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COMMENT

The devil's in the detail: cell-specific role of PPARγ in ILC2 activation by IL-33

Henry J. McSorley[1] and J. Simon C. Arthur[1]

PPARγ is a critical transcriptional regulator of adipogenesis and type 2 immune responses, however until recently its role in type 2 innate lymphoid cells had not been characterised. In two papers in this issue of Mucosal Immunology, PPARγ is shown to have a dominant role in ILC2 responses, mediating IL-33-responsiveness and activation.

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Type 2 innate lymphoid cells (ILC2) are innate counterparts to their adaptive cousins, Th2 cells. Like Th2 cells, ILC2 have roles in allergic inflammation, immunity to helminths, and the regulation of metabolism. Unlike Th2 cells, ILC2 lack antigen receptors, and are instead activated by epithelial cytokines such as IL-25, TSLP, and, perhaps most importantly, IL-33.[1] IL-33-mediated ILC2 activation in allergic asthma and metabolic disease has been an increasing focus of recent research. How ILC2 responses are controlled, and how these responses could be modulated, are likewise an important research question.

PPARγ is a sensor of lipid derivatives, and a critical transcriptional regulator of adipogenesis and type 2 immune responses. Over the past 30 years, its role in transcriptional regulation of adipocytes, epithelial cells, macrophages, dendritic cells, and Th2 cells has become understood. The role of PPARγ was initially discovered in stromal cells, where it is critical in the differentiation of adipocytes. Although total PPARγ-deficiency is embryonic lethal, a conditional total knockout was recently generated, which entirely lacks adipose tissue and suffers from severe metabolic dysfunction[2] supporting the essential role for PPARγ in adipogenesis. The first immune cell in which a role for PPARγ was identified was the macrophage, where PPARγ was found to be necessary for M2 macrophage polarisation. In macrophage-specific PPARγ knockouts, macrophages show defective M2 polarisation, and in contrast to global PPARγ-knockouts, develop increased adipose tissue and insulin resistance.[3] Comparatively, T-cell-specific PPARγ knockouts exhibit severely limited type 2 immune responses, while adiposity is decreased.[4,5] These studies highlight the range of cell-specific effects of this ligand-activated transcription factor, and the potential issues with modulating its effects.

The reduced allergic responsiveness seen in various cell-specific PPARγ-deficient mice, or with PPARγ antagonism, have led to a series of clinical trials of PPARγ ligands in asthma, however results have been disappointing due to a lack of efficacy and/or adverse events.[6] One potential reason for the lack of efficacy of PPARγ agonists are the complex and opposing functions of this pathway in different cell types.

While PPARγ plays important roles in type 2 immunity, its function in ILC2s had not been studied until recently.[7] Two papers, published in this issue of Mucosal Immunology,[8,9] expand on this initial report and examine the cell-intrinsic roles of PPARγ in ILC2s. Xiao et al.[9] and Fali et al.[8] characterise the role of PPARγ in controlling IL-33-regulated changes in cellular metabolism, proliferative capacity and cytokine production in ILC2s derived from the lung and adipose tissue.

These papers show that PPARγ is highly expressed in both murine and human ILC2, and that the expression of PPARγ (but not the related PPARα or PPARβ proteins) is further increased in ILC2s following IL-33 stimulation. While antagonism or deficiency of PPARγ shows either no[9] or only a small[8] reduction in ILC2 numbers at steady state, both Fali et al. and Xiao et al. showed a strong effect of PPARγ in ILC2 expansion following IL-33 stimulation, implying that the main role of PPARγ in ILC2 was in their activation, rather than their initial differentiation. When PPARγ was inhibited using the GW9662 antagonistic ligand, lung and adipose ILC2s showed reduced proliferation and cytokine production in response to IL-33 stimulation in vitro or in vivo. Conversely, delivery of the PPARγ agonist rosiglitazone, exacerbated ILC2 expansion and activation after IL-33 administration or allergen administration.[8,9] Furthermore, PPARγ-deficient mice had reduced airway inflammation in ILC2- and IL-33-dependent papain or Alternaria challenge models.[8,9] These effects are ILC2-intrinsic as reduced responses to IL-33 were seen in purified ILC2s in culture,[8] or following adoptive transfer of PPARγ-deficient ILC2s to ILC2-deficient mice.[9]

PPARγ, however, does not play a role in all ILC2s: Fali et al. showed that, unlike adipose or lung ILC2s, the ILC2s present in secondary lymphoid organs do not express high levels of PPARγ, while Xiao et al. showed that unlike the defective response of PPARγ-deficient ST2+ (the IL-33 receptor) ILC2s, KLRG1+ST2− PPARγ-deficient ILC2 expanded as normal. This suggests that the role of PPARγ is more important in the IL-33-responsive lung and adipose tissue-resident "natural" nILC2s compared to IL-25 responsive "inflammatory" iILC2s present in secondary lymphoid organs and the intestine.[10]

PPARγ, on ligand-mediated activation, acts as a transcription factor. Both Fali et al. and Xiao et al. identified PPARγ response elements in the *Il1rl1* promotor (which encodes ST2). Furthermore,

[1]Division of Cell Signalling and Immunology, University of Dundee, Dundee, UK
Correspondence: Henry J. McSorley (HMcsorley001@dundee.ac.uk)

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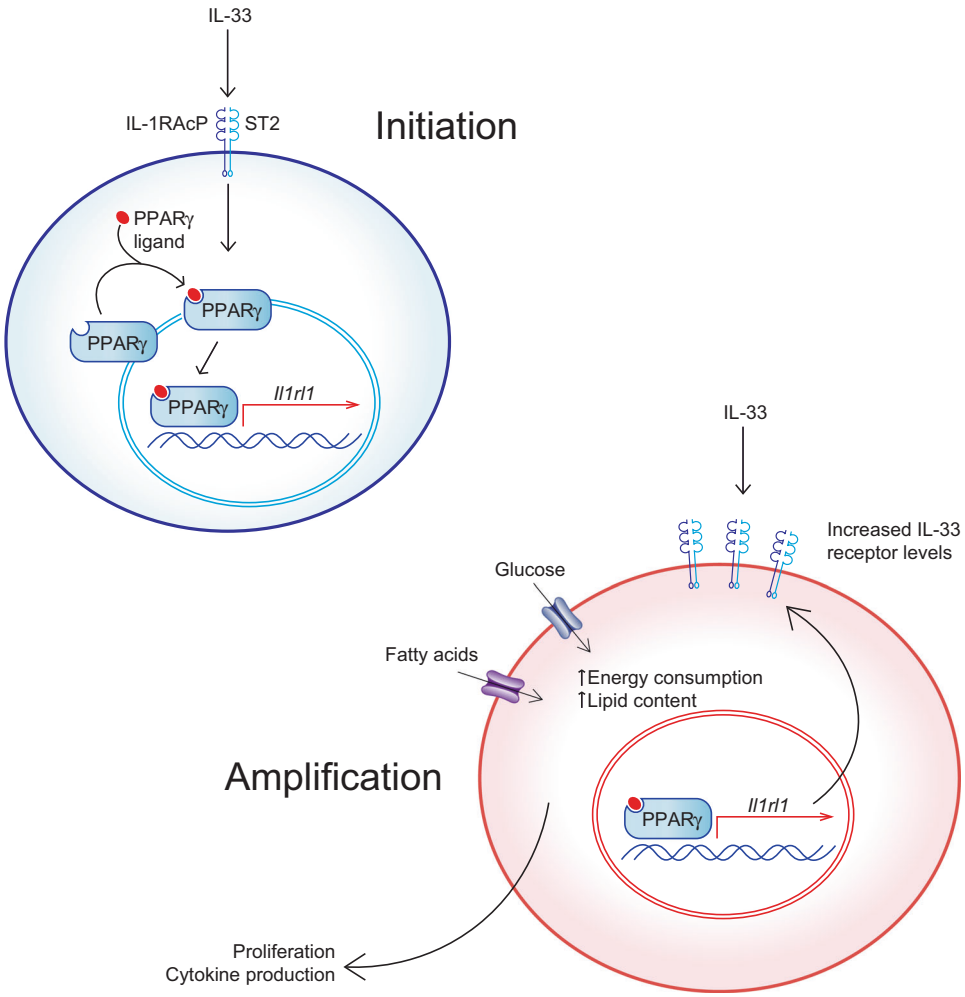

Fig. 1 When ST2+ ILC2 are activated by IL-33, upregulation of PPARγ and ligand-mediated activation of PPARγ results in increased expression of ST2, and glucose and fatty acid transporters (e.g., CD36). These changes increase sensitivity of ILC2s to IL-33, and increase uptake of glucose and fatty acids to fuel ILC2 proliferation and cytokine production.

PPARγ expression positively correlated with ST2 expression on ILC2s, and PPARγ deficiency or PPARγ antagonism reduced ILC2 ST2 expression (similar to the effect seen in Th2 cells[5]). Furthermore, PPARγ agonist treatment increased ILC2 ST2 expression (as shown previously[11]) leading to the conclusion that the effects of PPARγ could be to increase responsiveness of ILC2s to IL-33. In Xiao et al., this was elegantly demonstrated by retroviral transfection of ST2 into PPARγ-deficient ILC2s, resulting in restoration of responses to IL-33[9] (Fig. 1).

As PPARγ is implicated in cellular metabolism, Fali et al. then went on to investigate the effects of PPARγ in ILC2 metabolic changes on activation. They found that PPARγ deficiency or PPARγ antagonism reduced the uptake of fluorescent analogues of both glucose and fatty acids. The reduction in fatty acid uptake coincided with reduced expression of the fatty acid transporter CD36. Blockade of CD36 using sulfosuccinimidyl oleate suppressed ILC2 expansion and cytokine production in response to IL-33, similar to that seen in PPARγ deficiency. As well as mediating fatty acid uptake, CD36 can transport host-derived lipid mediators inside cells, such as eicosanoids (e.g., prostaglandins and leukotrienes). ILC2s express high levels of *Ptgs2* and *Alox5*, which are responsible for the production of prostaglandins and leukotrienes, respectively. When *Ptgs2* and *Alox5* were inhibited pharmacologically, ILC2 activation was decreased, consistent with a model of ILC2 activation where ILC2s intrinsically produce lipid ligands of PPARγ, allowing their activation. It is also possible that these eicosanoids act independently of PPARγ via the stimulation of receptors at the cell membrane, as has been shown previously.[1] Future work could investigate this, for example by testing the effects of *Ptgs2* and Alox5 inhibitors on PPARγ-deficient ILC2s, or by blocking leukotriene or prostaglandin cell membrane receptors to assess the direct effects of these lipid mediators on PPARγ.

These studies add to a recent publication showing that ILC2s regulate their metabolism following IL-33 stimulation via PPARγ activation,[7] however further work will be required to uncover the intracellular mechanisms controlling these changes. Proliferating cells need both a source of energy to generate ATP but also phospholipids and amino acids to allow the production of membrane and proteins. IL-33 acts via the Myd88-dependent signalling pathway and in macrophages this has been linked to a switch from oxidative phosphorylation (OxPhos) to glycolysis as a source of ATP, potentially freeing Acetyl-CoA to enter the fatty acid biosynthetic pathway.[12] Interestingly, ILC2 proliferation in vitro was reduced but not abolished in the absence of glucose, implying an additional source of energy.[7] Determining the fate of the fatty acids once taken up would therefore be relevant. Karagiannis et al. showed that in activated ILC2s, internalised fatty acids could be incorporated into phospholipids,[7] however it has also been suggested that fatty acid uptake in ILC2s can lead to an increase in OxPhos.[13] Further studies would be needed to determine the balance between these two options and determine how this is regulated in ILC2s.

An important message from these papers[7–9] is that these changes are specific to IL-33, and to IL-33-responsive ILC2s. Activation of ILC2s by IL-25 or TSLP likewise leads to ILC2 cytokine production, however cannot cause their proliferation or the metabolic changes described above, and IL-25-mediated responses were not dependent on PPARγ.[7,9] This begs the question of how IL-25-activated ILC2s fuel their responses.

Whether these findings on PPARγ ligand-mediated activation of ILC2 can be developed towards treatments for allergic or metabolic disease requires further understanding of the cell-specific pathways controlled by PPARγ. Previous trials of PPARγ ligands have shown a lack of efficacy and/or adverse effects, associated with a poor understanding of the role of PPARγ within multiple cell types. As with many findings in the immune system, the devil may be in the detail.

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ADDITIONAL INFORMATION

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