Post-Graduate Course: Lung innate immunity

Emerging roles of ILCs in mucosal barrier

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1. Introduction

Innate lymphoid cells (ILCs) are widely distributed in the body from hematopoietic organs such as the BM to secondary lymphoid tissues and non-lymphoid tissues. Among them, intestine, skin, lungs, and adipose tissues are relatively more enriched with ILCs, which account for ~2.5% of hematopoietic (i.e. $CD45^+$) cells in the intestinal lamina propria, ~3.5% in the skin and lungs, and ~12% in adipose tissues[1]. Various allergens such as house dust mite, cockroach, pollen, fungi, animal hair, and food allergens directly damage epithelium which located at the interface between internal and external environment. Moreover, damage to the barrier functions of the epithelium enhances mucosal permeability of foreign substances and releasing epithelial cytokines (e.g., TSLP, IL-25, and IL-33)[2]. Directly below the epithelium, numerous innate and acquired immune cells create a homeostasis, and ILCs act as gatekeepers to the mucosal compartment.

2. Innate Lymphoid Cells

Innate lymphoid cells (ILCs) are non-T, non-B effector cells that show high effector cell functions (mainly cytokine production) upon activation. ILCs were first described in the intestinal tract[3-5], and shortly after they were also identified in the lung[6-8]. ILCs were shown to be involved with tissue homeostasis, repair and remodeling and innate immunity[9]. As non-T, non-B effector cells, ILCs lack rearranged antigen-specific receptors (TCR: T cell receptor, BCR: B cell receptor) and are therefore antigen non-specific, but they react rapidly to a wide range of innate signals. Given that ILCs produce great variety and amount of cytokines, it is surprising that ILCs had been ignored earlier. But this might be due to limited focus of immunologist on adaptive immunology, and also due to technical limits to identify several cell populations at the same time.

The classification scheme of ILCs has been developed and this scheme suggests that ILCs can be further divided into three subsets — type 1 ILCs (ILC1s and NK cells), type 2 ILCs and type 3 ILCs (ILC3s and Lti (Lymphoid Tissue inducer) cells) — based on their cytokine productions (Table 1)[10]. The prototypic ILC is the natural killer (NK) cell, which is called ILC1 cell or type 1 ILC, and it produces IFN-γ and expresses T-bet (T-box transcription factor)[11,12]. The more recently discovered ILC subset is ILC2 cells or type 2 ILCs (previously called natural helper cells or nuocytes), which produce IL-5, 9 and 13 similar to the cytokines produced by Th2 cells[3,4]. ILC2 cells require the transcription factor RORα (retinoic acid receptor-related orphan receptor α)[13,14] and Gata3 (GATA transcription factor)[15]. Type 3 ILCs or ILC3 cells have also been described with at least three different subtypes: (a) lymphoid tissue inducer (LTi) cells, which produce IL-17 and IL-22[16]; (b) IL-17 producing ILC3 cells, which are active in the gut of colitis patient[17] and in obesity related forms of asthma[8], and (c) IL-22 producing ILC3 cells, which are present in the skin[18,19], and gut[20].

Although ILCs have various subsets, ILC family share a common lymphoid progenitor (CLP), which is identified as Lineage-IL7Rα^{+}KIT$^{+/low}$sca-1$^{+/low}$ and they also share common developmental pathways[9,14,21]. All ILCs require IL-7 signaling for survival, and Id2, a transcriptional repressor[22-25]. These findings indicate that ILCs might be developed from a common progenitor first and then they are differentiated into different subsets based on situation. However, several studies suggested that additional pathways and plasticity might exist in the ILC lineage establishment after all[25].

3. Innate lymphoid cells in allergic disease

ILCs in asthma

Asthma is one of the chronic inflammatory diseases, which affects about 300 million people worldwide[26]. The major symptoms of asthma are shortness of breathing, coughing and wheezing caused by airwayhyperreactivity (AHR), mucus overproduction, and airway remodeling[27]. Asthma is a heterogeneous disease which caused by many factors such as allergen exposure, virus infection, genetic background, excessive exercise and stress.

Allergic asthma was traditionally considered as T_H2-associated disease[28]. Likewise, the discovery of ILC2 cells which produce high levels of type 2 cytokines in the lungs expands our understanding of the pathogenesis of asthma[6,29,30]. In response to external stimuli, ILC2s accumulate in the lung and draining lymph nodes, and release large amounts of IL-5 and IL-13 which are important for the development of AHR[31,32]. Although most studies have focused on the role of ILC2s in acute asthma, the role of ILC2 cells in chronic asthma remains to be elucidated. For example, *Il1rl1*$^{-/-}$ mice had normal AHR responses after sensitization and challenge with OVA, and AHR preferentially triggered CD4^{+} Th2 cells rather than ILC2s[33,34]. Also, ILC2s can be involved in restoration of epithelial cells by producing amphiregulin (AREG),

one of the epidermal growth factor family at the chronic phase of asthma[35,36]. Therefore, the roles of ILC2s in the initiation of asthma versus the maintenance and resolution phases of allergic inflammation need to be investigated further. In human, ILC2s seems to contribute the development of eosinophilic asthma. The number of IL-5, IL-13 or IL-5 and IL-13 double positive ILC2s are increased in blood and sputum of patients with severe asthma than patients with mild asthma[37,38,39]. Interestingly, submucosal expression of IL-33 was increased in children with severe therapy-resistant asthma[40] (Saglani, 2013 #296), and the numbers of ILC2s also increased in children with STRA. Therefore, these results indicated that human ILC2s also played important roles in development of asthma. However, how human ILC2s are involved in asthma pathophysiology remains to be explored.

Although most researches focus on asthma mediated by type 2 responses, non-allergic forms of asthma, triggered by environmental factors such as air pollutants(Pichavant, 2008 #295), or obesity-associated asthma appear to occur independently of T_H2 cells[41]. Obesity has been shown to be a major risk factor for the development of asthma, particularly a severe, steroid-resistant form of asthma[42,43]. Recent studies have emphasized the role type 3 cytokine in the development of asthma in both mice and humans[44-46]. These studies have shown that direct administration of recombinant IL-17A into the lungs can induce airway inflammation and AHR by inducing contraction of smooth muscle cells, and the source of increasing IL-17A was pulmonary Th17 cells. Moreover, the level of IL-17A increased in the sputum or peripheral blood of asthmatics and the concentration of IL-17A is correlated with the severity of asthma[47,48]. In addition to Th17 cells, ILC3s can induce the development of AHR in obese mice[44]. Obese mice developed spontaneous AHR and numbers of $CCR6^+$ ILC3s producing IL-17A in the lung significantly increased. IL-1 β produced by classically activated macrophages stimulates IL-17A production from ILC3s, and blockade of IL-1R attenuate the development of obesity induced asthma[44].

All together, these studies indicate the previously unrecognized roles of ILCs in the development of various forms of asthma, and greatly expanded our knowledge of the pathogenesis of asthma.

ILCs in Atopic dermatitis

Atopic dermatitis (AD) is one of common chronic inflammatory skin diseases, which characterized by itching, dry skin, skin lesion and eczema[49]. The combined factors, such as environmental factors, genetic factors, and immunological factors, can induce AD. The immune mechanisms involved in the pathology of AD has been discovered, mostly type 2 immune response is dominant in AD lesion. Th2 cells and type 2 cytokines, IL-4 and IL-13, are increased in AD skin. Moreover, IL-25, IL-33 and TSLP are also increased in AD skin and these factors promote type 2 immune responses and also modulate the skin barrier functions. Recently, it is revealed that ILC2, responding IL-25, IL-33 and TSLP, also can enhance allergic inflammation in AD[50-52]. IL-33 transgenic (Tg) mouse show spontaneous phenotypes of AD at 6～8 weeks of age[53]. In the skin lesion of Tg mouse, eosinophilic inflammation is occurred in epidermis and mast cells

are also recruited in inflamed skin. Some studies show that ILC2s in skin is more responsive to TSLP than to IL-33 and IL-25[54]. Likewise IL-33 knockout and IL-25R knockout mice still develop AD. However, TSLP knockout mice, the development of AD significantly impaired. In human, there are several reports that IL-25 and IL-33 are predominant soluble factors that can enhance ILC2-mediated skin inflammation. ILC2s expresses the skin homing receptors, CCR4 and CCR10, and infiltrate into lesional skin in human[50,55]. Most recently, using in situ mapping of ILC in human skin, it is revealed that ILC resides epidermis near T cells, suggesting that ILC and T cells can interact and be involved in AD conditions cooperatively[56]. Moreover, AHR+ ILC3 is increased in skin biopsy of AD patients, meaning ILC3 also can be involved in AD conditions.

4. Future perspectives

Following the initial identification and characterization of ILC2s in 2010, our knowledge of this novel cell type has expanded rapidly. In mice, ILCs show both pathological and protective functions in response to various stimuli. Furthermore, ILCs network with many other immune cells, such as $CD4^+$ T-cells, macrophages, eosinophils, NKT cells as well as epithelial cells, to promote the development and persistence of allergic inflammation. However, the role of ILCs in chronic and recurrent airway inflammation in asthma and other allergic diseases, such as atopic dermatitis and chronic rhinosinusitis has not been fully characterized in human. Therefore, the contributions of ILCs and molecular mechanisms underlying their activation in allergic disease should be assessed further.

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