cytokines in pg/ml for different treatments:
- **TNFalpha**: Comparing DMSO, TGZ, DMSO+Curdlan, TGZ+Curdlan.
- **MIP3alpha**: Similar comparisons as above.
- **IL-1β**: Similar comparisons as above.
- **RANTES**: Similar comparisons as above.
- **IL-6**: Similar comparisons as above.
Figure 3
Figure 4A

Figure 4B
Figure 5A

Figure 5B

Figure 5C
Regulation of dectin-1–mediated dendritic cell activation by peroxisome proliferator-activated receptor-gamma ligand troglitazone

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