Immunology Letters 179 (2016) 2–8

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Human innate lymphoid cells

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ARTICLE INFO

Article history:
Received 11 January 2016
Accepted 25 January 2016
Available online 1 February 2016

Keywords:
ILC
ILC development
NK cells
NK cell differentiation
HSCT

ABSTRACT

The interest in innate lymphoid cells (ILC) has rapidly grown during the last decade. ILC include distinct cell types that are collectively involved in host protection against pathogens and tumor cells and in the regulation of tissue homeostasis. Studies in mice enabled a broad characterization of ILC function and of their developmental requirements. In humans all mature ILC subsets have been characterized and their role in the pathogenesis of certain disease is emerging. Nonetheless, still limited information is available on human ILC development. Indeed, only the cell precursors committed toward NK cells or ILC3 have been described. Here, we review the most recent finding on human mature ILC, discussing their tissue localization and function. Moreover, we summarize the available data regarding human ILC development.

1. Introduction

Innate lymphoid cells (ILC) are a heterogeneous population of cells that have been a subject of intense research during the past few years. ILC are innate lymphocytes that, unlike adaptive T and B lymphocytes do not express rearranged antigen specific receptors [1]. ILC effector function and transcription factor requirement partially resemble those of T lymphocytes [2]. Accordingly, ILC have been classified into killer-ILC and helper-ILC that mirror $CD8^+$ cytotoxic T cells and $CD4^+$ T helper cells, respectively [1]. Killer-ILC are represented by natural killer (NK) cells, the first ILC subset identified already in the 1970s [3]. NK cells display cytolytic activity and produce cytokines, primarily IFNγ, and mediate host defences against both tumor and virus-infected cells. NK cells express Eomesodermin (Eomes) and T-box transcription factor T-bet, required for their development and function. Helper-ILC are further classified into ILC1, ILC2, and ILC3. ILC1 express T-bet and secrete IFNγ, but different from NK cells, do not exert cytolytic activity. ILC1 have been shown to be involved in responses against protozoa and intracellular bacteria. ILC2 depend, for their development, on expression of GATA binding protein 3 (GATA3) and produce primarily IL-13 and IL-5. They contribute to the defence against helminthes and are involved in allergic responses. Finally, ILC3 express the retinoic acid receptor related orphan receptor (RORγt) and produce "type-17" cytokines, mainly IL-17 and IL-22. ILC3 include fetal lymphoid inducer (LTi) cells, which drive secondary lymphoid organ development during embryogenesis, and post-natal ILC3 that are involved in tissue homeostasis and defence against extracellular pathogens. The majority of studies that allowed the characterization of ILC function and development have been performed in mice [1,4,5]. Here we will review our current knowledge on ILC tissue distribution, function and development in humans.

2. Human ILC localization and function

2.1. *NK cells* vs *ILC1*

NK cells were the first ILC subset to be identified. Accordingly, they are the most widely characterized ILC population [6]. While helper-ILC are scarcely represented in peripheral blood (PB), NK cells may represent up to 15% of peripheral blood (PB) lymphocytes. NK cells include two main subsets, *i.e.* $CD56^{bright}CD16^-$ cells

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http://dx.doi.org/10.1016/j.imlet.2016.01.007
0165-2478/

and CD56^{dim}CD16^{+} cells, that differ in terms of phenotype, effector function, and tissue localization. CD56^{dim} cells account for ≈90% of PB NK cells. CD56^{dim} NK cells express the Fcγ receptor CD16, through which they can exert the antibody dependent cell-mediated cytotoxicity (ADCC). Moreover, NK cells mediate "natural cytotoxicity" *via* a set of activating receptors, which recognize their ligands on tumor or virus-infected cells. The main activating NK receptors are the natural cytotoxicity receptors (NCR, *i.e.* NKp46, NKp30 and NKp44), NKG2D, and DNAM-1 [6]. NK cells also express HLA-class I specific inhibitory receptors that prevent killing of autologous normal cells. In particular, CD56^{dim} NK cells express Killer Immunoglobulin Receptors (KIRs) and CD94/NKG2A [6]. CD56^{dim} NK cells express high levels of perforine and granzyme that mediate high cytotoxic activity. Moreover, CD56^{dim} cells produce cytokines in response to the engagement of activating receptors. On the contrary, CD56^{bright}(CD16^{-}KIR^{-}NKG2A/CD94^{+}perforine^{low}) cells are poorly cytotoxic and are major cytokine producer in response to cytokines, such as IL-12, IL-18, or IL-15. While CD56^{bright} NK cells constitute the minority of PB NK cells, they represent the large majority of NK cells in secondary lymphoid organs. Of note, CD56^{dim} and CD56^{bright} differ in the expression of chemokine receptors, and their tissue distribution in healthy tissues is in accordance with the pattern of expression of chemotactic factor in solid organs. However, most of NK cells present in normal tissues are CD56^{bright} [7].

Recent evidences in mice and humans have suggested that ILC1 represent a resident population within tissues while being barely detectable in PB. ILC1 differ from NK cells because they lack the expression of Eomes and do not exert perforin-dependent cytolytic activity. Studies in mice suggested that Tbet^{+}Eomes^{+} NK cells and Tbet^{+}Eomes^{-} ILC1 develop from distinct precursor cells of bone marrow and peripheral origin, respectively [8]. However, several phenotypic overlaps exist between NK cells and ILC1 [9–11]. Human CD127^{+}Tbet^{+}Eomes^{-}IFNγ^{+}(CD161^{+}CD56^{-}NKp44^{-}KIR^{-}Perforine^{-}) ILC1 have been identified in the gut and have been shown to be enriched in the intestine of Crohn disease patients [12]. Concomitantly, Fuchs et al. defined "intraepithelial ILC1" a population of CD127^{+} in the gastrointestinal epithelia and in tonsils, which was characterized by the CD56^{+}NKp44^{+}CD103^{+} phenotype, but also expressed Eomes. Subsequently, CD127^{+}CD161^{+}NKp44^{-} ILC1 have been identified also in the skin [13]. Interestingly, Marquardt et al. identified a population of intrahepatic CD3-CD56^{+}CD49a^{+}(NKp44^{-}CD103^{-}KIR^{+}CD57^{-}) that expressed Tbet, but not Eomes, similar to murine liver ILC1 [14]. The presence of CD127^{+}Tbet^{+}Eomes^{-}IFNγ^{+} ILC1 has also been reported in human decidua [15], however, Eomes^{+} NK cells represent the large majority of lymphocytes in this tissue [11]. Of note, most human decidual and endometrial Eomes^{+} NK cells express CD49a, as liver ILC1 [14]. Moreover, they can express CD103 and CD9 (markers of TGFb-exposure) and NKp44 similarly to the "intraepithelial ILC1" [10]. Thus, in humans, a clear distinction between ILC1 and NK cells is not easy. Tissue specific factors may influence the features of resident ILC populations and/or drive phenotypic changes in NK cells migrated from PB. An extensive transcriptional analysis would help defining whether ILC1 and NK cells indeed represent distinct developmental lineages as reported in mice.

2.2. ILC2

Group 2 ILC were initially identified in mice as an innate source of type-2 cytokines in 2001 [16], but they were fully characterized only in 2010 [17–19]. Later, Mjosberg et al. identified also humans ILC2 in fetal and adult gut and lung and in PB [20]. These cells are also present in the skin [21,22], in the adipose tissue [23], and in tonsils [12]. Experimental murine models have demonstrated that ILC2 contribute to anti-helminthic responses. However, they may also play a pathogenic role in experimental models of asthma, and of lung and skin diseases [24]. Although alterations of the ILC2 proportion have been reported in PB of patients experiencing helminthic infections, the actual involvement of these cells in human anti-helminthic responses has yet to be defined [25]. Of note, ILC2 accumulate in the skin of patients with atopic dermatitis (AD) [26,27] and in the nasal polyps of patients with chronic rhino sinusitis [20]. Similar to the murine counterpart, human ILC2 express GATA3 transcription factor. Moreover, they express CD161, CD127, CD25 (IL-2Rα), ST2 (IL-33R), and IL-17RA. ILC2 respond to IL-33 and IL-25 (IL-17E) by producing primarily the type-2 cytokines IL-13, IL-5, and IL-4. Nonetheless, ILC2 have been reported to produce also pro-inflammatory cytokines such as GM-CSF and IL-8 as well as IL-3, IL-9, and IL-21. ILC2 express the prostaglandin D_2 (PGD_2) receptor CRTH2, but its expression could be modulated by the tissue specific cell localization [28]. Of note, PGD_2 can induce the migration of PB and skin ILC2 as well as enhance their cytokine production [29]. All these findings suggest that human ILC2 may respond to epithelial derived cytokines and to mast cell derived metabolites. In turn, they would contribute to early type-2 responses trough the production of cytokines that can promote eosinophil recruitment and survival, and Th2 polarization. A recent report has also shown that *ex vivo* isolated and *in vitro* activated ILC2 express the NKp30 NCR. Moreover, B7-H6- mediated cell stimulation *via* NKp30 induced the production of type-2 cytokines [30]. Notably, B7-H6 has a widespread expression in the skin of patients with AD as compared to healthy subjects. This suggests that, in the skin of AD patients, ILC2 may be activated also trough NKp30.

2.3. ILC3

Both murine and human ILC3 are identified as Lin^{-}CD127^{+}RORγt^{+} cells. ILC3 include two main cell subsets, *i.e.* fetal LTi and postnatal ILC3. In mice it has been shown that the fetal LTi cells are among the first cells to populate the site of lymph node (LN) development. LTi can induce the up-regulation of adhesion molecules on stromal cells and the release of chemokines that promote the recruitment of T, B and dendritic cells that would colonize the LN, thanks to the interaction between lymphotoxin αβ and its receptor [31,32]. In humans, Lin^{-}CD45^{+}CD127^{+} LTi cells have been identified in fetal mesenteric LN and spleen during the first and second trimester of pregnancy [33,34]. Similar to murine LTi, they express *RORC, LTA, LTB,* IL-17, and IL-22, however, they lack CD4 and express CD161 and CD7. Human LTi cells co-cultured *in vitro* with stromal cells can induce the up-regulation of ICAM and VCAM [33], suggesting that they may play *in vivo* a lymphoid tissue inducing activity, similar to murine LTi cells.

After birth, murine ILC3 are required for the development of cryptopatches and isolated lymphoid follicles in the gut [35,36], in the remodelling/repair of LN after infection [37], and in the homeostasis of the intestinal epithelial barrier [38–40]. ILC3-derived IL-22 induces the release of antimicrobial proteins by intestinal epithelial cells, thus contributing to host protection against extracellular pathogens [41,42]. In humans, ILC3 have been identified in several organs. Human adult ILC3 were first described in intestine and tonsils as Lin^{-}CD56^{+} cells characterized by the expression of the typical NK cell receptor NKp44 and by IL-22 production and they were initially termed NK22 [43]. According to the proposed classification of adult ILC3 into NCR^{+} and NCR^{-}, NKp44^{+} ILC3 were thus re-termed NCR^{+}ILC3 [44], however subsets of NKp44^{-} ILC3 can express NKp46 [45]. Therefore, the definition of NCR^{+}ILC3 should not only rely on NKp44, but also on NKp46 expression. The different subsets of adult ILC3 differ in their cytokine profile. Besides IL-22, tonsil NCR^{+}ILC3