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CHAPTER ONE

Group 2 Innate Lymphoid Cells in the Lung

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Abstract

As the first line of defense, innate immunity plays an important role in protecting the host against pathogens. Innate lymphoid cells (ILCs) are emerging as important effector cells in the innate immune system and the cell type that regulate immune and tissue homeostases. Group 2 ILCs (ILC2s) are a subset of ILCs and are characterized by their capacity to produce large quantities of type 2 cytokines and certain tissue growth factors. In animal models, lung ILC2s are involved in allergic airway inflammation induced by exposure to allergens even in the absence of $CD4^+$ T cells and are likely responsible for tissue repair and recovery after respiratory virus infection. ILC2s are also identified in various organs in humans, and the numbers are increased in mucosal tissues from patients with allergic disorders. Further investigations of this novel cell type will provide major conceptual advances in our understanding of the mechanisms of asthma and allergic diseases.

1. INTRODUCTION

Innate immunity plays an important role in protecting the host against pathogens such as bacteria, viruses, and parasites. Innate lymphoid cells

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(ILCs) are emerging as important effector cells of the innate immune system that are involved in pathogen clearance, lymphoid organogenesis, and tissue remodeling. These cells are derived from a common lymphoid progenitor, exhibit lymphoid morphology, lack rearranged antigen receptors, and express no conventional lymphocyte or dendritic cell (DC) phenotypic markers.

Based on their cytokine production profiles and the transcription factors utilized for their development and functions, ILCs have been recently categorized into three groups: group 1 ILCs (ILC1s), group 2 ILCs (ILC2s), and group 3 ILCs (ILC3s) (Spits et al., 2013). ILC1s comprise IFN-γ-secreting ILCs that likely use transcription factor T-bet for lineage commitment. ILC2s comprise type 2 cytokine-producing ILCs that require transcription factor GATA3 for their development and function. ILC3s comprise IL-17- and/or IL-22-producing ILCs that are dependent on transcription factor RORγt for lineage specification. In this review, we will specifically focus on ILC2s, especially ILC2s in the lung, and discuss their functional roles in allergic airway diseases.

2. GENERAL FEATURES OF ILC2s

ILC2s are considered to be the counterpart of Th2-type $CD4^+$ T cells in the adaptive immune system. They characteristically produce type 2 cytokines, such as IL-5 and IL-13. ILC2s were first described in mice in the early 2000s as non-B/non-T cells that secrete IL-5 and IL-13 in response to IL-25 (Fort et al., 2001; Hurst et al., 2002). A subsequent study showed that these IL-25-responsive ILCs play important roles in *Nippostrongylus brasiliensis* worm expulsion (Fallon et al., 2006). In 2010, ILC2s were characterized in detail by three groups, and they were independently named as natural helper cells, nuocytes, and innate helper 2 cells (Moro et al., 2010; Neill et al., 2010; Price et al., 2010). They were later named as ILC2s in a consensus report (Spits et al., 2013).

Generally, mouse ILC2s are negative for classical cell surface markers for T cells, B cells, natural killer (NK) cells, myeloid cells, and DCs, including CD3, CD4, CD8, CD5, CD19, B220, TCR, NK1.1, Ter119, Gr-1, Mac-1, CD11c, CD14, and CD16/32; thus, they are designated lineage-negative (Lin^-). Mouse ILC2s do express ST2 (IL-33 receptor), CD127 (IL-7R α-chain), ICOS, CD117 (c-Kit), Thy1, IL-17RB (IL-25 receptor), CD44, and CD25 (IL-2R α-chain). Mouse ILC2s are widely distributed in the tissues, including fat-associated lymphoid clusters (FALC), mesenteric and mediastinal lymph nodes, liver, spleen, intestine, bone marrow, visceral

adipose tissue, and lung. Developmentally, ILC2s arise from common lymphoid precursors in the bone morrow and require IL-2 receptor common γ-chain (cγ), transcription factor inhibitor of DNA binding 2 (Id2), nuclear orphan receptor RORα, and transcription factor GATA3 for their development and differentiation (Hoyler et al., 2012; Moro et al., 2010; Wong et al., 2012; Yang, Saenz, Zlotoff, Artis, & Bhandoola, 2011). Mature mouse ILC2s are activated to produce type 2 cytokines, including IL-4, IL-5, IL-9, and IL-13, in response to the cytokines, such as IL-25, IL-33, and thymic stromal lymphopoietin (TSLP; Kim et al., 2013; Mjosberg et al., 2012; Moro et al., 2010; Neill et al., 2010; Price et al., 2010), that are derived from epithelial cells and certain immune cells.

Initial studies on mouse ILC2s demonstrated their critical roles in innate immunity against a variety of organisms. For example, ILC2s play critical roles in protective immunity against helminth infection (Moro et al., 2010; Neill et al., 2010; Price et al., 2010), in influenza-induced lung inflammation and airway hyperreactivity (AHR; Chang et al., 2011), and in respiratory epithelial repair after influenza infection (Monticelli et al., 2011). ILC2s and their cytokines also play pathological roles in allergen-induced airway inflammation (Barlow et al., 2012; Bartemes et al., 2012; Halim, Krauss, Sun, & Takei, 2012; Kim et al., 2012) and skin inflammation (Kim et al., 2013; Roediger et al., 2013). Some homeostatic and tissue remodeling roles for ILC2s have been reported, including eosinophil homeostasis (Molofsky et al., 2013; Nussbaum et al., 2013) and hepatic fibrosis (McHedlidze et al., 2013).

Multipotent progenitor type 2 (MPP^{type2}) cells are likely a special subset of ILC2s. These cells were originally discovered in the gut-associated lymphoid tissue of IL-25-treated mice (Saenz et al., 2010); they are also found in blood, lymph nodes, lung, and the peritoneal cavity (Saenz et al., 2013). Unlike other ILC2s, MPP^{type2} cells display a multipotent capacity to differentiate into monocyte/macrophage and granulocyte lineages (Saenz et al., 2010). In addition, MPP^{type2} cells can present antigens to T cells and promote Th2-type differentiation. A recent study demonstrated that MPP^{type2} cells are predominantly activated by IL-25, but not IL-33, and exhibit distinct transcriptional profiles and developmental requirements as compared to ILC2s (Saenz et al., 2013), suggesting that MPP^{type2} cells and classical ILC2s are distinct subsets.

3. LUNG ILC2s AT RESTING CONDITION

ILC2s are normally resident in the lungs of naïve animals. In the lungs of naïve mice, ILC2s are Lin^- and generally express various cell surface

markers, including CD117, CD122 (IL-2R β-chain), CD25, CD127, Ly5.2, Thy1, Sca-1, ST2, CD69, CD9, CD38, MHC class II, CD44, and ICOS (Bartemes et al., 2012; Halim et al., 2012; Monticelli et al., 2011; Price et al., 2010). Some heterogeneity in the expression of cell surface molecules is also observed among the studies, likely due to differences in the experimental models and housing conditions of the animals. Combinations of these cell markers are used to identify and isolate ILC2s among the Lin^- populations in the lung of naïve mice (Fig. 1.1A). Importantly, lung ILC2s are present in $Rag2^{-/-}$ mice and $ST2^{-/-}$ mice (i.e., deficient in IL-33R), suggesting that they do not require TCR recombination or IL-33 for their development. In contrast, mice that are deficient in IL-2 receptor cγ, IL-7R α-chain, or transcription regulator *Id2* lack mature ILC2s, consistent with their dependency on IL-7 and *Id2* for their development.

Lung ILC2s are a rare cell population. In wild-type C57BL/6 mice, lung ILC2s represent only 0.25–1% of total live cells in the lung. ILC2s are located in collagen-rich regions close to the confluence of medium-sized blood vessels and airways, but not in alveolar areas of the lung (Nussbaum et al., 2013). Resting lung ILC2s have morphology similar to that of resting lymphocytes, with no apparent intracellular granule structures (Bartemes et al., 2012). However, once they are stimulated with IL-33, lung ILC2s increase in size and display pronounced endoplasmic reticulum and Golgi apparatus (Fig. 1.1C).

Resting lung ILC2s also display a gene expression profile distinct from those of macrophages, DCs, $CD4^+$ T cells, NK cells, γδT cells, and regulatory T cells (Treg) in the lung. More specifically, ILC2s show high mRNA expression levels of *Gata3*, *Rora*, *Cd69*, *Il2ra*, *Il2rg*, *Il4ra*, *Il7r*, *Il17rb*, *Il1rl1*, *Il5*, and *Il13* (Halim et al., 2012). IL-5 and IL-13 transcripts were also detected in resting lung ILC2s in cytokine reporter mice (Ikutani et al., 2012; Nussbaum et al., 2013; Price et al., 2010). At the protein level, ELISAs could not detect IL-5 and IL-13 in the culture supernatants of naïve and resting lung ILC2s (Bartemes et al., 2012; Halim et al., 2012). However, ELISPOT assays revealed IL-5-producing lung ILC2s when cultured in medium alone (Nussbaum et al., 2013), suggesting constitutive but minimal production of IL-5 by resting ILC2s. Interestingly, this constitutive expression of IL-5 by ILC2s may play a role in regulating eosinophil homeostasis in various organs (Molofsky et al., 2013; Nussbaum et al., 2013). Some controversies exist as to the expression of IL-4. Although gene microarray analysis shows no or low expression levels of *Il4* (Halim et al., 2012), IL-4 transcripts were found in ILC2s in the lungs of IL-4 reporter mice (Price et al., 2010).

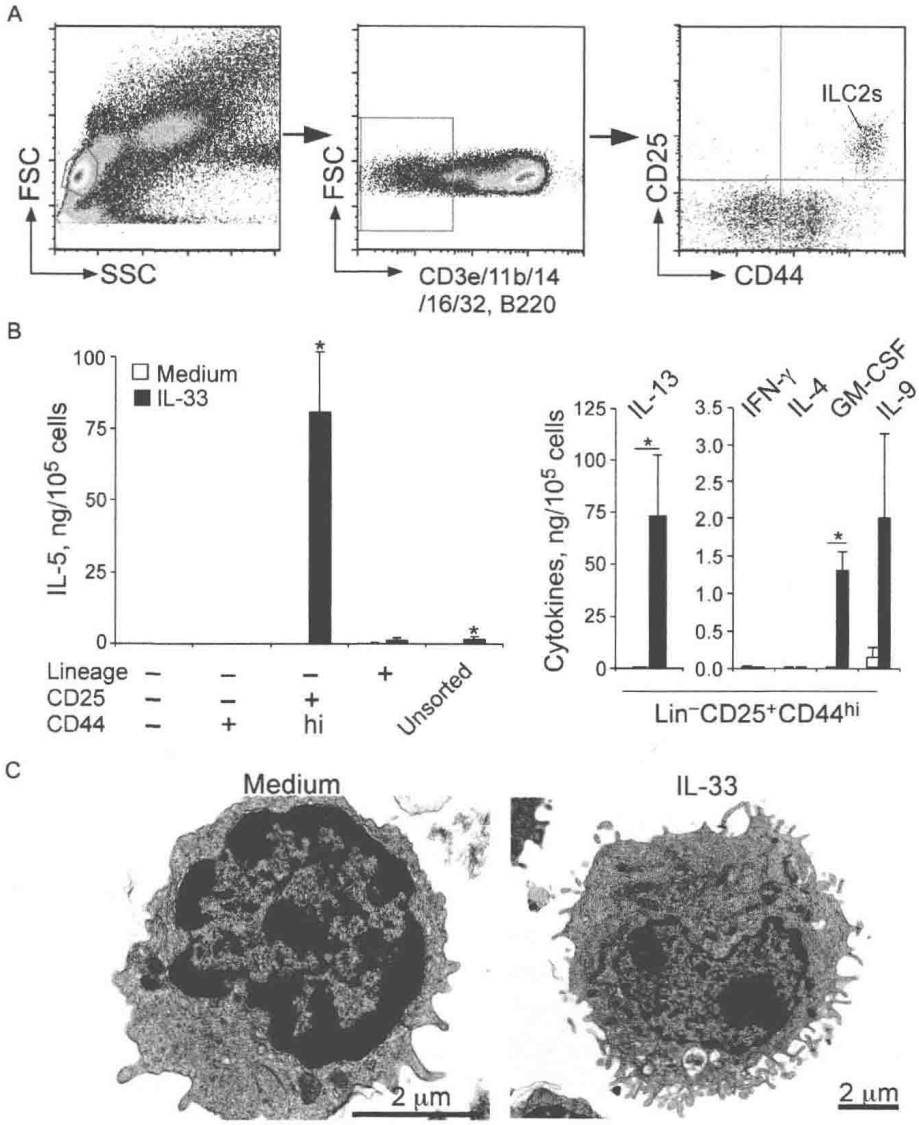

Figure 1.1 Lung ILC2s respond vigorously to IL-33 and produce a large quantity of IL-5 and IL-13 *in vitro*. (A) Gating strategy and identification of ILC2s in lung single-cell suspensions from naïve BALB/c mice. (B) Four populations of lung cells, including Lin^+ cells, $Lin^-CD25^-CD44^-$ cells, $Lin^-CD25^-CD44^+$ cells, and $Lin^-CD25^+CD44^{hi}$ cells (i.e., ILC2s), were isolated from naïve BALB/c mice by FACS sorting. Sorted and unsorted lung cells were cultured with medium alone or with IL-33, and the levels of cytokines in the supernatants were measured by ELISA. (C) Morphology of lung ILC2s. Lung ILC2s were cultured with medium alone or IL-33 and examined under electron microscopy. Original magnifications: 25,000 × (medium alone, left) and 12,000 × (IL-33, right). $^*p < 0.05$ compared to medium.

4. REGULATION AND FUNCTION OF LUNG ILC2s

Exposure of lung ILC2s to cytokines and other inflammatory mediators rapidly activates their effector functions. For example, IL-33 activates lung ILC2s to produce large quantities of IL-5 and IL-13 *in vitro* (Bartemes et al., 2012; Halim et al., 2012; Fig. 1.1B). While IL-25 and TSLP do not activate lung ILC2s by themselves, they synergistically enhance cytokine production by ILC2s (Halim et al., 2012). IL-25 and IL-33 also promote expansion and/or migration of lung ILC2s, as intraperitoneal or intranasal administration of IL-25 or IL-33 increased ILC2 cell numbers in lung tissues and draining lymph nodes *in vivo* (Barlow et al., 2012; Price et al., 2010). IL-33 is likely more potent than IL-25 in inducing ILC2 cell expansion (Barlow et al., 2013). While stimulatory effects of TSLP on lung ILC2s have not been demonstrated, TSLP has been shown to activate skin ILC2s (Kim et al., 2013), suggesting the specialization of ILC2s in different organs.

Lung ILC2 activities can also be regulated by IL-2-family cytokines. *In vitro*, neither IL-2 nor IL-7 alone induces significant IL-5 and IL-13 production by ILC2s. However, these two cytokines synergistically enhance IL-33- and IL-25-induced proliferation and type 2 cytokine production by lung ILC2s (Bartemes et al., 2012; Halim et al., 2012; Monticelli et al., 2011). Interestingly, IL-2 itself stimulated lung $CD25^+$ ILCs, which have phenotypes similar to those of ILC2s, to produce type 2 cytokines and IL-9 in culture (Wilhelm et al., 2011). IL-9 produced by ILCs may have a positive feedback effect on ILCs, since lung ILCs cultured with IL-9 increased the production of type 2 cytokines (Wilhelm et al., 2011). IL-9 might enhance ILC2 function by upregulating the anti-apoptotic protein BCL-3 in lung ILC2s, thereby promoting ILC2 survival (Turner et al., 2013). In addition, TL1A, a TNF superfamily member, has also been reported to induce ILC2 cell expansion (Yu et al., 2013).

Besides cytokines, lung ILC2s can be regulated by lipid mediators that are generated during allergic inflammation. *In vitro*, leukotriene D_4 (LTD_4) potently stimulates mouse lung ILC2s to produce not only IL-5 and IL-13 but also a large amount of IL-4; IL-4 is not generally produced by ILC2s when stimulated with IL-33 (Doherty et al., 2013). Intranasal administration of LTD_4 led to the expansion of IL-5-producing ILC2s in the lung *in vivo*. Furthermore, LTD_4 potentiated the proliferation and the accumulation of ILC2s in mice exposed to the fungus *Alternaria*