PPARγ activation drives Th17 cells into a Treg phenotype

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Introduction and Aims

The differentiation of T helper cells is intricately linked to the cytokine milieu that orchestrates activation of multiple intracellular signaling and transcriptional networks. Plasticity between Th17 and iTreg has been previously reported. At the molecular level, plasticity can be achieved by a cytokine-driven reprogramming of signaling pathways and targeted activation of master regulator transcription factors. Peroxisome proliferator activated receptor γ (PPARγ) regulates the CD4+ T cell differentiation process and it is a key contributor to the amelioration of gut inflammation. However, the role of PPARγ in controlling the modulation of Th17 differentiation and plasticity is incompletely understood. Herein, we created a computational and mathematical model of the CD4+ T cell differentiation process to provide a comprehensive understanding of the underlying mechanisms and modulation of CD4+ T cell differentiation and plasticity between Th17 and iTreg by PPARγ at the gastrointestinal mucosa., running *in silico* experimentation and confirming the predictions with *in vivo* validation studies.

Results

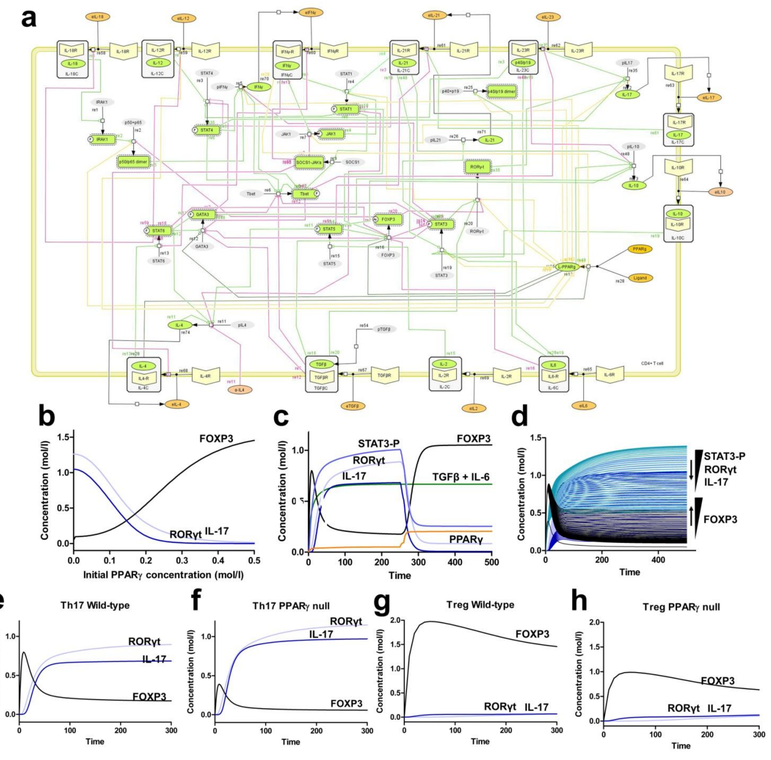

Figure 1. *In silico* modeling on the CD4+ T cell computational model

The CD4+ T cell differentiation model (Fig.1A) was induced from a naive state into Th17 adding IL-6 and TGFβ and demonstrated upregulation of RORγt, IL-17 and STAT-3. Increasing concentrations of PPARγ in the Th17 cell led to downregulation of RORγt and IL-17 and upregulation of FOXP3 (Fig.1B). The same trend was observed when after induction of Th17, we promoted PPARγ activation, thereby demonstrating a phenotypic switch from a Th17 to an iTreg phenotype (Fig.1C). A combination of time-course and scan reiterated the phenotype switch with increasing concentrations of PPARγ over time (Fig.1D). The effect on the deficiency of PPARγ *in silico* was tested by the creation of a PPARγ knock-out that showed increased expression of RORγt and IL-17 when differentiated to Th17 with IL-6 and TGFβ (Fig.1F) and decreased expression of FOXP3 in a TGFβ-only differentiated iTreg system (Fig.1H) when both compared to the wild type (Fig.1E and 1G), suggesting a regulatory role of PPARγ in initial CD4+ T cell differentiation from a naïve state.

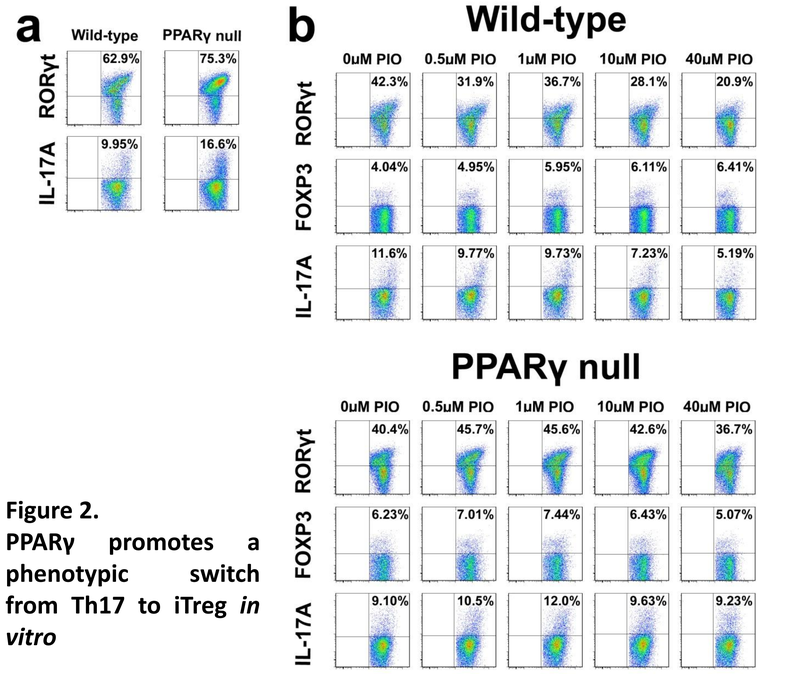

Figure 2.
PPARγ promotes a phenotypic switch from Th17 to iTreg *in vitro*

To validate the results of our computational simulation, we first isolated and sorted naïve CD4+ T cells from wild-type and T cell-specific PPARγ null spleens. Cells were polarized to Th17 under IL-6 and TGFβ presence during 60h, and then treated with a gradient of pioglitazone, a PPARγ agonist. Prior to the treatment, IL-17 and RORγt expression was significantly higher in PPARγ null Th17 differentiated cells when compared to wild-type Th17 cells (Fig.2A). Following pioglitazone treatment for 24h, Th17 cells from wild-type mice showed increasing levels of FOXP3 and downregulation of RORγt and IL-17A in wild-type but not in PPARγ null Th17 cells (Fig.2B and C). These results provide *in vitro* evidence that PPARγ drives iTreg differentiation from a Th17 phenotype through a PPARγ-dependent mechanism.

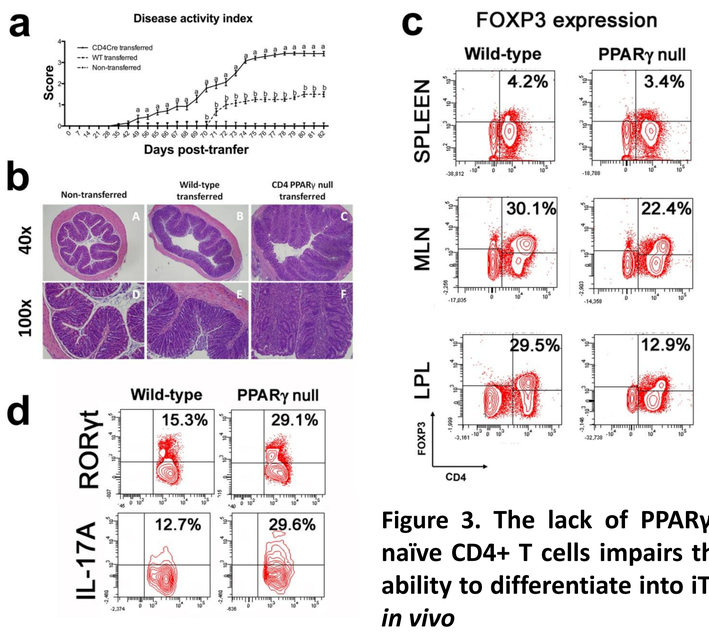

Figure 3. The lack of PPARγ in naïve CD4+ T cells impairs their ability to differentiate into iTreg *in vivo*

To determine whether the loss of T cell PPARγ leads to Th17 and impairs iTreg differentiation, we sorted CD4+ CD25- CD45RB^{high} naïve T cells from donor wild-type and T cell-specific PPARγ null spleens and adoptively transferred 4 x 10^5 viable cells to SCID recipients. Transfer of PPARγ null cells showed a significantly higher disease activity index when compared to wild-type transferred mice (Fig.3A). Histological examination demonstrated that colons recovered from recipients of PPARγ null CD4+ T cells had a significantly greater lymphocytic infiltration and crypt hyperplasia than those recovered from recipients of wild-type CD4+ T cells (Fig.3B). Cells isolated from colonic lamina propria (LP), spleen and MLN from recipient mice were assayed for expression of FOXP3, RORγt and IL-17A. The loss of PPARγ in CD4+ T donor cells resulted in greater accumulation of IL-17-producing Th17 cells and lower levels of FOXP3+ iTreg cells in spleen, MLN and colonic LP of recipient mice (Fig. 3C and D).

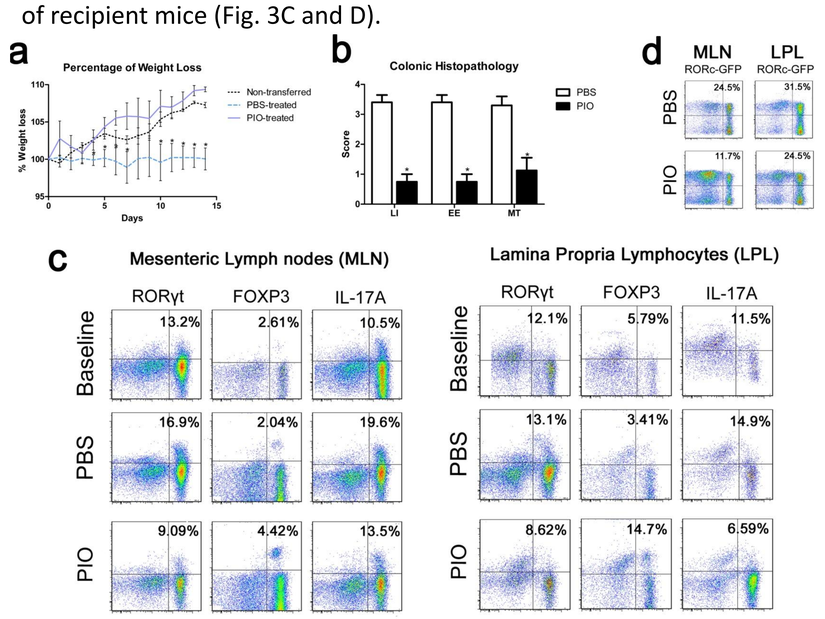

Figure 4. PPARγ activation modulates the plasticity between Th17 and iTreg cells *in vivo*

Based on the second prediction of our model, 4 x 10^5 viable sorted naïve T cells from donor RORc-GFP reporter mice spleens were adoptively transferred to RAG2$^{-/-}$ recipients. When clinical signs of colitis appeared, intracellular staining from a set of mice showed presence of FOXP3, IL-17A and RORγt. After verifying presence of Th17 CD4+ T cells, daily treatment with 70 mg/Kg of pioglitazone was performed via orogastric gavage to activate PPARγ. During the treatment period, disease activity scores significantly improved in pioglitazone-treated mice (Fig.4A). Histopathological examinations also showed how colons recovered from recipient mice treated with pioglitazone had a significantly lower lymphocytic infiltration and crypt hyperplasia than those recovered from non-treated recipients (Fig.4B). Untreated mice had a predominant Th17 phenotype characterized by increased levels of CD4+ T cells expressing RORγt and IL-17A. In contrast, pioglitazone-treated mice showed a predominance of an iTreg phenotype characterized by increased expression of FOXP3 and decreased IL17-A and RORγt in CD4+ T cells of the colon, MLN and spleen (Fig 4C and D). This data fully supports the *in silico* that activation of PPARγ in Th17 cells favors differentiation into iTreg cells.

Conclusion and Funding

- The plastic phenotype Th17 differentiates into Treg after PPARγ activation at the gastrointestinal mucosa.
- This phenotype switch mediated by PPARγ is associated with protection from CD4+ T cell-induced colitis in mouse adoptive transfer experiments.
- Computational and mathematical modeling on the CD4+ T cell differentiation process helps to better understand underlying mechanisms and unseen trends.

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