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Metabolic regulation by PPAR γ is required for IL-33-mediated activation of ILC2s in lung and adipose tissue

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SUMMARY

ILC2s are critical in the immune response to helminths, the development of allergies and homeostasis of adipose tissue. This study demonstrates that the metabolic sensor PPAR γ is central for ILC2s activation, proliferation and function in lung during airway inflammation as well as in adipose tissue.

ABSTRACT

Type 2 innate lymphoid cells (ILC2s) play a critical role early in the response to infection by helminths and in the development of allergic reactions. ILC2s are also involved in the physiologic regulation of adipose tissue and its metabolic response to cold shock. We find that the metabolic sensor PPAR γ is highly expressed in ILC2s of the lung and adipose tissue and increases responsiveness to IL-33. In turn, activation of ILC2 by IL-33 leads to increased expression of PPAR γ , a prerequisite for proliferation and expression of the effector cytokines IL-5 and IL-13. In contrast, pharmacological inhibition of PPAR γ leads to decreased fatty acid uptake, a necessary source of energy for ILC2s. As a consequence, treatment of mice with a PPAR γ antagonist modulated the development of an ILC2-dependent acute airway inflammation. Together, our results demonstrate the critical role of the metabolic sensor PPAR γ for ILC2s functions.

INTRODUCTION

Peroxisome proliferator-activated receptor gamma (PPAR γ), a ligand-activated transcription factor and metabolic “sensor”, regulates the expression of genes involved in the storage of fatty acids and the metabolism of glucose. PPAR γ is required for the differentiation of adipocytes, and its pharmacological inhibition or absence in mutant mice leads to a loss in functional adipocytes, failure to generate adipose tissue even in response to high fat diet, and hyposensitivity to insulin (Gilardi et al., 2019). PPAR γ is also involved in the regulation of immunity towards type 2 responses. In myeloid cells, it promotes the differentiation of type 2 macrophages (Bouhrel et al., 2007) and the capacity of dendritic cells (DCs) to induce the generation of Th2 cells (Nobs et al., 2017). In lymphoid cells, PPAR γ is critical for the generation of Th2 cells in the context of lung allergic inflammation and intestinal helminth infection (Chen et al., 2017), whereas it inhibits the generation of Th17 cells (Klotz et al., 2009). PPAR γ also promotes the accumulation of type 2 (Gata3⁺) regulatory T cells (Tregs) in adipose tissues where they regulate insulin sensitivity (Cipolletta et al., 2012).

Innate lymphoid cells (ILCs) are the innate counterpart of T cells and mirror the effector cytokine production of Th1, Th2 and Th17 cells (Eberl et al., 2015). Contrary to the time-consuming clonal selection and expansion process that T cells undergo upon antigen recognition, ILCs respond promptly to inducer cytokines expressed by myeloid or non-hematopoietic cells upon infection or injury. Therefore, ILCs play a unique role early in immunity in the regulation of the unfolding immune response. ILC2s have been first identified in the context of intestinal helminth infection (Moro et al., 2010; Price et al., 2010; Neill et al., 2010), and further shown to play an essential role in acute lung allergic inflammation (Halim et al., 2014; McKenzie et al., 2014). ILC2s are activated by the inducer cytokines interleukin (IL)-25, IL-33 and TSLP that are expressed by stromal, epithelial and endothelial cells in response to mechanical stress, structural injury or chemical cues that report infection by helminths (Eberl et al., 2015; McKenzie et al., 2014). ILC2s express the effector cytokines IL-5 and IL-13, which induce the recruitment of eosinophils and the production of mucus by epithelial cells, and prime DCs for the induction of Th2 cells. ILC2s are also involved in the IL-33-mediated response of adipose tissue to cold shock (Lee et al., 2014).

Even though ILC2s, like PPAR γ at the molecular level, play a prominent role in the development of type 2 immune responses, a role of PPAR γ in the function of ILC2s has only recently been reported through its role in the acquisition of fatty acids (Karagiannis et al., 2020). Here, we demonstrate that PPAR γ is highly expressed in ILC2s, and is required for their proliferation and effector functions through the control of energy metabolism and the

establishment of a positive feedback loop with the sensing of IL-33. As a consequence, pharmacological inhibition of PPAR γ blocks the development of an ILC2-dependent acute lung inflammation. Our data reinforce the notion that PPAR γ is a metabolic sensor of fatty acids that promotes type 2 immunity both at the innate and the adaptive levels.

RESULTS

ILC2s express high levels of PPAR γ in lung and adipose tissue

Among ILCs, ILC2s are best characterized by the expression of high levels of the transcription factor Gata3. Therefore, to facilitate the identification of ILC2s, we used heterozygous reporter mice that express EGFP under control of the *Gata3* gene locus (Fig. S1A). At the steady state, ILC2s in the lung and (visceral, VAT and subcutaneous, SC) adipose tissues expressed the highest levels of PPAR γ , as compared to ILC3s, B and T cells, and ILC2s in secondary lymphoid tissues (Fig. 1A and 1B). The expression of PPAR γ was further increased in lung and VAT ILC2s and ILC3s after intraperitoneal administration of recombinant IL-33 (Fig. 1C and 1D). Of note, transcripts for PPAR γ , but not for PPAR α or PPAR β , were increased following IL-33 treatment (Fig. S1B) and ILC3s frequency and number was not affected (Fig S1E). Together, these data show that the expression level of PPAR γ is highest in ILC2s as compared to other subsets of lymphoid cells, and further increases upon activation.

PPAR γ is required for the generation and expansion of tissue-resident ILC2s

In order to assess whether PPAR γ is involved in the generation of tissue-resident ILC2s, we examined the presence of ILC2s in lungs and adipose tissues of mice treated with the PPAR γ antagonist GW9662, as well as in PPAR γ -deficient mice (which also lack adipose tissues). The number and frequency of ILC2s was significantly reduced in mice treated with GW9662, as well as in PPAR γ -deficient mice, at the steady state (Fig. 1E and S1C). Furthermore, pharmacological inhibition of PPAR γ with GW9662 blocked the IL-33-induced increase in ILC2s in lungs and adipose tissues. Similarly, the IL-33-induced increase in lung ILC2s was dramatically reduced in PPAR γ -deficient mice (Fig. 1F, right panel). In contrast, rosiglitazone, a highly selective agonist for PPAR γ (Fig. S1B) potentiated the increase in ILC2 numbers (Fig. 1F and S1D). These data show that PPAR γ is required for the establishment of normal populations of ILC2s in lung and adipose tissues, as well as for their expansion in response to IL-33.

PPAR γ is required for ILC2s effector functions

We next assessed whether PPAR γ was required for the expression of effector cytokines by ILC2s. The IL-33-induced expression of IL-5 and IL-13 by ILC2s was significantly inhibited in mice treated with GW9662 and in PPAR γ -deficient mice (Fig. 2A and 2B, S3A and S3B).

Interestingly, the frequency of lung ILC2s expressing ST2, a component of the receptor for

IL-33, correlated with the mean expression level of PPAR γ at the steady state and after IL-33 challenge (Fig. 2C). The expression level of PPAR γ also correlated with the frequency of ILC2s expressing IL-5 and IL-13 (Fig. 2D). Together, these correlations suggest a positive feedback loop between IL-33- and PPAR γ -activated pathways. In accordance with this hypothesis, chromatin immunoprecipitation and sequencing (ChIP-Seq) data, available on GEO (GSE115505) and EMBL-EBI (E-MTAB-7255) servers, show the presence of PPAR γ binding sites in promoter and intronic regions of the *Ilr1l* gene coding for ST2, in macrophages and in T helper cells polarized by IL-4 (S2A and B). Furthermore, treatment with GW9662 lead to a decrease in the expression of ST2 (Fig. 2E), as well as in Gata3 (Fig. 2F), indicating that PPAR γ regulates at least two key factors involved in the generation and function of ILC2s. In order to assess whether PPAR γ controls the function of ILC2s in a cell-intrinsic manner, ILC2s were isolated from the lung of Gata3 reporter mice and cultured in the presence of IL-33 and GW9662. In agreement with a cell-intrinsic regulation of ILC2s by PPAR γ , GW9662 blocked IL-33-induced expression and production of IL-5 and IL-13 (Fig. 2G and 2H) whereas rosiglitazone potentiated the effect of IL-33 (Fig. S3C). Moreover, GW9662 prevented the IL-33 induced ILC2s proliferation while rosiglitazone synergized with IL-33, thus confirming the key role played by PPAR γ in proliferation of ILC2s, (Fig. 3A and 3B).

ILC2s may generate eicosanoid ligands of PPAR γ

The inhibition of effector functions and proliferation by a PPAR γ antagonist, in cultures of FACS-sorted ILC2s, suggests that these cells produce PPAR γ ligands. According to the Immgen database (www.immgen.org), genes involved in the production of eicosanoid ligands of PPAR γ , such as prostaglandin-endoperoxide synthase 2 (or cyclooxygenase 2, Ptg2) and arachidonate 5 lipoxygenase (Alox5), were expressed at higher levels in ILC2s as compared to the other ILC subsets (Fig. 3C). We therefore investigated the functional relevance of the expression of these enzymes in ILC2s. To this end, ILC2s were cultured in the presence of IL-33, as well as of the cyclooxygenase inhibitor, diclofenac, or an inhibitor of 5-lipoxygenase activating protein, Bay-X-1005. Both inhibitors blocked IL-33-induced proliferation of ILC2s as well as IL-5 and IL-13 production, indicating that ILC2s may produce their own eicosanoid PPAR γ ligands (Fig. 3D and 3E). Interestingly, *in vivo* treatment with rosiglitazone synergized with IL-33 to potentiate ILC2 proliferation (Fig. 1F, left panel and S1D) and cytokines production (Fig. S3C).

PPAR γ controls the energy metabolism of ILC2s

Fatty acids are required by ILC2s for the expression of effector cytokines in the context of helminth infection and lung allergy (Wilhelm et al., 2016; Karagiannis et al., 2020). As PPAR γ regulates the uptake and storage of fatty acids (Lee et al., 2018), it appears thereby to control the effector functions of ILC2s (Karagiannis et al., 2020). In accordance with this view, ILC2s from mice treated with IL-33 and GW9662 (Fig. 3F and S4A), or deficient in PPAR γ (Fig. 3G), showed significantly lower levels of fatty acids uptake as compared to ILC2s carrying normal PPAR γ activity. This deficit in fatty acid uptake correlated with a reduced expression by ILC2s of the fatty acid transporter CD36 in mice treated with GW9662 (Fig. 3H). Finally, inhibition or absence of PPAR γ also reduced the levels of glucose uptake (Fig. 3I and 3J, S4B), a likely consequence of glucose transporters (GLUTs) expression modulation by PPAR γ (Liao et al., 2007). Further confirming the role of fatty acid uptake in ILC2s expansion, CD36 inhibition by Sulfosuccinimidyl Oleate (SSO), partly blocked IL-33 induced expansion of ILC2s in lung and VAT (Fig 4A and Fig S5A) and their cytokine production (Fig 4B). Interestingly, pharmacological blockade of CD36 resulted also in decreased PPAR γ and CD36 expression in ILC2 cells (Fig 4C and 4D), while had no effect in T and B cells from lung and VAT (Fig S5B and S5C) suggesting that PPAR γ ligand production and fatty acid uptake by CD36 form a positive feedback loop restricted to ILC2 subset.

Prevention of acute allergy by pharmacological inhibition of PPAR γ

As PPAR γ controls the expansion and effector functions of ILC2s, we assessed whether pharmacological inhibition of PPAR γ was effective in the prevention of acute airway inflammation induced by papain, shown previously to depend on ILC2s (Verhoef et al., 2016) (Fig. S6A). In this context, PPAR γ expression was increased in ILC2s and to a lesser degree in ILC3s, but not in B and T cells (Fig. 5A). In Gata3 reporter mice treated with both papain and GW9662, the frequencies of ILC2s and their production of IL-5 and IL-13 were reduced in the lung, as expected (Fig. 5B and 5C, S6B). PPAR γ expression correlated with the frequency of ST2⁺ ILC2 in the lung of papain challenged mice (Fig. 5D). Measurements of lipid and glucose uptakes in ILC2s indicated that lung inflammation induced by papain drives metabolic changes similar to that observed with IL-33 challenge. Papain treatment induced lipid and glucose uptake was reverted by GW9662 treatment (Fig. 5E and 5F). As a consequence, the number of eosinophils, the main mediators of the pro-allergic airway response that are recruited by IL-5, were decreased 10-fold in the lung of GW9662-treated mice (Fig. 5G, S6C). The number of lung macrophages was also reduced, while neutrophils

were not (Fig. 5H and 5I, S6D and S6E). Of note, alveolar macrophages express high levels of PPAR γ and are therefore likely to be directly affected by GW9662 (Ginhoux, 2014; Schneider et al., 2014). A decrease in IL-5 and IL-13 secretion was observed in the BAL fluid from mice treated with GW9662 after papain challenge (Fig. S6F).

Altogether, our results demonstrate that PPAR γ is a critical regulator of ILC2 activity through the control of Gata3 and ST2 expression levels, as well as of CD36 and fatty acid

uptake. Therefore, PPAR γ may be targeted to modulate the activity of ILC2s during allergic inflammation and thereby preventing or reducing the severity of allergy.

DISCUSSION

Our data demonstrate that PPAR γ directly controls the effector functions of ILC2s. Among ILCs, ILC2s expressed the highest level of PPAR γ , which was further increased upon activation by the type 2 inducer cytokine IL-33. Inhibition of PPAR γ , or its absence in mutant mice, lead to decreased numbers of ILC2s in tissues, decreased expression of Gata3 (a critical transcription factor for the differentiation and function of ILC2s), decreased expression of IL-33 receptor and a consequent decrease in the expression of the effector cytokines IL-5 and IL-13. This effect of PPAR γ on ILC2s was cell intrinsic as it was recapitulated in cultures of isolated ILC2s, presumably because ILC2s could produce eicosanoids ligands for PPAR γ . Finally, pharmacological inhibition of PPAR γ modulated the acute allergic airway inflammation induced by papain, an inflammation that is dependent on the activation of ILC2s (Halim et al., 2014; McKenzie et al., 2014).

These data are in agreement with earlier results showing that PPAR γ is involved in different elements of type 2 immunity. In particular, PPAR γ is required for the generation and effector functions of Th2 cells in the context of lung allergy and helminth infection by *Heligmosomoides polygyrus* (Chen et al., 2017). PPAR γ is also required for the accumulation of type 2 Tregs in VAT, which contribute to maintain VAT insulin sensitivity (Cipolletta et al., 2012), as well as in the generation and maintenance of type 2 macrophages (M2) while antagonizing type 1 macrophages (M1) (for a review on the role of PPAR γ in macrophage polarization see Olefsky and Glass, 2010). In contrast, the activation of PPAR γ inhibits the generation of Th17 cells (Klotz et al., 2009), in accordance with a broad role for PPAR γ in the promotion of type 2 immune responses and inhibition of competing responses.

We have observed that the frequency of ILC2s expressing ST2, as well as the frequency of ILC2s expressing IL-5 and IL-13, were correlated with the mean expression level of PPAR γ . In addition, the expression level of Gata3 was decreased in the absence of PPAR γ or in the presence of a PPAR γ inhibitor. Together, this indicates that PPAR γ , a transcription factor, is directly or indirectly involved in the regulation of the genes coding for ST2 and Gata3. In support of this view, CHIP-seq data obtained with polarized M2 macrophages and Th2 cells show that PPAR γ binds to promoter and intronic regions of *Il1rl1* (coding for ST2) (Daniel et al., 2018; Henriksson et al., 2019) (Fig.S2). PPAR γ also regulates gene

expression by affecting the epigenetic code and local chromatin accessibility. In the process of macrophage polarization by IL-4, PPAR γ facilitates the expression of a network of genes involved in extracellular matrix remodeling through recruitment of the coactivator P300 and the DNA repair protein RAD21 (Daniel et al., 2018). It will therefore be interesting to investigate whether such mechanisms operate in the regulation of ILC2 differentiation and activity by PPAR γ .

We observed that the treatment of isolated ILC2s with a PPAR γ antagonist affected their proliferation and function, suggesting that ILC2s produce PPAR γ ligands, such as eicosanoids. Interestingly, according to the Immgen database, ILC2s express several genes coding for enzymes involved in the manufacture of eicosanoids, such as *Ptgs2* and *Alox5*. We found that inhibition of these enzymes decreased the activation of ILC2s, suggesting that PPAR γ , in ILC2s, is activated in a cell-intrinsic fashion. This finding sheds new light on the potential mechanisms involved in the efficacy of leukotriene neutralization for the treatment of asthma (Claesson and Dahlén, 1999). It will be interesting to assess whether the expression and activity of these enzymes is induced by IL-33, and to isolate PPAR γ ligands produced by ILC2s. Nevertheless, it is also possible that the activity of PPAR γ in ILC2s is induced by pairing with the nuclear receptor RXR, and therefore is ligand-independent yet sensitive to PPAR γ antagonists (Daniel et al., 2018).

A common trait of ILC2s, Th2, VAT Tregs and type 2 macrophages is their reliance on fatty acid oxidation as a source of energy (Pelgrom and Everts, 2017). To this end, uptake of triacylglycerol *via* CD36, and the subsequent elevation in oxidative phosphorylation, are critical steps in the generation of M2 macrophages (Huang et al., 2014). Similarly, we find that ILC2s activated by IL-33 upregulate their expression of CD36 and increase their storage of fatty acids. As reported earlier in macrophages and hepatocytes, our data demonstrate that PPAR γ is required for CD36 expression and fatty acid storage, which also leads to increased cellular levels of glucose. The storage of lipids into cytoplasmic droplets, a PPAR γ -dependent mechanism, has recently been shown to be necessary for proper function of ILC2s. However, the role of these lipid droplets remains to be elucidated (Karagiannis et al., 2020). It remains also to be understood whether fatty acid uptake and oxidative phosphorylation are driving a type 2 immune program, or whether PPAR γ **orchestrates these metabolic and immune changes concomitantly**. Therefore, elucidating the contribution of PPAR γ to ILC2 biology in additional models of ILC2 activation (such as helminth infection or bleomycin-induced lung injury) will be very informative. Further studies are also required to

elucidate how IL-33 increases PPAR γ expression. Our data suggest, but do not demonstrate, that IL-33 directly regulates PPAR γ expression.

In summary, we show here that PPAR γ is a central regulator of ILC2 function through the modulation of proliferation and activation pathways, as well as through regulation of energy metabolism. Thus, PPAR γ is a potential target for the modulation of ILC2 activity in order to mitigate acute allergic reactions, or, on the contrary, to boost ILC2 function in the context of helminth infection. Given the association of PPAR γ with fatty acid storage and energy metabolism, our results also support the notion that management of lipid intake, through tailored diets for example, is a potential avenue to modulate ILC2 activity in particular, and type 2 immunity in general.

Materials and Methods

Mice

C57BL/6 mice were purchased from Charles River and exposed to the environment of the local animal facility for at Institut Pasteur for at least for 2 weeks before mating. Gata3-GFP reporter mice were generated by Meinrad Busslinger (Grote et al., 2006), bred at Institut Pasteur, and used in all experiments as heterozygotes. PPAR γ -deficient mice were generated by breeding Sox-2Cre and PPAR γ -floxed mice as described in (Nadra et al., 2010). Experiments were performed on 8-9 weeks old animals of both genders. All animal experiments were approved by the committee on animal experimentation of the Institut Pasteur and by the French Ministry of Research.

In vivo treatments

Mice were treated with IL-33 injected into the peritoneum (10 μ g/kg/d) and/or GW9662 (10 mg/kg/d) and/or Rosiglitazone (10 mg/kg/d) and/or **Sulfosuccinimidyl Oleate (SSO)** (10 mg/kg/d) for one week (single treatment) or 2 weeks (double treatment).

For the experimental model of acute airway inflammation, mice were exposed, under anesthesia, to intranasal inhalation of papain every 3 days, (150 μ g in PBS per mouse, Wako Pure Chemical, >0.5 units/g) by placing a drop in the nasal cavity of the mouse, for a total of five challenges, and then treated with GW9662 injected into the peritoneum (10 mg/kg/d) for one more week.

Flow cytometry

Lungs and adipose tissues were minced and digested with Liberase-TM and Liberase-TL respectively (Roche) and DNase (Roche) for 1h at 37 °C. After filtration through a nylon mesh (40 μ m cell strainer; Greiner), single cells were collected. Cells were stained for 10 min at 4°C with LIVE/DEAD fixable blue dead stain kit (Invitrogen). Cells were further incubated with anti-mouse CD16/CD32 mAb (93; eBioscience) for FcR blocking for 15 min on ice and stained for 20 min with antibodies to surface markers. For intracellular staining, cells were fixed and permeabilized with a commercially available fixation/permeabilization kit (eBioscience). Intracellular staining was performed with antibodies specific for Gata3 (FITC), ROR γ t (PE) or PPAR γ (APC) (validated in PPAR γ KO mice) or for IL-5 (BV421) or IL-13 (PE). Briefly, cells were stimulated or not with 50 ng/ml PMA and 500 ng/ml ionomycin for

4h in RPMI 1640 (Invitrogen) containing 10% FCS, 25 mM HEPES, 0.05 mM 2-mercaptoethanol and 100 U/ml of penicillin and streptomycin. For measurements of glucose and fatty acid uptake, cells were washed in PBS and incubated in 100 μ M of 2-(n-(7-nitrobenzo-2-oxa-1,3-diazol-4-yl)amino)-2-deoxyglucose (2-NBDG; Invitrogen) or in 10 μ M of BODIPY™ FL C16 probe for 20 min at 37°C. Cells were then washed with cold PBS and stained with surface markers. All stainings were analyzed on an LSR Fortessa flow cytometer (Becton Dickinson) and data analyzed using FlowJo v10.6 (Tree Star, Inc).

In vitro ILC2 culture assays

ILC2s were sorted from Gata3 reporter mice (using a BD FACS ARIA III) using a gate on live Gata3-GFP⁺ CD45⁺CD19⁻CD3⁻CD127⁺ST2⁺ cells and directly plated into 96 well plate at a density of 5000 cells per well. Cultures were maintained for 72h in complete RPMI 1640 (Invitrogen) containing 10% FCS, 25 mM HEPES, 0.05 mM 2-mercaptoethanol, 100 U/ml penicillin and streptomycin and 10ng/ml of rIL-2 and rIL-7 (R&D system). The following molecules were then added in the different experiments: rIL-33 at 10ng/ml (Biolegend); PPAR γ antagonist GW9662 (10 μ M); Cox inhibitor, Diclofenac at 1.5 μ M; Lox inhibitor, Bay-X-1005 at 100 nM, and PPAR γ agonist Rosiglitazone (ROSI) at 10 μ M. For proliferation assays, sorted ILC2 cells were first stained with Violet proliferation dye (VPD) (BD Bioscience, 562158) following manufacturer instructions and seeded into 96 well plate.

ELISA

Cytokines (IL-5 and IL-13) were measured by ELISAs according the manufacturers' recommendations (R&D System).

Quantitative PCR

Sorted ILC2s were immediately stored in Lysing solution from Single Cell RNA Purification Kit (NORGEN) followed by RNA isolation. cDNA synthesis and purification were performed using the reverse transcriptase SuperScript IV kit (Invitrogen). Real-time quantitative PCR cDNA was performed using the SybrGreen Mix and primers from Biorad. Ct values were normalized to the mean Ct obtained with the two housekeeping genes *Gapdh*, and *Hprt* in each sample.

Statistical analysis

Graph generation and statistical analysis were performed using GraphPad Prism software (GraphPad Software, San Diego, California, U.S.A.). Each data point represents an individual mouse or *in vitro* replicates. Error bars show S.D. p values were calculated using the tests indicated in figure legends.

AUTHOR CONTRIBUTIONS

G.E. and D.M. designed the study and supervised the project; T.F. and T.A. designed parts of the study and performed most of the experiments with the help of M.F., J.Y.J. and M.B. generated and provided Gata3 reporter mice. All authors were involved in writing the manuscript.

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Figure Legends

Figure 1. **ILC2s express high levels of PPAR γ in lung and adipose tissue and is required for the generation and expansion of tissue-resident ILC2s**

(A) Expression of PPAR γ in ILC2s, ILC3s, total T and B lymphocytes isolated from lung, visceral adipose tissue (VAT). (B) Expression of PPAR γ (mean fluorescence intensity, MFI) in ILC2s, ILC3s, T and B lymphocytes from lung, VAT and subcutaneous (SC) adipose tissue, lymph nodes (LN) and spleen of Gata3 reporter mice. (n=10 mice, in 3 independent experiments). (C) PPAR γ expression levels in lung ILC2s from Gata3 reporter mice injected into the peritoneum with IL-33 or PBS and PPAR γ -deficient mice. (D) PPAR γ MFI in ILC2s, ILC3s, T and B lymphocytes from lung and VAT of Gata3 reporter mice injected into the peritoneum with IL-33 or PBS. (E) Frequency of ILC2s in CD45⁺ cells in lung, VAT and SC of Gata3 reporter mice treated for 2 weeks with the PPAR γ antagonist GW9662 or PBS, or in the lung of PPAR γ -deficient mice. (F) Frequency of ILC2s in CD45⁺ cells in lung, VAT and SC of Gata3 reporter mice treated with IL-33 + vehicle, in combination with the antagonist GW9662, or with the agonist rosiglitazone, or from the lung of PPAR γ -deficient mice treated with IL-33 only. Data are from two or three independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B, F) one-way Analysis of variance ANOVA. (D, E) Mann-Whitney *U* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. ns, not significant.

Figure 2. **PPAR γ is required for ILC2 effector functions**

(A) Frequency of IL-5⁺IL-13⁺, IL-5⁺ and IL-13⁺ ILC2 cells from Gata3 reporter mice treated with PBS, IL-33 or IL-33 and the PPAR γ antagonist GW9662 (data from 3 independent experiments). (B) Frequency of IL-5⁺IL-13⁺ ILC2 cells in PPAR γ -deficient mice treated with PBS or IL-33. (C, D) Correlation between MFI of PPAR γ expression and frequency of ST2⁺ cells (C) or frequency of IL-5⁺IL-13⁺ ILC2 cells (D) from lung and VAT of mice treated with PBS or IL-33. (E, F) MFI of ST2 expression (E) and of Gata3 expression (F) in lung and adipose tissue ILC2s from Gata3 reporter mice treated with PBS, IL-33 or IL-33 and the PPAR γ antagonist GW9662. (G, H) Intracellular cytokine staining for IL-5 and IL-13 in ILC2s (G) sorted from the lungs of Gata3 reporter mice, and (H) production of these cytokines

after 72h of cell culture treated with PBS, IL-33 or IL-33 and GW9662. Data are from one or two independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (A, B, E, F) one-way Analysis of variance ANOVA. (G, H) Mann-Whitney *U* test. Spearman's rank test was used to determine correlations. (C, D).*, $P \leq 0.05$; **, $P \leq 0.01$; ****, $P \leq 0.0001$. ns, not significant.

Figure 3. PPAR γ and eicosanoids are required for ILC2 proliferation and controls the energy metabolism of ILC2s

(A) Proliferation measured by dilution of VPD fluorescence intensity of ILC2s isolated from Gata3 reporter mice and cultured in plain medium (NS), or in the presence of IL-33 or IL-33 and GW9662. Representative overlay FACS plot (left panel) and percentage of number of cell division (D0, D1, D2, D3) (right panel). (B) Proliferation index (PI) measured after 72h of culture (Ratio of VPD MFI for each condition by VPD MFI at day 0). (C) Expression of *Ptgs2* and *Alox5* by lymphoid cells, from the RNA Seq Immgen database. (D, E) Proliferation of (D) and cytokine expression by (E) ILC2s isolated from Gata3 reporter mice and cultured in plain medium (NS), or in the presence of IL-33, or IL-33 and the cyclooxygenase inhibitor Diclofenac, the inhibitor of 5-lipoxygenase activating protein Bay-X-1005, or the PPAR γ agonist rosiglitazone (left panel) and representative cytometry dot plot (right panel). (F, G) Levels of fatty acids uptake, as measured by incorporation of bodipy FLC16, in ILC2s isolated from lung and VAT of Gata3 reporter mice treated with PBS, IL-33 or IL-33 and GW9662 (F) and in ILC2s isolated from the lung of PPAR γ -deficient mice treated with PBS or IL-33 (G). (H) Expression of CD36 in ILC2s from lung and VAT of Gata3 reporter mice treated with IL-33 or IL-33 and GW9662. (I, J) Glucose uptake measured by 2NBDG incorporation, in ILC2s isolated from lung and VAT of Gata3 reporter mice treated with PBS, IL-33 or IL-33 and GW9662 (I) and in ILC2s isolated from the lung of PPAR γ -deficient mice treated with PBS or IL-33 (J). Data are from one or two independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B, D) Mann-Whitney *U* test (F, G, H, I, J) One-way Analysis of variance ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$; ****, $P \leq 0.0001$. ns, not significant.

Figure 4. PPAR γ controls tissue-resident ILC2s expansion and effector functions in lung and adipose tissue through CD36 pathway.

(A) Frequency of ILC2s in CD45⁺ cells in lung and VAT tissues of Gata3 reporter mice treated for 1 weeks with Sulfosuccinimidyl Oleate (SSO) CD36 inhibitor and with or without IL-33. (B) Frequency of IL-5⁺IL-13⁺ ILC2 cells from lung and VAT of Gata3 reporter mice treated with vehicle, IL-33 alone, SSO alone or IL-33+SSO. (C) Expression of PPAR γ (mean fluorescence intensity, MFI) in ILC2s isolated from lung and VAT of Gata3 reporter mice treated with vehicle, IL-33 alone, SSO alone or SSO in combination with IL-33. (D) Expression of CD36 in ILC2s from lung and VAT of Gata3 reporter mice treated with SSO, IL-33 or IL-33 and SSO. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B, F) one- way Analysis of variance ANOVA. (D, E) Mann-Whitney *U* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. ns, not significant.

Figure 5. Modulation of acute allergy by pharmacological inhibition of PPAR γ

(A) PPAR γ expression in ILC2s, ILC3s and total T and B lymphocytes isolated from the lung of Gata3 reporter mice challenged with PBS or papain. (B, C) Frequency of (B) and cytokine expression by (C) ILC2s in the lung of mice treated with PBS, papain, GW9662 or a combination of papain and GW9662. (D) Correlation between PPAR γ expression and frequency of ST2⁺ cells from the lung of Gata3 reporter mice treated with PBS or Papain. (E) Levels of intracellular fatty acids, as measured by incorporation of bodipy FLC16 in lung ILC2s of mice treated with PBS, papain, GW9662 or a combination of papain and GW9662. (F) ILC2 Glucose uptake measured by 2NBDG incorporation in lung ILC2s of mice treated with PBS, papain, GW9662 or a combination of papain and GW9662. (G-I) Absolute numbers of eosinophils, macrophages and neutrophils in the lungs of mice as in B. Representative data are from two or three independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (A) Mann-Whitney *U* test, (B-H) one-way Analysis of variance ANOVA, and (D) Spearman's rank test was used to determine correlations.

*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. ns, not significant.

Supplementary Figures

Figure S1. PPAR γ inhibition or deficiency affects lung and adipose tissue ILC2s generation

(A) Gating strategy for the cytometric analysis of ILC2s in Gata3 reporter mice (lung). **Of note, ILC2s were gated on CD45⁺CD127⁺RORgt⁻Gata3⁺ whereas ILC3s were gated on CD45⁺CD127⁺RORgt⁺Gata3⁻. Percentage of ILCs in CD45⁺ cells.** (B) mRNA levels of PPARs (α , β , γ) expressed genes in sorted lung ILC2 from mice treated for 2 weeks with the PPAR γ antagonist GW9662, agonist rosiglitazone or PBS. (C) Absolute numbers of ILC2s in the lung, VAT and SC of Gata3 reporter mice treated for 2 weeks with the PPAR γ antagonist GW9662 or PBS, or in the lung of PPAR γ -deficient mice. (D) Absolute numbers of ILC2s in the lung, VAT and SC of Gata3 reporter mice treated with PBS, IL-33, IL-33 and GW9662, or IL-33 and rosiglitazone. Representative data are from two or three independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B) Mann-Whitney *U* test (C) one-way Analysis of variance ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.

Figure S2: Analysis of ChiP seq and ATAC seq data on the binding of PPAR γ to the *Il1rl1* gene promoter in polarized macrophages and CD4⁺ T cells.

(A) Data has been downloaded from GEO:GSE115505 (Daniel et al., 2018) and shows binding sites for PPAR γ and P300, as well as accessible DNA regions in the *Il1rl1* gene (coding for ST2), during M2 polarization (determined by sequencing of the chromatin immunoprecipitated with specific antibodies and Tn5 tagged DNA (ATAC-seq)). (B) Data has been downloaded from EMBL-EBI:E-MTAB-7255 (Henriksson et al., 2019) and reports bindings site of PPAR γ in the *Il1rl1* gene as determined by sequencing of the chromatin immunoprecipitated with a FLAGged form of PPAR during Th2 polarization.

Figure S3. PPAR γ inhibition or deficiency affects lung ILC2s effector functions

(A) Gating strategy for the cytometric analysis of ILC2s in Gata3 reporter mice treated with IL-33 or IL-33 and GW9662, and in PPAR γ -deficient mice. (B) Gating strategy and frequency of IL-5⁺IL-13⁺ ILC2 cells in PPAR γ -deficient mice. (C) Frequency of ILC2s in the lung and VAT of Gata3 reporter mice treated with PBS, IL-33, IL-33 and GW9662, or IL-33 and rosiglitazone. Representative data are from one or two independent experiments. Each

data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (C) one-way Analysis of variance ANOVA. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. ns, not significant.

Figure S4. PPAR γ differentially affects the metabolism of ST2⁺ and ST2⁻ ILC2s

(A) Levels of intracellular fatty acids, as measured by incorporation of bodipy FLC16, in ST2⁺ and ST2⁻ ILC2s isolated from lung and VAT of Gata3 reporter mice treated with PBS, IL-33 or IL-33 and GW9662. **(B)** Levels of intracellular glucose uptake, as measured by incorporation of 2NBDG, in ST2⁺ and ST2⁻ ILC2s isolated as in A. Representative data are from one or two independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (A, B) Mann-Whitney *U* test; *, $P \leq 0.05$; **, $P \leq 0.01$, ns, not significant.

Figure S5. PPAR γ controls tissue-resident ILC2s expansion and effector functions in lung and adipose tissue through CD36 pathway.

(A) Absolute numbers of ILC2s in lung and VAT tissues of Gata3 reporter mice treated for 1 weeks with Sulfosuccinimidyl Oleate (SSO) CD36 inhibitor and with or without IL-33. **(B)** Expression of PPAR γ (mean fluorescence intensity, MFI) in Lung T cells (LT) and B cells (LB) isolated from Gata3 reporter mice treated with vehicle, IL-33 alone, SSO alone or SSO in combination with IL-33. **(C)** Expression of PPAR γ (MFI) in VAT LT and LB isolated from Gata3 reporter mice treated with vehicle, IL-33 alone, SSO alone or SSO in combination with IL-33. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B, F) one- way Analysis of variance ANOVA. (D, E) Mann-Whitney *U* test. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$. ns, not significant.

Figure S6. Modulation of acute allergy by pharmacological inhibition of PPAR γ

(A) Gating strategy for the cytometric analysis of Eosinophils, Macrophages and Neutrophils from lung Gata3 reporter mice. **(B-E)** Absolute numbers of ILC2s (B) and frequencies of eosinophils (C), macrophages (D) and neutrophils (E) in the lung of mice treated with PBS, papain, GW9662 or a combination of papain and GW9662. **(F)** Cytokines analysis (IL-5 and IL-13) in the BAL fluid from mice treated with PBS, papain, GW9662 or a combination of papain and GW9662. Representative data are from one or two independent experiments. Each data point represents an individual mouse. Data represent mean (\pm SD). Statistical analysis: (B-D) one-way Analysis of variance ANOVA.; *, $P \leq 0.05$; **, $P \leq 0.01$.