Editorial

Pathways to limit group 2 innate lymphoid cell activation

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Group 2 innate lymphoid cells (ILC2s) are a recently discovered population of lymphocytes that produce large amounts of T_H2 cytokines.[1] Unlike conventional T cells, ILC2s are not antigen specific and also lack specific lineage markers that identify other lymphocytes, including T, B, natural killer, and natural killer T cells. Mouse models of asthma have shown that ILC2s contribute to eosinophilic inflammation and hyperresponsiveness, and human studies have revealed increases in ILC2 counts in patients with asthma, allergic rhinitis, eosinophilic esophagitis, and atopic dermatitis.

The epithelial cytokines IL-33, thymic stromal lymphopoietin, and IL-25, as well as the lipid mediators prostaglandin D_2 (PGD_2) and cysteinyl leukotrienes, promote rapid ILC2 activation.[1-3] Although there is considerable evidence of a multitude of mediators that activate ILC2s, there are fewer reports demonstrating ways to suppress ILC2 function, which could prove to be an important strategy to limit pathology in patients with type 2 inflammatory diseases. One attractive strategy to reduce the burden of disease in allergic patients is to increase suppressive regulatory T (Treg) cell counts to reduce responses mediated by T_H2 cytokine–producing cells.

Treg cell subsets include natural Treg cells that arise from the thymus and inducible Treg (iTreg) cells that arise in the periphery. Treg cells are established regulators of adaptive immune responses through the suppressive activities of the cytokines IL-10 and TGF-β, as well as through direct cell contact mechanisms. In this issue of the *Journal*, Rigas et al[4] have demonstrated that Treg cells directly suppress ILC2 function *in vitro* and *in vivo*. The authors show that mouse ILC2s cocultured with iTregs, but not natural Tregs, had reduced T_H2 cytokine production after stimulation with IL-33. TGF-β and IL-10 contributed to mouse ILC2 inhibition, and inducible costimulator (ICOS)/ICOS ligand interactions promoted suppression of human and mouse ILC2 activation.

Finally, IL-33–challenged NOD-SCID IL-2 receptor (IL-2R) γ–null mice lacking lymphoid cells, including Treg cells and ILC2s, were adoptively transferred with human ILC2s with or without Treg cells in order to isolate the effect of Treg cells on ILC2 responses *in vivo*. Mice transferred with both Treg cells and ILC2s showed reduced airway hyperresponsiveness and lung eosinophil counts compared with mice given only human ILC2s. Importantly, the suppressive effect of iTreg cells was dependent on ICOS/ICOS ligand interactions.

These investigations demonstrate an important direct role of iTreg cells in the suppression of ILC2s, as well as highlight a critical contribution of ICOS/ICOS ligand interactions between iTreg cells and ILC2s. Because the current study[4] demonstrates that ILC2s are also targets of Treg cell–mediated suppression, reductions in T_H2 responses in prior Treg cell adoptive transfer studies might not only have been mediated by inhibition of $CD4^+$ cells but also by inhibition of ILC2s. Notably, the same group recently reported that ICOS/ICOS ligand interactions promote proinflammatory ILC2 responses.[5] Because the current study[4] examined the effects of Treg cells and ILC2s on acute inflammation, further studies are also needed to examine the effects of ICOS/ICOS ligand on ILC2 responses during chronic phases of airway inflammation in which the balance between the effector and suppressive effects of ICOS/ICOS ligand on ILC2s are unknown.

Protein inhibitors of DNA binding Id2 and Id3 transcriptionally regulate T-lymphocyte lineage development, and Id2 is required for ILC2 development.[1] The importance of Treg cells on ILC2 levels has recently been demonstrated in studies using Cre-Lox techniques to inactivate the transcription factors Id2 and Id3 in forkhead box protein 3 (FOXP3)–positive Treg cells.[6] These studies demonstrated that Treg cells deficient in Id2 and Id3 had a spontaneous increase in ILC2 counts, as well as accumulation of eosinophils in the lungs, esophagus, and eyelid, highlighting the normal suppressive role of FOXP3-positive Treg cells in suppressing ILC2 responses. Overall, these investigations support the importance of strategies to induce iTreg cells in patients with allergic disease for suppression of both ILC2-driven innate and CD4-driven adaptive responses.

Recent work by other investigators has suggested that several cytokines also suppress ILC2 responses.[7-9] Type 1 interferons, including IFN-β, as well as the T_H1-associated cytokines IL-12, IFN-γ, and IL-27, have recently been shown to inhibit mouse and human ILC2 responses. Importantly, IL-12 reduces ILC2 GATA-3 expression and transforms ILC2s into IFN-γ–producing ILCs with increased T-bet expression.[9] Interestingly, the pro-inflammatory cytokine IL-1β appears to both activate ILC2 cytokine production and prime ILC2s to produce IFN-γ in response to IL-12. Thus, the presence of a surprisingly high level of ILC2 plasticity in tissues suggests that these potent T_H2 cytokine–producing cells could possibly be modulated in terms of which profile of cytokines they produce by means of exposure to different combinations of cytokines.

In addition to cytokines, lipid mediators, including PGD_2 and cysteinyl leukotrienes, such as leukotriene D_4, promote ILC2

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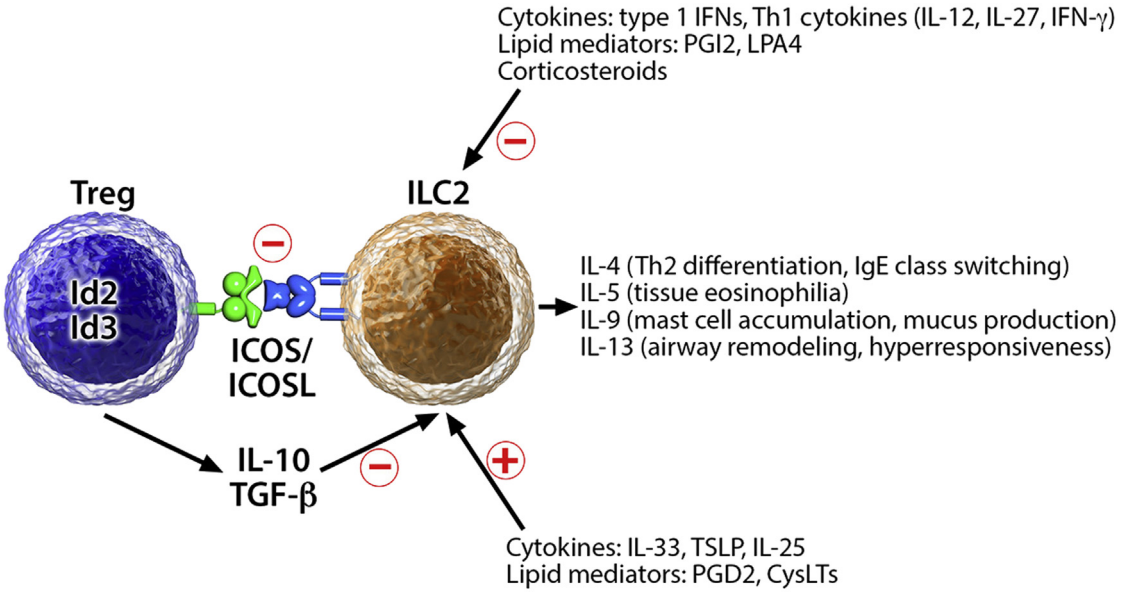

FIG 1. Multiple pathways of ILC2 inhibition. ILC2s are stimulated by epithelial cytokines (IL-33, thymic stromal lymphopoietin *[TSLP]*, and IL-25) and lipid mediators (cysteinyl leukotrienes *[CysLTs]* and PGD_2) to produce IL-4, IL-5, IL-9, and IL-13, resulting in type 2 inflammation. Treg cells inhibit ILC2 activation through ICOS/ICOS ligand *(ICOSL)* interactions, as well as through production of IL-10 and TGF-β. Inhibition of the transcription factors Id2 and Id3 in Treg cells results in increased numbers of ILC2s. Other mechanisms promoting ILC2 suppression include cytokines (type 1 interferons and T_H1 cytokines), lipid mediators (prostaglandin I_2 *[PGI_2]* and lipoxin A_4 *[LXA_4]*), and corticosteroids.

activation. Thus, activated mast cells and eosinophils that produce PGD_2 and leukotriene C_4 can rapidly induce activation of ILC2s in a pathway independent of cytokines.[1-3] Inhibitory responses of ILC2s to lipid mediators have recently been demonstrated, suggesting the occurrence of ILC2 fine-tuning by a wide array of these mediators. Barnig et al[10] first showed that lipoxin A_4 inhibited human blood ILC2 IL-13 production induced by the combination of IL-33, IL-25, and PGD_2. More recently, prostaglandin I_2 was shown to markedly inhibit human and mouse ILC2 cytokine production induced by IL-33 *in vitro* and by the fungal allergen *Alternaria alternata in vivo*.[11] Importantly, the prostaglandin I_2 analogue iloprost is currently used clinically to treat pulmonary hypertension, and perhaps similar strategies could be used to dampen ILC2-driven responses.

Corticosteroids are a mainstay of treatment of chronic allergic diseases, and recent work has shed some light on the effect of corticosteroids on ILC2 responses. Initial studies demonstrated that corticosteroids could inhibit IL-33–driven ILC2 activation, although thymic stromal lymphopoietin induced partial corticosteroid resistance, as reviewed by Doherty.[1] A subsequent study showed that dexamethasone-treated mice that were administered *Alternaria* species had increased ILC2 apoptosis and markedly reduced lung ILC2 numbers, cytokine production, and eosinophilic lung inflammation.[12] Thus the inhibitory response of ILC2s to corticosteroids might be dependent on the cytokine milieu. Whether human ILC2s are directly inhibited by corticosteroids remains to be determined.

Overall, ILC2s have been shown to promote type 2 responses in animal models and are present in human tissues of patients with asthma, chronic rhinosinusitis, eosinophilic esophagitis, and atopic dermatitis. The past 6 years of ILC2 investigation has brought considerable excitement toward understanding the potential contribution of ILC2s to the pathogenesis of these diseases, although we are likely in the infancy in understanding how ILC2s initiate and propagate allergic diseases. Studies have demonstrated ways to reduce ILC2-driven pathology that include induction of iTreg cells and administration of cytokines and lipid mediators (Fig 1). In addition, nicotinic receptor signaling and histone deacetylase inhibition might reduce ILC2 responses.[13,14] The surprising level of ILC2 plasticity opens the way for potential treatments to manipulate these cells and inhibit their untoward effects. However, caution is prudent with such strategies because ILC2s also promote healthy tissue repair responses and normal metabolic pathways in animal models, supporting the complex roles of these cells in health and disease.

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