Turning on ILC2s: Diet control

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Abstract: New research by Fali and colleagues shows that PPARγ is a central metabolic regulator of ILC2 controlling the functional activation of these potent innate immune initiators in lung & adipose tissue.

Commentary: Since their discovery 10 years ago in the context of helminth infection, ILC2s have come a long way. ILC2s were first shown to play a critical role in the response to parasite infection, tissue repair and the induction of allergic inflammation[1,2]. Their action is principally mediated by the early and robust secretion of IL-5 and IL-13. Their function has now been expanded to adipose tissue where they play a central role in the maintenance of adipose tissue homeostasis and the induction of adipose tissue beiging[3]. PPARγ, master regulator of adipocyte differentiation, has recently emerged as a key regulator of type 2 immune cells in allergy and helminth infection[4,5]. In a recent study in *Mucosal Immunology*, Fali et. al establish that PPARγ plays a critical role in the activation of ILC2s both in the lung and adipose tissue, increasing fatty acid and glucose uptake to respond to increase in energy demand upon activation[6].

ILC2s are discreet populations of lymphoid cells, found in relatively higher proportions in the lung and adipose tissue than other sites. Here the authors used a simple experimental design to analyse in mice challenged with IL-33 the role of PPARγ in ILC2 activation using PPARγ antagonists or agonists. They found that ILC2s isolated from the lung and adipose tissue expressed high levels of PPARγ compared to ILC2s from secondary lymphoid organs. The activation of ILC2s, as assessed by proliferation and secretion of IL-5 and IL-13, required PPARγ which confirmed recent findings by Karagiannis and collaborators[5]. In addition, the authors found that IL-33 enhanced the expression of PPARγ in ILC2s, indicating that PPARγ was part of a positive feedback loop reinforcing ILC2 activation (Figure 1). Another recent report showed that PPARγ promotes the expression of PD-1, which is important for sustained production of IL-5 and IL-13 by ILC2s[7]. Strikingly, the insulin-sensitizing drug Rosiglitazone, a selective PPARγ agonist, potentiated the effect of IL-33 on ILC2s increasing their number and frequency in both adipose tissue and lung. PPARγ is also required for the accumulation of type 2 Treg in adipose tissue and Rosiglitazone promotes Treg function in adipose tissue, contributing to the positive effect of Rosiglitazone on insulin sensitivity[8]. It may well be that PPARγ in ILC2s also contributes to the beneficial effect of rosiglitazone on insulin sensitivity.

PPARγ is thus emerging as a universal regulator of adipose tissue resident type 2 immune cells where it exerts a beneficial action on glucose metabolism.

PPARγ is activated by a number of agents including fatty acids (such as arachidonic acid and its metabolites) and eicosanoids. This raised the question of the origin of the PPARγ ligands driving the activation of ILC2s. Interestingly, when ILC2s were exposed to IL-33 *in vitro*, their activation as assessed by IL-5 and IL-13 secretion was dependent on PPARγ, indicating that PPARγ ligands may be produced by ILC2 themselves. ILC2s do express *Ptgs2* and *Alox5*, genes involved in the production of eicosanoid ligands of PPARγ. In support of a role for these ligands, ILC2s cultured with IL-33 *in vitro* in presence of the cyclooxygenase inhibitor, diclofenac or an inhibitor of 5-lipoxygenase activating protein, Bay-X-1005 inhibited ILC2 activation.

The nuclear receptor PPARγ is a key regulator of adipocyte differentiation, inducing the expression of genes involved in lipid and glucose uptake as well as lipid storage[9]. Importantly, ILC2s were shown to require fatty acids to produce effector cytokines in the context of helminth infection and lung allergy[10]. Here, the authors analysed *in vitro* the uptake of fatty acids and glucose using fluorescently labelled FL-C16 and 2NBDG by purified ILC2s stimulated with IL-33. They found that pharmacological inhibition of PPARγ or genetic deletion of PPARγ led to defective uptake of fatty acids and glucose by ILC2. These findings thus indicate that PPARγ allows ILC2s to adapt to higher energy requirements for activation by increasing nutrient uptake, confirming the recent report from Karagiannis et al. demonstrating the importance of PPARγ for the uptake of fatty acids[5]. CD36 is a major fatty acid transporter and is required for the uptake of fatty acids by macrophages for example. Fali *et al.* showed that pharmacological inhibition of CD36 limited the expansion of ILC2s in response to IL-33 and prevented the induction of IL-5 and IL-13 expression. Strikingly, the induction of PPARγ was blunted as was the induction of CD36, indicating that CD36, uptake of fatty acids and PPARγ were all part of the same amplification loop priming ILC2s for action (Figure 1). Such a feedback loop is reminiscent of that reported two decades ago for macrophages in the context of IL-4 activation[11].

What are the physiologic consequences of PPARγ inhibition in ILC2s? ILC2s are key drivers of allergic airway inflammation. Fali and collaborators found that papain induced lung inflammation was prevented by pharmacological inhibition of PPARγ. Papain led to increased PPARγ expression by, and lipid content in, ILC2s but this was blocked by the use of a PPARγ inhibitor. Glucose uptake was also increased upon papain challenge but a PPARγ antagonist decreased glucose uptake. These results are in agreement with the recent findings of Karagiannis et al., showing that PPARγ regulated the uptake of fatty acids by lung ILC2s upon exposure to papain[5].

While it has become clear that fatty acids represent an important energy source for type 2 immune cells, in particular in tissues were availability of glucose may be low, how these lipids are used remains unclear. In the context of helminth infection, fatty acid oxidation is critical to support ILC2 energy requirements and function[10]. Karagiannis et al. showed that

during allergic airway inflammation, ILC2s transiently form lipid droplets, a phenomenon also regulated by PPARγ[5]. There are two main reasons why immune cells form lipid droplets: to prevent lipotoxicity or to maintain a pool of triglycerides to support energy needs in times of nutrient restriction. However, there are currently no evidence that ILC2s are capable of lipolysis of triglycerides as shown during macrophage alternative activation[12]. Direct usage of fatty acids and transient storage in lipid droplets may not be exclusive processes, it is entirely possible that ILC2 may rely more on one or the other depending on the context and availability of nutrients. B1a B cells also form lipid droplets and uptake exogenous lipids; such cells are reliant on autophagy for their metabolic homeostasis[13]. It will be interesting for future studies to address whether ILC2s are also dependent on autophagy for their metabolic adaptability.

Lipid droplet formation in ILC2s was found to be dependent on glucose and activation of the mTOR pathway during airway allergic inflammation[5]. Glucose levels are kept low in the airway epithelium[14]. This is important to limit bacterial infections but may drive the hyporesponsiveness of alveolar macrophages to IL-4[15] suggesting that low glucose in the lung epithelium may also keep ILC2s in check. Interestingly, a study of patients with chronic obstructive pulmonary disease, showed glucose levels are increased in the airway[16]. Is the dependency on glucose a feature of pathological activation of lung ILC2s during allergic airway inflammation? Is glucose also required for ILC2 function in adipose tissue to maintain healthy adipose tissue? Answers to these questions may help us understand what happens in obesity. Indeed, obesity is an important risk factor for asthma and ILC2s contribute to worsened allergic inflammation in obese mice[17,18]. Reliance on different energy sources in the lung versus the adipose tissue may be key to understand why ILC2s are lost in obesity but overactivated in the lungs.

In conclusion, observations by Fali *et al.* elegantly demonstrate how PPARγ couples functional activation with metabolic priming, enabling ILC2s to increase fatty acid and glucose uptake in the lung and adipose tissue. In ILC2-dependent acute allergic airway inflammation, pharmacological inhibition of PPARγ reduced nutrient uptake and the severity of lung inflammation. Future work will elucidate how local nutrient availability in the tissues regulate the function of ILC2s and how this is altered in different disease status such as obesity or asthma.

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Author contributions

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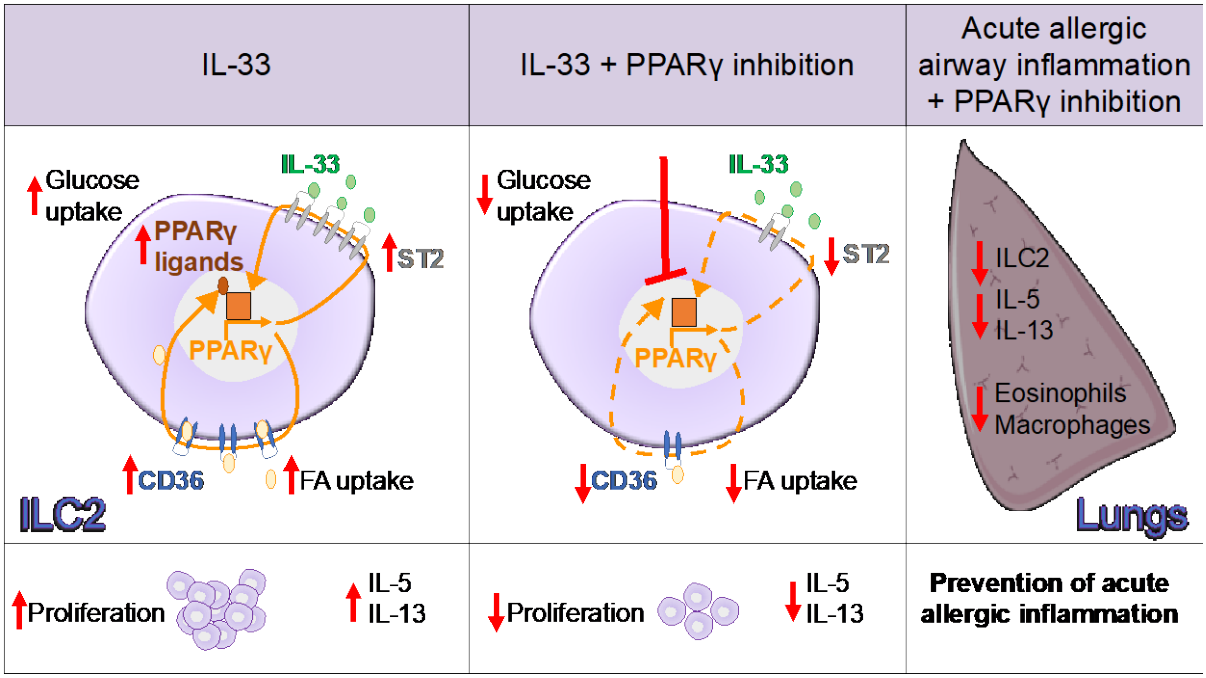

Figure 1: PPARγ regulates the activation of ILC2 in the adipose tissue and lungs

IL-33 leads to proliferation of ILC2s in lungs and adipose tissue as well as the release of IL-5 and IL-13. IL-33 induces increased expression of PPARγ, which in turns leads to increased expression of ST2 revealing the existence of a positive feedback loop between IL-33 and PPARγ-activated pathways. PPARγ activation also induces increased expression of CD36 and fatty acid (FA) uptake. ILC2s may then be able to convert FA into PPARγ ligands, driving PPARγ activation. PPARγ antagonists inhibit IL-33 induced proliferation and IL-5 and IL-13 release by limiting ST2 and CD36 expression. During acute allergic airway inflammation, PPARγ antagonist inhibit ILC2 proliferation and IL-5 and IL-13 secretion leading to decreased recruitment of eosinophils and macrophages in the lung. Targeting PPARγ could thus be explored as a treatment for asthma.