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RESEARCH HIGHLIGHT

Enabling anti-tumor immunity by unleashing ILC2

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Group 2 innate lymphoid cells (ILC2s) are tissue-resident lymphocytes involved in type 2 immune responses to worm infections and in the pathogenesis of allergies. Balachandran and colleagues now reveal that IL33-driven expansion of ILC2 unleashes T cell-mediated tumor immunity and that therapeutic modulation of ILC2 is a promising immunotherapeutic strategy for the treatment of pancreatic cancer, in particular when combined with PD1 checkpoint blockade.

The immune system is an important barrier to tumorigenesis.[1] While much insights have been gained into harnessing the power of T cells and natural killer (NK) cells to defeat cancer, the roles of other components of the immune system, in particular those of its innate arm, are less clear. This is much warranted as some of these are important "enablers" of adaptive, antigen-specific immunity.[2] Innate lymphoid cells (ILCs) are a recently discovered population of tissue-resident innate lymphocytes that are mainly found in barrier tissues and in organs associated with metabolism (i.e., liver, pancreas and adipose tissue).[2] Based on distinct effector programs, three main groups of ILC can be discerned, ILC1, ILC2 and ILC3, which are striking mirror images of the three main T helper cell effector states (Th1, Th2 and Th17).[3] However, there are profound and intriguing differences. In contrast to T cells, ILCs are tissue-resident cells that are often deposited into tissues early during ontogeny and become an integral part of their fabric, deeply integrated into the regulation of organ function and into the adaptation of tissue function to changing environmental demands.[4,5]

Given their extreme sedentary lifestyle in tissues, ILCs are positioned at sites where cancer initiation occurs, but our understanding of their roles in tumor immunity is still in its infancy. While ILC1 and conventional NK cells have direct anti-tumor function, the roles of ILC3 are more complex. Both tumor-restraining and tumor-promoting functions have been reported for ILC3.[6] ILC2s are a component of the type 2 module of immunity and they have been linked to immunity to parasite infections and the pathogenesis of allergies.[3] Previous data suggested that ILC2 may represent an important checkpoint for the initiation of T cell responses in the context of worm infections, but any such role in coordinating adaptive anti-tumor immunity has not been explored.[7] While pulmonary ILC2 seemed to be endowed with anti-tumor functions, tumor-infiltrating ILC2 in breast, hepatic and skin cancers were correlated with cancer progression.[8] In a recent issue of *Nature*, Balachandran and colleagues identified a new pathway by which ILC2 enables anti-tumor immunity in pancreatic cancer.[9] Pancreatic cancer is the seventh leading cause of death worldwide, according to data from the World Health Organization and still has a relatively poor prognosis. Thus, new therapeutic approaches are urgently needed.

As already reported for prostate cancer,[10] Moral et al. observed an accumulation of tumor-infiltrating ILC2 in human pancreatic ductal adenocarcinoma (PDAC).[9] Conversely to what has been observed in prostate cancer though, higher infiltration of ILC2 in PDAC was correlated with increased survival. In addition, patients with high-level expression of IL33 (a cytokine stimulating ILC2 proliferation and effector function) in tumors showed longer survival, which was correlated with higher intratumoral cytolytic activity. In spontaneous and orthotopic mouse models of PDAC, the authors observed a striking, completely IL33-dependent infiltration of PDAC by ILC2, suggesting that these murine models can be used to mechanistically dissect the role of ILC2 in pancreatic cancer.

RNA-seq analysis of tumor-infiltrating hematopoietic cells in *Il33*$^{-/-}$ mice showed that they are characterized by diminished signatures of T cell activation and of class I MHC antigen processing. Indeed, frequencies and activation of intratumoral CD8$^+$ T cells were reduced in *Il33*$^{-/-}$ animals, suggesting a possible defect of T cell responses in the absence of IL33 and ILC2. In fact, depletion of T cells abrogated the differences in tumor size observed between *Il33*$^{-/-}$ and *Il33*$^{+/+}$ mice, establishing that T cells are the effector arm of IL33-driven anti-tumor immunity (Fig. 1a). To understand whether ILC2s were required, the authors assessed the expansion of antigen-specific CD8$^+$ T cells in PDAC-bearing mice allowing for the specific ablation of ILC2. Indeed, ILC2 deficiency led to reduced priming of tumor antigen-specific T cells and an increased tumor burden, suggesting that the effect of IL33 on CD8$^+$ T cell effector programs in PDAC is mediated via ILC2.

Could then recombinant (r) IL33 be of therapeutic use in the treatment of PDAC? Treatment with rIL33 improved survival of mice with PDAC, which was correlated with ILC2 expansion in the tumor and increased function of tumor antigen-specific CD8$^+$ T cells (Fig. 1b). These data raised the question of how ILC2s mechanistically enhance CD8$^+$ T cell function. IL33 application had no direct effect on CD8$^+$ T cells but led to increased recruitment of CD103$^+$ dendritic cells (DCs) to the tumor bed, suggesting that the IL33-ILC2 axis promoted DC recruitment which may enhance priming of tumor antigen-specific T cells. By combining single-cell RNA-seq (scRNA-seq) analysis of tumor-infiltrating ILC2 with in vitro migration assays, the authors identified ILC2 as producers of CCL5 which was responsible for CD103$^+$ DC recruitment. Indeed, administration of rIL33 to ILC2-deficient mice had no effect on tumor progression, CD103$^+$ DC recruitment and activation of CD8$^+$ T cells. Conversely, rIL33 treatment of mice genetically lacking CD103$^+$ DCs increased the

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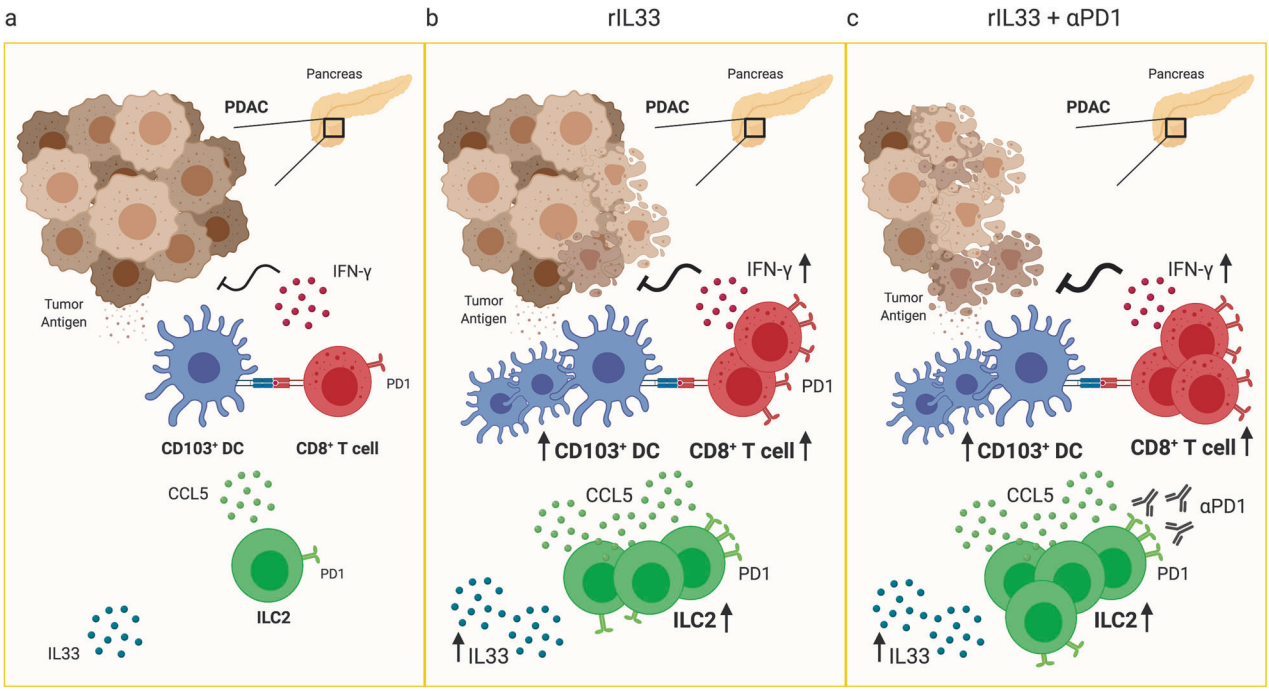

Fig. 1 ILC2-mediated orchestration of anti-tumor immunity against PDAC. a ILC2 infiltration in PDAC is dependent on (endogenous) IL33. **b** Tumor immunotherapy with rIL33 induces ILC2 proliferation, activation, and CCL5 production by ILC2. High levels of CCL5 promote the recruitment of $CD103^+$ DCs to the tumor bed, enhancing the priming of $CD8^+$ T cells and thus their anti-tumor functions. **c** Combination of rIL33 treatment with anti-PD1 checkpoint blockade synergistically enhances anti-tumor immunity most likely by further unleashing ILC2 function. Illustrations were prepared with BioRender (BioRender.com).

numbers of ILC2 but did not result in $CD8^+$ T cell activation and reduced tumor growth. Collectively, the data suggest that IL33 controls the pool of tumor-resident ILC2. In turn, ILC2s recruit $CD103^+$ DCs via CCL5 to the tumor, supporting the priming of tumor antigen-specific $CD8^+$ T cells (Fig. 1b).

Since IL33-mediated activation of ILC2 leads to the activation of anti-cancer immunity, Moral and colleagues searched for possible therapeutic approaches that could further increase ILC2 activity. Using scRNA-seq, PD1 was identified as the only co-inhibitory molecule selectively expressed by tumor-resident ILC2 after rIL33 injections. Indeed, combination treatment with rIL33 and anti-PD1 was significantly more effective than either treatment alone (Fig. 1c). Tumor regression induced by the combination of rIL33 and anti-PD1 required the presence of ILC2, arguing for a pivotal role of ILC2 in mediating the efficacy of the combination treatment. Interestingly, PD1 blockade had a dominant effect on ILC2, and PD1-deficient ILC2 reduced tumor burden and amplified anti-tumor $CD8^+$ T cell responses more efficiently compared to PD1-proficient ILC2. Importantly, the combined treatment with rIL33 and anti-PD1 did reduce the volume of murine $IL33^{low}$ PDAC that resembled human pancreas tumors resistant to PD1 checkpoint blockade. As there was a significant correlation between the co-occurrence of $PD1^+$ ILC2 and $PD1^+$ T cells in human PDAC, such dual therapy should be considered as a potential candidate for clinical applications.

The advent of checkpoint blockade has demonstrated that the power of the immune system can be unleashed to fight cancer. Much of these efforts have focused on enhancing T cell function, but it is increasingly clear that other immune cell types also contribute to anti-tumor immunity and may be important future targets for cancer immunotherapy. In the current report, Moral and colleagues identify ILC2 as an important immunoregulatory hub for coordinating and enabling anti-tumor T cell responses against pancreatic cancer, which still has a comparably poor prognosis. They demonstrate in mice and humans that boosting ILC2 function by providing rIL33 may be a promising therapeutic approach, in particular when combined with the blockade of PD1. Such immunotherapy of PDAC may interfere with the immune-escaping characteristics of established pancreatic cancer and can unleash the anti-cancer activity of ILC2, allowing for control of pancreatic cancer.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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