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Innate Lymphoid Cells (Non-NK ILCs)

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Abstract

Until a decade ago, natural killer (NK) cells constituted the major—if not the sole—player of innate lymphoid cell populations. The discovery of the presence and execution of curial functions by lymphoid tissue inducer-like cells (LTi) in adults is followed by the discovery of Th2-like innate cells and later Th1-like helper group 1 ILCs. With these findings, the innate lymphocyte family has expanded and a new paradigm has emerged. Apparently, innate versions of helper subsets of CD4⁺ T cells existed in humans and mice. These cells, unlike their adaptive counterparts, lack CD3, T and B cell receptors, do not rearrange their antigen receptors and get activated by microbial products or cytokines. Furthermore, these cells rely on similar transcription factors that helper CD4⁺ T cells use for their development and functions (such as T-bet, Gata3 and Ror γ t); they produce similar effector cytokines (such as IFN- γ ; IL-5, IL-13, IL-4; IL-17A, IL-22, GMCSF, respectively). Moreover, these cells assume crucial functions as an immediate, first line source of cytokines/chemokines against pathogens during protective immune responses. Lastly, very much like their adaptive counterparts, they are present and contribute to pathogenesis in various chronic inflammatory diseases of mice and humans in several tissues.

Keywords: ILC1, ILC2, ILC3, innate lymphoid cell, LTi cell

1. Introduction

In this review, recently described ILC subsets (group 1, 2 and 3 innate lymphoid cells,) which are phenotypically and functionally distinct from NK cells, are introduced (**Figure 1**). The origin of these cells, their development, the genes that are necessary for their generations as well as functions are described. The signature cytokines produced by ILC subsets and the parallels between their adaptive counterparts, namely T helper lineages (Th1, Th2, Th17) are discussed. For each ILC type, their phenotypic diversity and subsets, unique and discriminating surface markers are explained. For each cell type, their role in protective immunity, as well as their

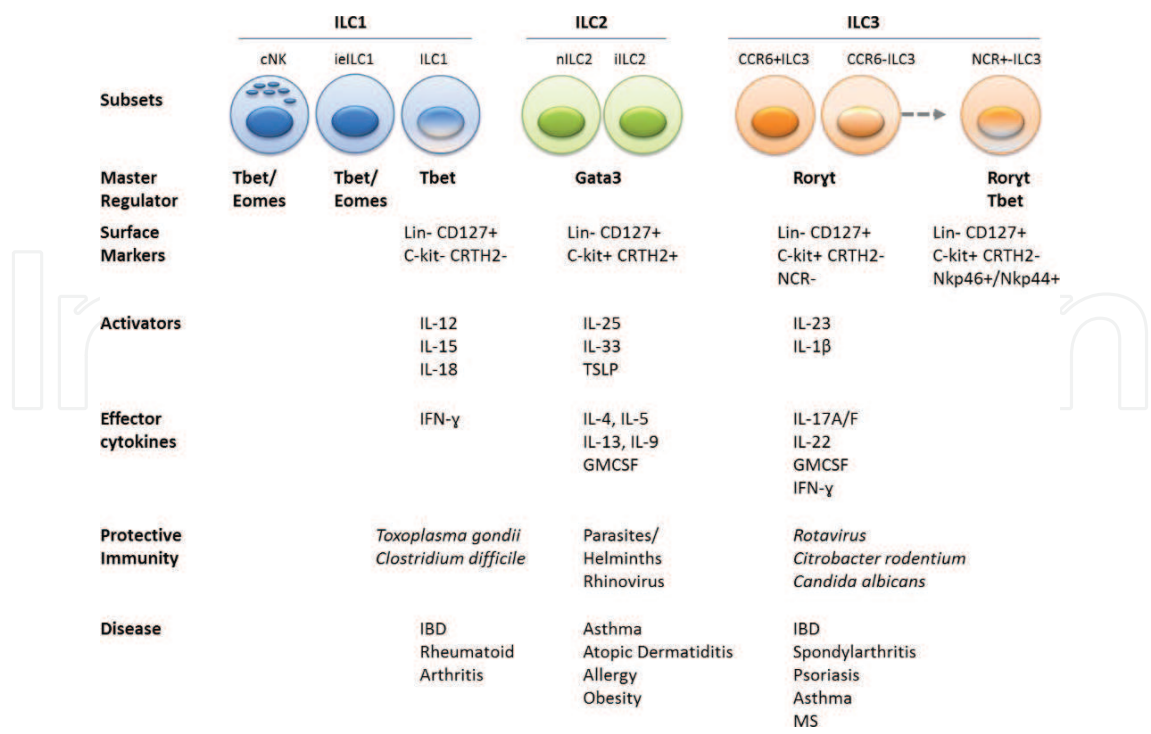


Figure 1. Human innate lymphoid cell classification, signature cytokines and immune processes they are involved in.

involvement in the pathogenesis of various inflammatory diseases, is discussed based on the various data from animal models and human patients. Finally, the plasticity of these cells and the experimental evidence indicating a transition between each lineage are laid out.

2. Group 1 innate lymphoid cells (ILC1)

Group 1 ILCs include Natural killer cells and non-NK ILC1s [1]. We will not be describing NK cells, but concentrate non-NK ILC1s in this review. The distinction between NK cells and non-NK ILC1s is a difficult one to make due to shared use of many markers across tissues [2, 3]. Furthermore, a criterion valid for discrimination of NK cells from non-NK ILC1s in one tissue does not appear to hold for other tissues. Having said that, conventional NK (cNK) cells differ from ILC1 in some regards. ILC1s are found in liver, intestines, uterine tissue, lung, tonsils, peritoneum, spleen and blood [2, 3]. Unlike circulating NK cells, non-NK ILC1s are considered to be mostly tissue resident [4, 5]. ILC1s, like other ILC subsets, express CD127 (IL-7R) whereas mature NK cells do not. T-bet deficiency does not impact cNK cells ontogeny (although it is important in mature NK cells). In contrast, ILC1s are missing in T-bet-deficient mice. Moreover, cNK cells need eomesodermin (Eomes) in addition to T-bet for their function; ILC1s, however, do not express Eomes, though this may change with the tissue of interest. Eomes could also be expressed in low quantity by some NK cells, thus it may not always be a unique NK cell definer.

In humans and mice, both CD127+ and CD127- ILC1s have been described; however, the difference of the latter subset from the cNK cells have been still debated [2, 3, 6]. CD127+ ILC1s

were described as Lineage-ckit-CD161+ NKp44+ cells which lacked NK cell markers CD56, CD94, granzyme B and perforin and responsive to IL-12 to produce IFN- γ [7]. CD127+ ILC1s described in the intestinal lamina propria, tonsils and blood as well. These cells are Eomes- and T-bet+, which enforced the idea that they are different than cNK cells. In mice, similar T-bet+ NKp46+ ILC1 were identified by Vonarbourg et al. [8].

CD127- ILC1s, however, are phenotypically closer to cNK cell. They were described in the human intestinal intraepithelial region (named as ieILC1) and tonsils; they express CD56, also CD103 allowing them to interact with epithelial cell [9]. ieILC1s express CD160 in mice. ieILC1s express Eomes and T-bet-like cNK cells but develop from distinct progenitors [10, 11]. In the liver of humans and mice, ILC1s have been reported. CD3 ϵ -NK1.1+ DX5- CD49a+ cells are considered to be liver non-NK ILC1s and lack Eomes; in humans, a small fraction of CD49+ Eomes- ILC1 is presently likely to be the human equivalent of mouse liver ILC1s [3].

In salivary glands, ILC1s are described; however, they express DX5 and Eomes like cNK cells and produce low levels of IFN- γ , and thus, it is unclear if they are truly different than NK cells or ILC1s [3, 12, 13].

2.1. ILC1 development and activation

Hematopoietic common lymphoid progenitors (CLP) differentiate into, first, early innate lymphoid progenitor (EILP) that later give rise to more specialized progenitor positive for TCF-1 [14]. EILP can make both NK cells and ILCs [15]. EILP further differentiates into an ID2+ common helper innate lymphoid progenitor (CHILP) which has lost potential to make NK cells and can generate all helper ILC subsets. CHILP further differentiates into transcription factor promyelocytic leukemia zinc finger (PLZF)+ precursors, which can make, again all ILC subsets with the exception of true LT α cells [16, 17]. PLZF is not expressed in ILC1s; however, ILC1s come from progenitors who expressed PLZF for a period of time during their development [16]. Similar to other ILC subsets, ID2 and Tox are needed for ILC1 development [18, 19]. Nfil3 requirement for ILC1 is controversial [20–23]. Nfil3-independent ILC1s have been reported in breast and prostate tumor tissues, salivary glands, skin, uterus and kidney [3, 12, 13, 24]. Runx3 was also shown to regulate ILC1 as well as ILC3 development [25, 26]. Lastly, at least some of the ILC1s arise as a result of the conversion of ILC3s or ILC2s to ILC1. Ex-ILC3 ILC1s develop from CCR6- ILC3s through upregulation of NKp46 and gradual loss of Ror γ t upon IL-12 and IL-15 stimulation [6, 8]. ILC2s can also give rise to ILC1 upon IL-12 and IL-1 β exposure, thus a fraction of ILC1 would be ex-ILC2s [27–29].

IFN- γ is the signature cytokine of ILC1s. And ILC1s are activated by IL-12, IL-18 or IL-15 to produce those cytokines.

2.2. ILC1s in protective immunity

ILC1s are thought to be important in immunity against viruses, intracellular bacteria or protozoans owing to their production of IFN- γ [30]. Several infectious agents have been used to assess the role of ILC1s in protective immunity.

A study from Diefenbach's laboratory showed that during *Toxoplasma gondii* infection ILC1s contribute to IFN- γ and TNF- α production and thus, to protective immunity; in T-bet-deficient mice, in which ILC1s are missing, the infection progresses more severely [31]. Though T-bet also functions in NK cells, this study suggests that ILC1s might be important in immunity to *Toxoplasma gondii*. Another study by the same group showed that ILC1s substantially contribute to IFN- γ production during *Salmonella enterica* infection, and the bacteria-induced colitis is alleviated if these cells were depleted [6].

ILC1-based protective immunity to another pathogen was tested by Eric Pamer's group. They showed that due to the absence of ILC1 (but not ILC3), Rag2-/-Il2R γ c-/- mice are more susceptible to *Clostridium difficile* infection. Accordingly, those mice are protected after adoptive transfer of ILC1s, suggesting that ILC1s may play a crucial role in protective immunity to *C. difficile* infections [32].

2.3. ILC1s during inflammatory diseases

Synovial fluids of psoriatic arthritis have been reported to be enriched in ILC1 content [33]. Although a similar increase in NCR+ ILCs in rheumatoid arthritis patients' synovial fluid has been reported, it is unclear whether these cells were conventional NK cells or not.

ILC1 enrichment, particularly in the intestines, has been reported in inflammatory bowel diseases (IBD) patients as well as in various murine models of IBD than found in any other disease [7, 8, 34, 35]. These ILC1s appear to be ex-ILC3s who lost Ror γ t expression over time and became a major source of IFN- γ .

3. Group 2 innate lymphoid cells (ILC2)

ILC2s are found in mucosal surfaces, such as lungs and intestines, and the mesentery, fat-associated lymphoid clusters (FALC) as well as blood. They are identified by various groups at about the same time under different names as nuocytes, natural helper cells, innate type 2 helper (Ih2) cells, or multipotent progenitor type 2 (MPP^{type2}) cells [36–38]. Although these innate cells have been previously reported by McKenzie's group and others as a source of Th2 cytokines, naming and a thorough characterization of them came after these initial reports [39, 40].

Currently, ILC2s in mice can be categorized into two subsets. Natural ILC2s (nILC2) and inflammatory ILC2s (iILC2). nILC2 was shown to be regulated by IL-33 and rely more on IL-33R (IL-1 β LR), whereas iILC2 reportedly express IL-25R and is regulated by IL-25 [41]. nILC2s produce Th2 cytokines IL-5 and IL-13. iILC2, however, can produce IL-13 and IL-17 together.

Human ILC2s that can produce both IL-13 and IL-22 with low Ror γ t expression has been defined [42].

Although T-bet is a Th1-specific transcription factor important in driving IFN- γ expression (in NK cells and ILC1), its deletion in ILC2s revealed that it also assumes important functions

particularly suppressing IL-9 production by ILC2s. In IL-33-induced airway inflammation mouse models, Tbx21 deficiency lead to exacerbated eosinophilic inflammation mediated by unleashed IL-9 production [43].

3.1. ILC2 development and activation

All the ILC subsets differentiate from a common lymphoid progenitor (CLP) that can also give rise to adaptive lymphoid cells. Further specialization of this progenitor proceeds to a branching point after which inhibitory DNA binding protein 2 (Id2) is derepressed in newly formed progenitors. This branching by upregulating Id2 is believed to commit the precursor to a helper ILC lineage. This Id2⁺ precursor is named as common helper innate lymphoid progenitor (CHILP), and so far, lacks potential to make cNK cells. Although Id2 is also reported to be required for cNK cells development, its upregulation occurs after Id2-NK cell precursor branches off to an NK cell fate before the CHILP stage. In other words, current literature suggest that NK lineage branches off from CLP before CHILP does. Phenotypically, CHILP is defined to be Lin⁻Id2⁺Flt3-IL-7Rα⁺ CD25⁻ or more recently Lin⁻Flt3-IL-7Rα⁺α₄β₇⁺ PD-1^{high} and have Tox2, Tcf-1, Gata3 expression and differentiate into all ILC subsets after adoptive transfer [44].

Deletion of Gata3 in hematopoietic cells blocks development of all helper ILC subsets, but spares cNK cells [45]. However, spatiotemporal deletion driven by stage-specific promoters revealed that Gata3 deletion after Id2 is turned on, only blocks ILC2 generation, whereas its deletion after the initiation of Ncr-1 expression impacts ILC1 [46].

Notch signaling has also been shown to be required for the development of all three ILC subsets to varying degrees both *in vitro* and *in vivo* [47].

Bcl11b is particularly required for specialization into ILC2, its absence blocks ILC2 generation [48].

Rora is also crucial in ILC2 development. As such Rora^{-/-} mice lacks ILC2, whereas Th2 cells appear to develop normally. Furthermore, Rora^{-/-} mice fail to mount protease-induced asthma consistent with the role of ILC2s in this process [49, 50].

T-cell factor 1 (TCF1) is another transcription factor required for ILC2 development. TCF-1 is produced by its gene *Tcf7*. TCF-1 is crucial in early thymic T cell development and is also expressed by ILC2s [51, 52]. Studies show that its deletion impairs ILC2 development and that TCF-1 works downstream of Notch signaling during ILC2 development. Thus, papain-induced inflammation or protective immunity is diminished in Tcf7^{-/-} mice [15].

Nuclear factor IL-3 (*Nfil3*), also known as *E4BP4*, is a transcription factor previously reported to be required for generation of some of the hematopoietic cells, including CD8⁺ DCs and, although controversial, NK cells. Nfil3^{-/-} mice have been shown to lack Peyer's patches, both ILC3 and ILC2 cells have been shown to be greatly reduced in the Nfil3^{-/-} mice. Consistent with these results, Nfil3^{-/-} mice mounts a weak immune response to *Citrobacter rodentium* infection and a reduced airway inflammation to papain-induced allergy [53].

Gfi1 (growth factor independent 1) is also another gene reported to regulate ILC2 development and function. Its genetic deletion results in reduced number and function of ILCs, and render mice more susceptible to worm infection and more resistant to papain-induced lung inflammation [54].

ILC2 cells interact with Th2 cells, via costimulatory molecules OX40/OX40L, MHCII as well as cytokines such as IL-4; by doing so, they allow generation of a robust immunity [55].

IL-25, IL-33, TSLP, leukotriene D4 and IL-4 are the most notable activators of ILC2s.

Activation of Th2 cells through TCR leads to activation and translocation of NFAT to the nucleus, in addition to mobilization of AP1 and NF- κ B, all of which lead to the expression of Th2 cytokines. The latter two were described for ILC2s, how NFAT gets activated was not known. More recently, leukotriene receptors have been shown to activate NFAT in ILC2s [56].

Another protein important for Th2 activation is PKC- θ . PKC- θ was also expressed by ILC2 and its absence results in reduced ILC2 numbers, in addition to Th2 cell reduction. Moreover, IL-5, IL-13 as well as IRF4 production by ILC2s are regulated by PKC- θ , thus its deletion or inhibition blocks their production in HDM allergen-induced airway hypersensitivity model [57].

IL-33 in the lung is produced by epithelial cells (mostly pneumocytes) and antigen presenting cells (DCs and macrophages) [58].

ILC2s also express IL-4R and expand in response to IL-4 (produced by basophils) during atopic dermatitis [59].

3.2. ILC2s in protective immunity

ILC2s are important in defense against helminths/worms and rhinoviruses. *Nippostrongylus brasiliensis* or *Strongyloides venezuelensis* infection models are widely used in ILC2 studies, the infection of mice with these pathogens results in accumulation and activation of ILC2s in the lungs [60, 61]. Using IL-33KO mice, it was shown that ILC2 expansion and IL-13 production by ILC2s are important in immunity against hookworm *N. brasiliensis* in mice [62].

ILC2s has also been shown to expand during rhinovirus infection in mice in an IL-25-dependent fashion [63].

ILC2s also support the generation of a robust Th2 response. ILC2s function as a IL-4 source and by providing IL-4 they support Th2 differentiation or maintenance. Thus, during *H. polygyrus* infection in mice ILC2 specific deletion of the IL-4 result in diminished Th2 response [64].

3.3. ILC2s during inflammatory diseases

ILC2s are implicated in various chronic inflammatory conditions, including asthma, atopic dermatitis (AD) and chronic rhinosinusitis.

AD patient skins have increased ILC2 (as well as other ILC subsets) [65]. Studies with mouse models of AD also showed that ILC2 cells can induce AD symptoms in the absence of T cells

in Rag^{-/-} mice. IL-5, IL-13 and/or IL-2 may be important in driving pathology in these contexts [66–68].

Chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2) is expressed by Th2, ILC2, eosinophils and basophils. A humanized antibody targeting CRTH2 can deplete these cells along with ILC2 and have been shown to effectively ameliorate airway inflammation developed by various means in a humanized mouse model [69].

The contribution of ILC2 to the development of asthma in various mice models have been shown. Even in the presence of Th2 cells, ILC2s make a substantial contribution to pathogenesis through the production of IL-4, IL-5 and IL-13 during IL-25/IL-33 induced as well as house dust mite-induced asthma models [70]. The contribution of ILC2s to allergic inflammation was assessed most notably in Rag^{-/-} mice which lack adaptive immune cells. House dust mite (HDM), as well as papain protease-induced models of asthma/airway inflammation, was ameliorated in Rag1^{-/-} animals when ILC2s were deleted, and disease was restored when ILC2s were adoptively transferred in to Rag2^{-/-}/IL2rgc^{-/-} animals which lack ILC2s [49].

Furthermore, virally induced airway inflammation via H3N1 (within 5 days of infection) has also been shown to be dependent on ILC2 [71] and manifested itself in either the WT or Rag1^{-/-} mice. As expected, the inflammation was dependent on IL-33 and the downstream IL-13.

Tuft cells in the intestine constitutively produce IL-25 and regulate ILC2 homeostasis. After helminth infection, ILC2 activation relies on tuft cell-derived IL-25 which also further stimulates ILC2 for IL-13 production leading eventually to epithelial regeneration [72].

In a mouse model of cutaneous injury, ILC2 was suggested to promote wound healing through their activation by IL-33 [73]. ILC2s were found enriched in the nasal polyps of chronic rhinosinusitis patients as well [74].

For the eosinophilia associated with asthma or other allergen induced asthma models, ILC2-derived IL-13 was shown to be necessary. Additionally, ILC2s were proposed to cause the eosinophilia observed in some patients with autoimmune disease who are receiving experimental IL-2 therapy. This is shown to be mediated via IL-5 rather than IL-13 [75].

ILC2s were reported to be involved in obesity in various reports. ILC2s are present in the adipose tissue both in humans and mice and were shown to regulate adipocyte differentiation directly via ILC2-derived methionine-enkephalin peptides that act on adipocytes or indirectly by mobilizing eosinophils which eventually leads to adipocyte beiging. Thus, modulating ILCs or activatory cytokines IL-25 or IL-33 presents themselves as potential therapeutic approaches [76–79].

4. Group 3 innate lymphoid cells (ILC3)

Various subsets of RORγt⁺ group 3 ILCs have been reported both in humans and mice [80–82]. ILC3s are characterized as lineage—(CD3-Tcrab-Tcrγδ-CD11b-Cd19-) CD45⁺ CD127⁺ Ckit⁺ Rorγt⁺ IL-23R⁺ in both mice and humans. Currently, the ILC3 community categorizes them

into two major groups (LTi cells/NCR-ILC3s/CCR6+ ILC3s and NCR+ ILC3s); however, these two major groups do not represent final states, likely vary in their expression of various cell surface markers upon exposure to different microenvironment-specific stimuli.

LTi cells (CCR6+ ILC3s or NCR-ILC3s): Lymphoid tissue inducer (LTi) cells are the prototype of group 3 ILCs. They were found initially in the fetal liver of mice and were reported to be required for the generation of peripheral secondary lymphoid organs such as lymph nodes and Peyer's patches via their interactions with stromal cells. In the absence or dysfunction of LTi cells (shown through genetic deletion of the genes including *Roryt*, *IL-2R γ c*, *IL-7R* (CD127) and *LT α* , or *LT β R*), these organs fail to develop. LTi cells are also important for the development of cryptopatches and isolated lymphoid follicles in the intestine whose formation take place early after birth [83, 82]. Fetal LTi cells are characterized by the expression of CD127 (IL-7Ra), CCR6, *Roryt*, IL-23R and lymphotoxin $\alpha 1\beta 2$ [84]. LTi cells do not express natural cytotoxicity receptor (NKp46 or NKp44). Adult human and mice also harbor such LTi-like cells (adult LTi cells) with potential to induce lymphoid organogenesis and with similar surface markers [44, 85].

LTi cells also have subsets. Neuropilin+ subset is identified which mainly reside in tissues and nearby high endothelial venules [86]. LTi cell can also be categorized based on a CD4 expression.

NCR+ ILC3s (CCR6-ILC3s): The second major ILC3 subset express natural cytotoxicity receptors NKp44 or NKp46 in humans and mice, respectively [81, 87, 44]. It was debated whether these cells develop through a separate lineage than that of LTi cells. The most current evidence indicates that a branching occurs in the developmental pathway allowing progress of two different lineage pathways one leading to the precursors of NKp46+ ILC3, which do not give rise to true LTi cells. The other branch, on the other hand, leads to LTi development. NKp46+ ILC3, unlike LTi cells, express low levels of CCR6 and interestingly co-express the Th1 master regulator T-bet and *Roryt*. NCR+ ILC3s, in mice, have been shown to derive from CCR6-NCR- ILC3s, which expand shortly after birth in the intestines upon exposure to various dietary ligands. CCR6-NCR- ILC3s then can give rise to NCR+T-bet+ILC3s; further conditioning of these cells with IL-12 pushes them to ILC1 phenotype. Upregulation of NKp46 in CCR6-NCR- ILC3 requires T-bet and Notch signaling, thus in *Tbx21*^{-/-} mice, although CCR6+ and CCR6- ILC3 are present, upregulation of NKp46+ and transition from NCR-CCR6- state to NKp46+ ILC3 does not occur due to a block [6, 8, 88].

4.1. ILC3 development and activation

ROR γ t transcription factor is a common requirement for all ILC3 subset's development. As such, its genetic deletion results in complete absence of all ILC3 subsets and lack of lymph nodes and Peyer's patches as well as crypto patches and isolated lymphoid follicles in mice [89–91]. Like all other ILCs, *Id2*, *tox2* and *IL-7* are crucial for the development of ILC3s. Indeed, genetic deletion of any of these genes in mice results in a reduction in the ILC3 population [92]. *Tox 2* is transcription factor is necessary for upregulation of *Id2*. Also, TCF-1, NFIL3, *Gata3* were also shown to regulate ILC3 development [44]. AhR is another transcription factor required for the generation of CCR6- ILC3 subset. Genetic deletion of AhR in mice or removal

of AhR ligands from the diet result in a reduction in CCR6-ILC3 subsets [6, 44]. These studies also showed that CCR6- ILC3 subsets are involved in crypto patches and isolated lymphoid follicle formation in the small intestine. Our studies revealed that cytoskeleton protein dedicator of cytokinesis 8 (Dock8) is required for the generation/maintenance and function of adult ILC3s [93]. In the Dock8^{-/-} mice ILC3 cells are missing in the adult mice despite the fact that Dock8^{-/-} mice have normal lymph nodes and Peyer's patches suggesting that fetal LTi cells are spared. Lastly, as alluded above, T-bet is necessary for the development of NCR⁺ ILC3s; thus, in Tbx21^{-/-} mice these cells are missing [6].

All ILC3 subsets express the IL-23 receptor (IL-23R) and are activated by IL-23 produced by antigen presenting cells upon activation with various PAMPs [94]. IL-23 also regulates the expansion of ILC3s [95–97]. IL-23R signaling activates STAT3, which subsequently translocates to the nucleus, and turns on several ILC3 signature cytokine genes (IL-22, IL-17A/F etc.). In addition, IL-1 β was also shown to activate and expand ILC3s [97].

4.2. ILC3s in protective immunity

In the steady state, ILC3s assume critical functions in the mucosal surfaces for the containment of the commensals at an arms distance of epithelial cells [98, 99]. This is mainly achieved via ILC3-derived IL-22, which act on epithelial cells, in turn resulting in the production of various anti-microbial molecules, including Reg3 γ , Reg3 β , S100A8, S100A9 and mucins. Indeed, in the absence of ILC3s, microbial translocation across intestinal epithelium and detection of microbes in the distant organs/tissues has been reported [100].

Viral, bacterial, as well as fungal infection models have been used to dissect the role ILC3s during these infections. Rotavirus infection is cleared much more efficiently with the help of IL-22 coming from the innate sources (mainly ILC3). IL-22 was shown to synergize with IFN- γ to boost the production IFN- γ -mediated expression of antiviral genes [101, 102].

ILC3s also fight some enteric bacteria. The most notable example is attaching-effacing *Citrobacter rodentium*, which is widely used by the ILC3 community in murine models. ILC3s and innate IL-22 are necessary for protection from infection in mice lacking adaptive cells [93, 103, 104]. In the lymphoreplete mice, it was controversial whether ILC3s are absolutely required owing to the presence of Th17 cells, and data indicate in the immunocompetent mice that the redundant mechanisms may eventually save the mice [104].

To dissect the role of ILC3s in fungal immunity *Candida albicans* has been used in murine models. Rag1^{-/-} mice became susceptible when ILC3s are depleted, or when *Rorc* is deleted [105, 106]. Given that absence of Th17 axis leads to susceptibility to *Candida* infections, ILC3s are likely important, however, how essential they are, whether Th17 and ILC3 redundantly control the immunity is unknown.

4.3. ILC3s during inflammatory diseases

ILC3 are implicated in several chronic inflammatory diseases based on data obtained from murine models and human patients. Accumulation of ILC3-like cells (defined as NK-22 in the

paper with ability to produce IL-22 and TNF- α) or LT α i cells in the synovial fluid of rheumatoid arthritis patients have reported [107, 108].

IL-23 pathway and IL-17 has been shown to drive the pathogenesis of spondylarthritis. Antibodies against IL-17 are being tested in trials. In the synovial fluid of psoriatic arthritis patients, increased IL-17 $^{+}$ NKp44 $^{+}$ ILC3s were reported; augmented amounts of CCL20 in the synovial fluid imply that CCR6 $^{+}$ ILC3s may be attracted through this ligand [30, 33, 109].

NKp44 $^{+}$ ILC3s enrichment in the tissues (ileum, synovial fluid, blood bone marrow) of ankylosing spondylitis have been reported. Some of the studies found IL-17 production by these ILC3s, others reported them as an IL-22 source [30].

NKp44 $^{+}$ ILC3 are also implicated in systemic sclerosis and systemic lupus erythematosus [30, 110].

In various mouse models of IBD, ILC3s were shown to play critical roles [111]. In infection-induced colitis (by *Citrobacter rodentium* or *Helicobacter pylori*), these cells mediate pathology via IL-17 and IFN- γ [95]. ILC3s, via IL-22, have also been shown to drive pathology in some IBD models [96, 104], whereas in other murine models ILC3-derived IL-22 was shown to be protective [112]. In human Crohn's disease patients' intestines, ILC3s were enriched. CD56 $^{+}$ ILC3s were shown to produce IL-22, whereas CD56 $^{-}$ ILC3s produced IL-17 and IFN- γ [113]. Others, however, reported enrichment of IFN- γ $^{+}$ ILC1s, rather a reduction in NCR $^{+}$ IL-22 ILC3s [7, 114]. IFN- γ $^{+}$ ILC1s presence in the intestine appear to be a common theme in both human IBD and murine IBD models, in fact, by fate map experiments, IFN- γ $^{+}$ ILC1s have been shown to derive from ILC3s via gradual loss of Ror γ t [6, 8, 35].

ILC3s have also been shown to be crucial for the induction of peripheral tolerance to commensal antigens via MHCII molecules they express along with low levels of costimulatory molecules. Deletion of MHCII in ILC3s break this tolerance and results in IBD-like disease in mice models [115, 116].

Increased ILC3 number and/or activity have been reported in other autoimmune diseases [30]. In psoriasis patients, both in the skin and blood, elevated frequency of ILC3s either producing IL-22 or IL-17A has been described [117, 118]. Similarly, in the mouse model of multiple sclerosis, EAE, ILC3 presence in the brain was reported. More importantly, ILC3 number or activity is also described in MS patients blood and CSF [119–122]. In both of these autoimmune conditions, how actually ILC3s impact the disease progression is yet to be defined.

5. Plasticity of ILCs

Most recent research indicates that ILCs could modify their transcriptional program and convert to another type in the presence of environmental cues that favor the effector functions of one over the other. This was proposed to occur between ILC3 and ILC1, via modulating the availability of IL-23 or IL-12. Similar plasticity has been reported between ILC2 and ILC1. A summary and discussion of the current information regarding the plasticity between ILC subsets are presented below.

5.1. ILC2 to ILC1 or ILC2 to ILC3 plasticity

A few intracellular molecules that maintain ILC2 identity have been defined. Bcl11b and Gfi1 maintain the expression of ILC2 genes associated with ILC2 identity, the deletion of either gene in mice blocks ILC2 master regulator Gata3 expression and, subsequently, IL-5 and IL-13 production [54, 123]. Bcl11b or Gfi1 KO ILC2 can also produce IL-17. Lysine methyltransferase G9a gene was shown to also suppress ILC3-related genes' expression in ILC2. Additionally, Zhang et al. showed that Notch signaling can promote Rorc and IL-17 production by iILC2 cells that are primarily responsive to IL-25 [124].

External stimuli that drive phenotypic switch from ILC2 to other innate lineages have been demonstrated recently in murine models [27, 29, 125]. IL-12 and/or IL-18 have been shown to push ILC2 to an ILC1-like phenotype (with increased production of IFN- γ and reduced Gata-3 expression). This conversion appears to be driven by viruses (influenza, RSV) and bacteria (*S. aureus*). Observations in human patients of chronic obstructive pulmonary disease (COPD) or chronic rhinosinusitis with nasal polyps (CRSwNP) have been shown to harbor elevated levels of ILC1 and ILC2 cells, and their signature cytokines imply that this phenotypic conversion may occur in humans as well. A detailed examination of ILC2 fate in human hematopoietic cells-engrafted IL2 γ ^{-/-}Nod/scid mice suggested that these cells indeed can assume an ILC1 phenotype with IL-12 exposure [27]. IL-1 β was shown to prime ILC2s at high concentrations and potentiate the IL-12 driven phenotypic switch to Gata3⁺ T-bet⁺ ILC1 fate [125].

5.2. ILC3 to ILC1 plasticity

The plasticity of ILC3 lineage was first described by Vonarbourg et al. in a study which employed Ror γ t fate map mice [8]. In this work the authors demonstrated that Ror γ t⁺ ILC3s downregulated this transcription factor, gradually upregulated T-bet and NKp46, this eventually led to a cell population termed as "ex- ILC3". This transition requires T-bet, notch signaling. IL-12 and IL-15 were shown to promote this transition. Conversion of ILC3s to ILC1s has been observed by many other investigators in mice. More importantly, ILC3-to ILC1 conversion has been shown to operate for human ILC3s in a reversible fashion, dictated by the presence of IL-12 in ILC3 to ILC1 direction and by IL-23 in the other direction [7, 35, 114].

6. Conclusions

The discovery of ILCs brought a paradigm shift in our understanding of both innate and adaptive immunology. From a developmental standpoint, understating the lineage specification in ILCs, their similarities to and differences from helper T cells need to be worked out in better detail moving forward. Ambiguities regarding ILC1-NK cell classifications require more studies. Various gene expression studies have been done to identify such unique and distinctive molecules, and hopefully, more functional studies will remove the confusion. More importantly, the extent and nature of the involvement of these cells in the inflammatory diseases and tissue homeostasis will be further evaluated beyond diseases described for the past 7 years. Also, the discovery of more specific surface markers and inhibitors that will

target exclusively ILCs are needed for both understanding the possible redundancy between ILCs and helper T cells and for potential use as therapeutics.

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