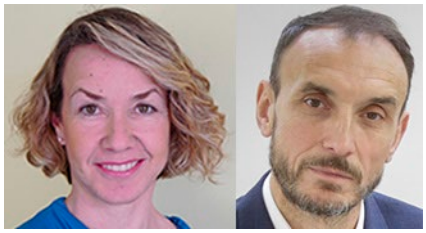
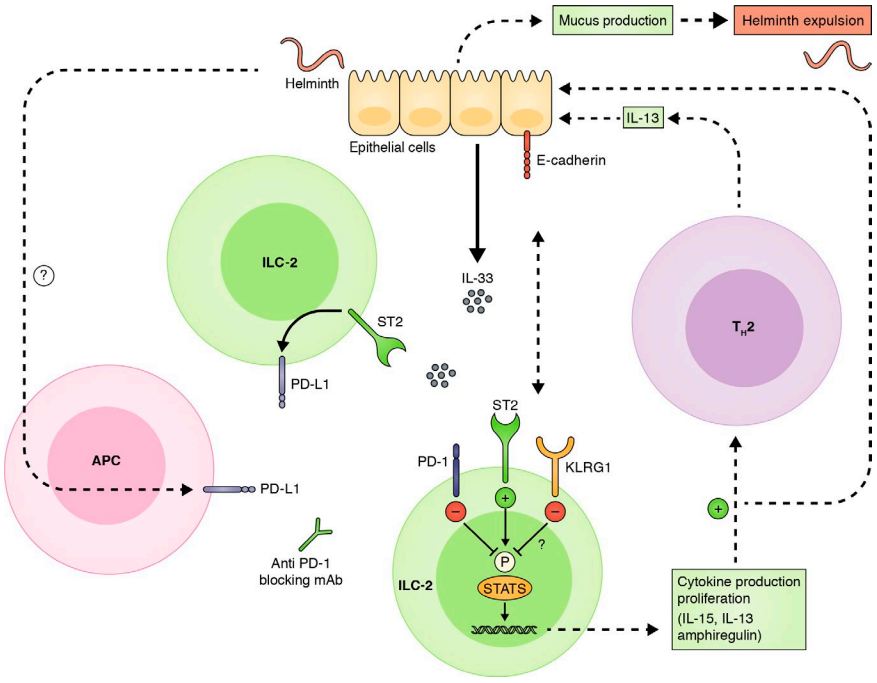
Immune checkpoints on innate lymphoid cells

In this issue of JEM, Taylor et al. (https://doi.org/10.1084/jem.20161653) describe PD-1 as a critical negative regulator of group 2 innate lymphoid cells (ILC-2s). PD-1 intrinsically controls proliferation and cytokine production of both mouse and human ILC-2s. PD-1 signaling inhibits STAT5 phosphorylation and the removal of this brake by knocking down PD-1 expression or by using anti–PD-1 blocking antibodies, translated in vivo into better clearance of helminth worm infection in mice.



Insight from Laura Chiossone and Eric Vivier

Innate lymphoid cells (ILCs) are the most recently identified immune cell types, and the regulation of their responses is still not completely understood. ILCs are tissue-resident cells mainly found at mucosal surfaces of intestine and lungs and in the skin (Klose and Artis, 2016). Thanks to these strategic locations, they are among the first immune cells to react to pathogens. In contrast to myeloid cells, the expression of a large panel of receptors sensing microbes has not been described on ILCs (Hammad and Lambrecht, 2015). The current knowledge on ILC regulation has been mainly focused on the impact of soluble factors released by myeloid or epithelial cells. Among ILCs, ILC-2s are defined as lineage negative (Lin^-), $CD127^+$ $CD25^+$ $KLRG1^+$ $GATA\text{-}3^{high}$ cells. Cytokines such as IL-33, IL-25, TSLP, IL-2, IL-4, and IL-7 and inflammatory mediators such as prostaglandin D2 and leukotriene D4 stimulate ILC-2 expansion and effector functions. Upon stimulation, ILC-2s secrete IL-5, IL-13, and the epidermal growth factor–like molecule amphiregulin, making them central regulators of type 2 immune responses (Hammad and Lambrecht, 2015; Klose and Artis, 2016). In particular, ILC-2s have been shown to actively control parasitic worm infections, epithelial repair, and mucosal tissue homeostasis in several mouse models. However, if deregulated, ILC-2s can also induce tissue fibrosis and trigger type 2 immunopathologies such as allergies, asthma, and atopic dermatitis (Klose and Artis, 2016). Therefore, the dissection of the mechanisms leading to the regulation of ILC-2 functions is of great interest.



PD-1 is a negative regulator of ILC-2 responses. PD-1 provides a negative signal that controls proliferation and cytokine production of mature ILC-2s, thus limiting type 2 immune responses and contributing to maintain local Th1/Th2 balance. During helminth infection or other inflammatory conditions, PD-1 signaling is detrimental as it reduces the control of the infection. Anti–PD-1 blocking antibodies can restore ILC-2 activation and by consequence improve helminth expulsion.

Besides cytokine receptors, only few cell surface receptors interacting with membrane-bound ligands and regulating ILC-2 effector functions have been described (Salimi et al., 2013, 2016; Huang et al., 2015; Maazi et al., 2015). In this issue, Taylor et al. demonstrate that PD-1 is an important negative checkpoint of ILC-2s, both in mice and in humans (see first figure). PD-1 is an inhibitory receptor that binds PD-L1 and PD-L2 that are expressed on several tumors, on infected cells, and on antigen-presenting cells present in inflammatory foci. PD-1 is a well-known checkpoint of T cell activation, and more recently, it has been described to control also NK cell functions (Beldi-Ferchiou et al., 2016; Pesce et al., 2017). Deficiency in PD-1 induces the suppression of tumor growth and metastasis in mice (Okazaki et al., 2013). Checkpoint inhibitors, such as anti–PD-1 blocking

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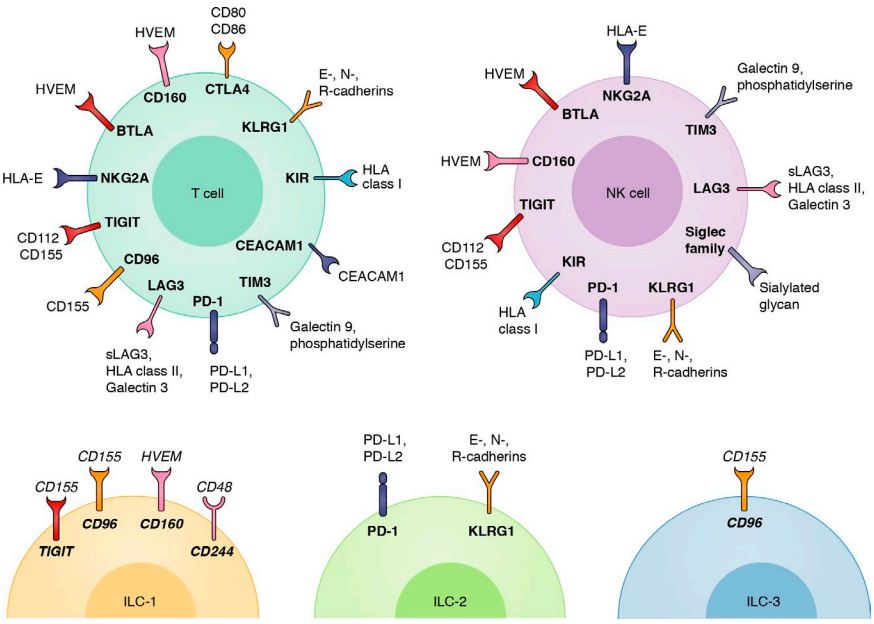
The Journal of Experimental Medicine

antibodies, are currently used in the treatment of different advanced solid tumors with some unprecedented successes that are revolutionizing the standard of care in these clinical conditions (Mahoney et al., 2015).

Taylor et al., 2017 show that mouse ILC-2s express PD-1 in different percentages depending on their tissue origin and that its expression is enhanced by IL-33 stimulation. PD-1$^+$ ILC-2s display a reduced capacity to release cytokines as compared with the PD-1$^-$ ILC-2 population. The analysis of PD-1–deficient mice (*Pdc1*$^{-/-}$) revealed a positive correlation between the lack of PD-1 and the ILC-2 frequencies in tissues, suggesting a role of PD-1 in the control of ILC-2 expansion. Recently, two papers described that PD-1 identifies ILC committed progenitors (Seillet et al., 2016; Yu et al., 2016), but although Yu et al. (2016) showed that its expression was required for ILC-2 differentiation, Seillet et al. (2016) demonstrated that PD-1 was not essential for the generation of ILC precursors and mature ILCs. Here, using transfer experiments, Taylor et al., 2017 show that lack of PD-1 does not inhibit ILC-2 development but intrinsically regulates ILC-2 proliferation and IL-13 production. Moreover, with a series of in vitro and in vivo cytokine stimulation assays, they provide mechanistic evidence that PD-1 inhibits STAT5 phosphorylation. STA5 is an important transcription factor, whose activation is mediated by IL-2 family cytokines, and that regulates the expression of genes involved in cell proliferation, differentiation, and survival of lymphocytes. ILC-2s from *Pdc1*$^{-/-}$ mice display much higher STAT5 phosphorylation upon IL-33 stimulation, as compared with ILC-2s from WT mice. Clearly, additional studies are needed to precisely dissect the impact of PD-1 and its ligands on ILC-2s. Nonetheless, gene expression profiling revealed that PD-1–deficient ILC-2s have an increased expression of a set of genes involved in immune response, such as cell activation, proliferation, adhesion, and chemotaxis, suggesting that PD-1 can inhibit these functions in ILC-2s, similar to what has previously demonstrated in CD8$^+$ T cells (Duraiswamy et al., 2011).

Additional data came from experiments performed in mice infected with *Nippostrongylus brasiliensis*, a gastrointestinal roundworm that infects rodents. ILC-2s were found to undergo massive expansion in *Pdc1*$^{-/-}$ mice upon *N. brasiliensis* infection, near the site of infection (mesenteric lymph nodes), to produce high levels of IL-5 and IL-13 and were also more efficient that parental ILC-2s in clearing worm burden, even without the cooperation of adaptive immune cells (Taylor et al., 2017). The same results were indeed reproduced in *Rag1*$^{-/-}$ infected mice by using anti–PD-1 blocking antibodies, suggesting also that PD-1–targeted immunotherapy can enhance ILC-2 responses (Taylor et al., 2017). These data provide new clues for therapeutic intervention, not only in helminth infections for which less expensive antiparasitic drugs are currently used, but also to restore type 2 immunity in disorders that result from excessive type 1 immune responses, such as allograft rejection, contact dermatitis, or other chronic inflammatory disorders. Importantly, human ILC-2s also express PD-1. Similar to what was demonstrated in mice, human PD-1$^+$ ILC-2s produce lower amounts of IL-5 and IL-13 and display lower proliferative potential after cytokine stimulation, as compared with PD-1$^-$ ILC-2s. Furthermore, antibody-mediated blocking of PD-1 can restore these functions, both in vitro and in vivo in a model of humanized mice (NSG mice reconstituted with human PBMCs).

Collectively, these results demonstrate that it is possible to modulate ILC-2 effector functions, and by consequence type 2 immune responses, by using PD-1 blocking antibodies. However, it remains to be understood how PD-1 signaling is triggered at the site of helminth infection. Do helminths release soluble factors that trigger PD-L1 expression on immune cells present in tissue microenvironment? Such a mechanism could be a strategy for helminth to silence immune response, somewhat



Inhibitory checkpoints expressed by human T lymphocytes and ILCs. This figure was generated based on literature for receptors whose expression has been demonstrated by surface staining (T cells, NK cells, and ILC-2s) and on microarray data available in public databases for relative quantification of transcripts (italics) when no information of surface expression has been reported (ILC-1 and ILC-3). This listing does not preclude the expression of other inhibitory receptors, for instance under various stimulation conditions. Receptors are in bold, whereas their cognate ligands are in normal characters.

similarly as cancer cells do in their microenvironment. In vitro culture with IL-33 up-regulated PD-L1 expression on ILC-2s, suggesting that ILC-2s could inhibit each other. These findings need to be demonstrated in vivo to show whether epithelial cells activated during helminth infection can indeed inhibit ILC-2 responses by releasing IL-33. Alternatively, do amphiregulin or other cytokines released by ILC-2s induce PD-L1 expression on surrounding cells? If valid, this scenario would represent a negative feedback triggered during ILC-2 activation.

Besides parasitic infections, the results by Taylor et al., 2017 may also help to clarify the cellular interactions and the molecular mechanisms taking place during treatment with checkpoint inhibitors targeting the PD-1 axis. In particular, these findings prompt the monitoring of ILC-2s in cancer patients treated with anti–PD-1 antibodies. That PD-1 can act as a negative checkpoint on ILC-2s is indeed particularly relevant today in the new expanding field of immunotherapy. Along this line, several immune checkpoints are inhibitory receptors containing one or more immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domain. A bioinformatic search across the entire genome revealed the existence of more than 300 integral membrane proteins that contain at least one ITIM domain (Daëron et al., 2008). Of these receptors, only a few are targeted in therapeutic approaches. Increasing evidence suggests the employment of combination therapies with antibodies that block several negative regulators, with the expectation of their additive or synergistic effects on antitumor response. As highlighted by the expression of PD-1 on NK (Beldi-Ferchiou et al., 2016; Pesce et al., 2017) and on ILC-2s (Taylor et al., 2017), several inhibitory checkpoints are not restricted to T lymphocytes (see second figure). Interestingly, human and mouse ILC-2s express the inhibitory molecule KLRG1 (Salimi et al., 2013; Huang et al., 2015). KLRG1 is a ITIM-bearing receptor that is also shared by NK cells, T cells, mast cells, basophils, and eosinophils, and its expression varies with cell activation (Huntington et al., 2007). Experiments in mice showed that in vivo administration of IL-25 elicits the expansion of a subset of ILC-2s referred to as "inflammatory" ILC-2s that are characterized by high expression of KLRG1 and IL-25 receptor and a high activity in the control of helminth infection (Huang et al., 2015). The interaction of KLRG1 and its E-cadherin ligand has been shown to inhibit human ILC-2s in vitro, but its function in vivo remains to be established (Salimi et al., 2013). Thus, the dissection of the role of inhibitory receptors on ILCs will be critical for the full understanding of the regulation of ILC functions and of the mode of action of immunotherapies using checkpoint inhibitors. In particular, it will be key to dissect whether unleashed ILCs participate in the inflammatory/autoimmune disorders that are associated with the treatments with checkpoint inhibitors.

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