Human Innate Lymphoid Cells

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Abstract

Innate lymphoid cells (ILC) are lymphoid cells that do not express rearranged receptors and have important effector and regulatory functions in innate immunity and tissue remodeling. ILC are categorized into three groups, based on their distinct patterns of cytokine production and the requirement of particular transcription factors for their development and function. Group 1 ILCs (ILC1) produce interferon gamma (IFNγ) and depend on Tbet, group 2 ILC (ILC2) produce type 2 cytokines like IL-5 and IL-13 and require GATA3, and group 3 ILC (ILC3) include lymphoid tissue inducer (LTi) cells, produce IL-17 and/or IL-22 and are dependent on RORγt. Whereas ILC play essential roles in the innate immune system, uncontrolled activation and proliferation of ILC can contribute to inflammatory autoimmune diseases. In this review we provide an overview of the characteristics of ILC in the context of health and disease. We will focus on human ILC but refer to mouse studies if needed to clarify aspects of ILC biology.
Introduction

Innate lymphoid cells (ILC) constitute a recently identified family of mononuclear hematopoietic cells with key functions in the preservation of epithelial integrity and tissue immunity throughout the body. They are defined by their lymphoid morphology (Figure 1A) and the absence of rearranged antigen specific receptors.1 Two prototypic members of the ILC family are Natural Killer (NK) cells and Lymphoid Tissue inducer (LTi) cells. NK cells were discovered in the mouse in 1975,2 and are operationally defined by the capability to kill certain target cells in the absence of antigen specific priming. LTi cells, identified in 1997,3 are essential for the formation of lymph nodes during embryogenesis and are also present in the post-natal gut, where they are important for the formation of cryptopatches and intestinal lymphoid structures, also called isolated lymphoid follicles (ILF). NK cells and lymphoid tissue inducer (LTi) cells are developmentally related, as both cell types require the common gamma (γc) chain of the IL-2 receptor and the transcriptional repressor Id2 for their development.4

Recently, several distinct ILC populations have been identified that also depend on the γc chain5–7 and Id2 for their development.6,8 Each of these ILC populations show distinct patterns of cytokine production that mirror the cytokine-secreting profiles of T helper cell subsets.9 Recent studies demonstrated that ILC populations have important effector functions during the early stages of the immune response against microbes,5,6 in tissue repair,10,11 in the anatomical containment of commensals,12 and in maintaining epithelial integrity at barrier surfaces.13 ILC function needs to be
tightly regulated as uncontrolled activation and proliferation can contribute to severe inflammation and damage in gut,\textsuperscript{14} lung,\textsuperscript{15,16} skin\textsuperscript{17,18} and liver.\textsuperscript{19}

A group of researchers has proposed a classification of these ILC populations, on the basis of their phenotypical (Figure 1B) and functional characteristics.\textsuperscript{20} The nomenclature is based on the T helper cell nomenclature and categorizes the ILC subsets into three groups (Figure 2): Group 1 ILCs (ILC1) comprise ILCs that produce interferon gamma (IFN\textgamma); group 2 ILC (ILC2) produce type 2 cytokines, in particular IL-5 and IL-13, and group 3 ILC (ILC3) produce IL-17 and/or IL-22. In this model NK cells were classified in group 1 ILC because of their capacity to produce IFN\textgamma.\textsuperscript{20} Recent information on the developmental pathways of mouse ILC, however, suggest that NK cells and CD127\textsuperscript{+} ILC should be considered as the innate forms of CD8\textsuperscript{+} and CD4\textsuperscript{+} T cells, respectively.\textsuperscript{21} Development of NK cells and CD127\textsuperscript{+} ILC may be driven by the transcription factors Nfil3\textsuperscript{22} and GATA3,\textsuperscript{23,24} respectively (Figure 2).

In this review we provide an overview of the characteristics of distinct ILC subsets with emphasis on human ILC. Because of space constraints we will not extensively review human LTi cells, but refer to reviews that are published elsewhere.\textsuperscript{1,25} In addition, some excellent recent reviews provide more detailed information on mouse ILC biology.\textsuperscript{26,27}

**Key features of group 1, 2 and 3 ILC**

ILC1 are not yet well defined. Both in humans and mice several populations have been described that produce IFN\textgamma but not other signature cytokines such as IL-17, IL-22 or IL-5. The developmental relationship of these ILC1 is not yet clarified and
the mouse equivalents of currently identified human ILC1 and vice versa are not precisely known. Human group 1 ILC include two ILC1 subsets that can be distinguished from NK cells and each other based on their intestinal anatomical location and the expression of certain surface markers. Intraepithelial CD127\textsuperscript{low} ILC1 express CD103, CD56, CD94 and NKp44, and are responsive to IL-12 and IL-15.\textsuperscript{28} The intraepithelial CD127\textsuperscript{low} ILC1 bear resemblance to NK cells as these cells are CD56\textsuperscript{+} and also express perforin which is essential for cytotoxicity.\textsuperscript{28} The equivalent of CD127\textsuperscript{low} ILC1 in the mouse are claimed to be intraepithelial CD160\textsuperscript{+} ILC (CD160 is also expressed on human CD127\textsuperscript{low} ILC1), which, like NK cells, are dependent on the transcription factor Nfil3 but, in contrast to NK cells, are independent of IL-15 for their development.\textsuperscript{28} It has been argued that the CD127\textsuperscript{low} ILC1 are the equivalents of intraepithelial CD8\textsuperscript{+} T cells\textsuperscript{28} which would fit with the emerging concept that Nfil3-dependent ILC are innate equivalents of CD8\textsuperscript{+} T cells.\textsuperscript{21} Human CD127\textsuperscript{high} ILC1 are predominantly located in the lamina propria, lack CD56, CD94 and NKp44 expression, and respond to IL-12 and IL-18 by producing IFN\gamma.\textsuperscript{29} It is likely that human CD127\textsuperscript{high} ILC1 depend on IL7 for their development but this has yet to be confirmed.

Both human CD127\textsuperscript{low} and CD127\textsuperscript{high} ILC1 populations highly express Tbet and it is likely that Tbet is important for development. Recently a Tbet-dependent ILC1 population was identified in the mouse liver that displays some cytotoxic activity\textsuperscript{30} but differs from NK cells by lacking expression of the transcription factor Eomes and develops from a precursor that is unable to differentiate into NK cells.\textsuperscript{21,30,31} The human equivalent of these cells have yet to be identified.
Group 2 ILCs (ILC2) are responsive to IL-25, IL-33, thymic stromal lymphopoietin (TSLP) and produce type 2 cytokines, predominantly IL-5 and IL-13 but also amphiregulin, that is important for tissue repair. GATA3 is essential for development and function of human ILC2, whereas NOTCH signaling stimulates development of human ILC2. Likewise mouse ILC2 are dependent on GATA3 and Notch, and in addition to TCF-1 and RORα for their development and function.

Group 3 ILC (ILC3) respond to IL-23 and IL-1β by secreting IL-22. ILC3 can be divided into natural cytotoxicity receptor (NCR) (NKp46 in mice and NKp44 and NKp46 in humans) and NCR- ILC3. In the mouse ILC3 depend on the transcription factors Notch, TCF-1 and RORγt for their development and function, but the requirement of these factors for human ILC3 development has yet to be confirmed. Mouse ILC3 that express the NCR NKp46 require also Tbet for their development, but it is unknown whether this is also the case for human NKp44+ILC3. It has been observed that upon stimulation with IL-1β and IL-23, human RORγt+ NKp44+ ILC3 can differentiate in vitro into NCR+ ILC3 and under influence of IL-12 into CD127+ ILC1. NCR+ILC3 can also differentiate into ILC1 upon stimulation with IL-12. During this process these cells down-regulate RORγt and up-regulate Tbet. A similar transition of NCR+ILC3 into INFγ-producing Tbethigh RORγtklow ILC has been observed in vivo in a mouse model. These data indicate that ILC3 are plastic cells that can adopt an ILC1 fate depending on environmental cues. An overview of the ILC subsets, their phenotype, the signature
cytokines they produce, their critical transcription factors and their developmental relationships is provided in Table 1, Table 2 and in Figure 2.

**Developmental relationship of ILC and NK cells**

Since human CD127\(^+\) ILC express many NK cell markers including CD56, NKp44, and CD161\(^+\), the question was raised what the relationship was of NK cells and CD127\(^+\) ILC. The developmental pathway of NK cells in the mouse has been studied in detail and has been reviewed extensively recently,\(^{48}\) but the developmental pathways of human NK cell development and the transcription factors that control this process are less well defined.\(^{49,50}\) Earlier studies have led to a model in which four stages of human NK cell development can be distinguished on the basis of expression of CD34, cKit, CD94 and CD56.\(^{51,52}\) Stage 1 cells are CD34\(^+\)CD56\(^-\) cells that most likely overlap with the human common lymphoid progenitor (CLP); stage 2 cells co-express CD34 and CD117 (cKit); stage 3 cells, called immature NK cells (iNK), express CD117 and CD56, and stage 4 and 5 cells are more mature CD56\(^+\) CD94\(^+\) CD16\(^-\) and CD56\(^+\) CD94\(^+\) CD16\(^+\) NK cells, respectively.

When it was found that iNK cells produced IL-22,\(^{53}\) it was speculated that IL-22 production was a property of iNK cells which then loses this capacity upon maturation to conventional NK cells. However, more recent analyses have revealed that stage 3 iNK cells comprise a heterogeneous population. A substantial proportion of these cells were ROR\(\gamma\)\(^+\), expressed CD127 and produced IL-22 and were unable to differentiate into mature NK cells *in vitro*, strongly suggesting that these cells within the iNK population are in fact mature ILC3 and not immature NK
Concurrently the minority of iNK cells lacked expression of CD127 and those cells were able to differentiate into mature NK cells \textit{in vitro}.\textsuperscript{54} In another study it was confirmed that human CD56\textsuperscript{+} ILC3 generated from cord blood hematopoietic stem cells are unable to differentiate into conventional NK cells.\textsuperscript{55} In line with these observations, experiments with ROR\gamma t fate mapped mice demonstrated that ROR\gamma t is not expressed during the development of NK cells.\textsuperscript{56,57} In addition, whereas NK cells are IL-7 independent, both human and mouse ILC3 require IL-7 for optimal development.\textsuperscript{56,57} These data clearly demonstrate that ROR\gamma t\textsuperscript{+} ILC and conventional NK cells belong to different lineages. Since 50\% of all ILC3 cells express CD56,\textsuperscript{40} CD56 cannot be used as a defining marker for NK cells, as previously thought. Other markers such as KIRs, CD94 and CD16, which are not expressed on CD127\textsuperscript{+} ILCs, should be used to distinguish between CD127\textsuperscript{+} CD56\textsuperscript{+} ILC3 and conventional NK cells. LFA-1 was found to be selectively expressed on human NK cells, but not on ILC3, qualifying LFA-1 as another marker to distinguish these two populations.\textsuperscript{55} With the recent identification in mice of committed precursor subsets that gives rise to group 1, 2 and 3 ILC but not to NK cells (or T and B lymphocytes), it becomes clear that NK cells indeed represent a subset of ILC that are developmentally related to but distinct from ILC1, ILC2 and ILC3.\textsuperscript{21,31} These ILC precursor subsets expresses the transcription factors PLZF, Id2, GATA3 and TOX and are phenotypically defined as cells that are lineage negative, IL7R\alpha\textsuperscript{+} ckit\textsuperscript{+} \alpha4\beta7\textsuperscript{+}. Human ILC progenitor subsets that are committed to develop into ILC1, ILC2 or ILC3 but have lost the potential to develop into NK cells have yet to be identified.
Tissue distribution of human ILC

In healthy individuals, about 0.01 to 0.1% of circulating lymphocytes express a CD127+ ILC phenotype. The majority of CD127+ ILC found in peripheral blood are group 2 ILC, whereas NKp44+ ILC3 and CD127- ILC1 are nearly absent (Figure 1B). Peripheral blood ILC subsets from healthy individuals do not express cytokine transcripts indicating that they are not activated. The composition of human ILC subsets in tissues depends on the tissue type. For instance, whereas group 2 ILC and NKp44+ ILC3 are the most prevalent ILC subsets in healthy human skin tissue, in other tissues such as thymus, tonsils and bone marrow and in the gut, NKp44+ ILC3 is the prominent ILC subset. In the following paragraphs we review our knowledge of human ILC in intestine, lung and skin. Mouse ILC have also been identified and characterized in adipose tissue, where they promote accumulation of eosinophils and alternatively activated macrophages which are implicated in metabolic homeostasis. Moreover, these cells are resident in liver and when stimulated with IL-33 they can mediate liver fibrosis. Thus far there is no information available about ILC in the human liver and adipose tissue. Recently, ILC3 were detected in the human spleen, where they interact with stromal cells for survival signals and with innate B cells to produce antibodies.

ILC in the intestine

In 2009, in parallel with the identification of human fetal LTi cells and post natal tonsillar LTi-like cells, the first report appeared demonstrating the presence of human IL22 producing ILC3 in the healthy gut (Figure 3A). These NKp44+ ILC3,
which were originally called NK22 cells, produce IL-22 that signals to epithelial cells where it promotes proliferation, IL-10 and antimicrobial peptide production, and mucus production.\textsuperscript{5} In vitro, NKp44\textsuperscript{+} ILC3 were responsive to IL23, IL1\(\beta\), IL-2, and IL-7 by producing IL-22,\textsuperscript{43,44,63} which is enhanced in the presence of TLR2\textsuperscript{63} and NKp44 ligands.\textsuperscript{64} Furthermore, human gut ILC3 express transcripts for leukemia inhibitory factor (LIF),\textsuperscript{5} which induces proliferation of epithelial cells, and IL-26,\textsuperscript{5} a cytokine that, like IL-22, belongs to the IL-10 cytokine family. IL-26 negatively modulates proliferation of intestinal epithelial cells and induces secretion of pro-inflammatory cytokines TNF and IL-8 by these cells.\textsuperscript{65} Also, gut resident NKp44\textsuperscript{+} ILC3 produce cytokines that act on T and B cells including IL-2,\textsuperscript{63} the B cell activating factor B cell activating factor (BAFF) that supports survival and expansion of mature B cells,\textsuperscript{44} and the chemokine CCL20 that directs the migration of T and B lymphocytes and ILC into the gut.\textsuperscript{44}

The observation that TLR agonists can activate NKp44\textsuperscript{+} ILC3 raised the question whether microbiota regulate not only the function but also the development of these cells. However, analysis of ILC3 in the human fetal gut revealed that the development of NKp44\textsuperscript{+} ILC3 is a programmed event independent of commensals.\textsuperscript{66} These data are consistent with mouse studies demonstrating that IL-22 producing ILC3 are present in the gut during fetal life, before intestinal colonization.\textsuperscript{12,67}

Studies in mouse models showed the importance of IL-22 producing NCR\textsuperscript{+} ILC3 in the protection against colitis induced by the attaching and effacing pathogenic bacterium \textit{Citrobacter rodentium},\textsuperscript{5,7,41} and in the anatomical containment of commensal intestinal bacteria.\textsuperscript{12} Recently, another role of ILC3 in the maintenance
of gut homeostasis in mice was revealed. It was demonstrated that NCR- ILC3 express MHC class II antigens and present microbial antigens to gut CD4+ T cells. This did not result in activation, presumably because ILC3 lack co-stimulatory molecules, but in inactivation of gut commensal bacteria specific T cell responses. Of note, also human ILC3 were found to express MHC class II molecules in this study, suggesting that this class II MHC dependent control of T cells is also operational in the human gut. ILC3 have in addition been shown to regulate Th17 cells via the aryl hydrocarbon receptor (AHR) in mice. AHR is a ligand dependent transcription factor; the ligands include environmental toxins such as dioxin derivatives, dietary components and endogenous ligands such as the tryptophane metabolite. Qiu et al. observed that Th17 cells were strongly increased in Ahr-/- mice. This effect was indirect: the absence of AHR resulted in diminished IL-22 production which in turn caused segmented filamentous bacteria, known to stimulate Th17 cells, to expand. Whether this type of control of Th17 cells is also functional in human gut remains to be established.

Collectively, these studies show that in mice, ILC3 play a crucial role in gut immunity, by directly inducing epithelial cell proliferation, promoting epithelial derived production of anti-inflammatory cytokines and anti-microbial peptides, preventing dissemination of commensal bacteria, and by suppressing microbiota specific pro-inflammatory CD4+ T cell responses, and suggest that in humans gut ILC3 may have similar functions.

The human gut also contains CD127^{high} ILC1 and CD127^{low} ILC1. CD127^{low} ILC1 respond to danger signals originating from epithelial cells and myeloid cells
suggesting that they play a role in the immune response against pathogens that elicit these danger signals, but the specific pathogens that provoke a response of these cells have yet to be identified. Also the function of human CD127\textsuperscript{high} ILC1 cells has yet to be firmly established. Since there is uncertainty about the exact equivalent of these cells in mice, a precise determination of the \textit{in vivo} function of CD127\textsuperscript{high} ILC is not yet possible. However, because in mice Tbet dependent, IFN\gamma producing ILC were found to be important in the immune response against \textit{Salmonella enterica}, we speculate that human CD127\textsuperscript{high} Tbet\textsuperscript{high} ILC1 also play a role in the immune response against pathogenic gut bacteria. This hypothesis is supported by the observation that in contrast to ILC3 and ILC2, CD127\textsuperscript{high} ILC1 are not present in the human fetal gut, suggesting that colonization with bacteria triggers the appearance of these cells. Interestingly, a pronounced accumulation of IFN\gamma-producing CD127\textsuperscript{low} ILC1 and CD127\textsuperscript{high} ILC1 was observed in inflamed intestinal tissues of Crohn’s disease patients, whereas the frequency of NKp44\textsuperscript{+} ILC3 was diminished, possibly through IL-12 dependent differentiation of ILC3 towards ILC1. Of note, other ILC subsets such as IL-17 producing CD56\textsuperscript{-} ILC3 are also expanded in Crohn’s disease patients. The importance of IL-12 and IL-23 responsive lymphocytes in the pathobiology of Crohn’s disease has been emphasized recently by the observation that blockade of the IL-12/IL-23 axis by Ustekinumab, an inhibitor of the subunit dimer p40 that is shared between IL-12 and IL-23, led to disease remission in TNF antagonist resistant patients. The role of ILC2 in gut homeostasis and immunity has received considerably less attention. ILC2 are also present in the human gut, most prevalently in the fetal gut;
in adult human intestinal tissues CRTH2+ ILC2 account for only a very small minority of the total CD127+ ILC pool. Fetal gut ILC2 express transcripts for IL-5 and IL-13 in situ but the role of these cytokines in fetal gut is unknown. It is possible that ILC2 play important roles in tissue generation in the gut. In the mouse ILC2 are the source of IL-5 that regulate eosinophil homeostasis and IL-13 that may induce alternatively activated macrophages (AAM). Upon infection with nematodes, murine gut ILC2 contribute to clearance of worms by producing IL-9 and IL-13. The anti-helminth, eosinophil- and AMM-regulating activities of ILC2 in the human gut remain to be confirmed.

**ILC in the respiratory system**

ILC2 are most thoroughly studied in the context of lung immune cell homeostasis, immunity and inflammation (Figure 3B). Studies in the mouse have demonstrated that ILC2 are involved in the immune response against nematodes such as *Nippostrongylus brasilienis*, and in the repair of lung tissue damage inflicted by infection with pathogens through the production of the epidermal growth factor family member amphiregulin. CRTH2+ ST2+ ILC2 have been identified in healthy human lung but the role of human ILC2 in lung homeostasis is currently unknown.

Inflammatory conditions of the lung are characterized by a type 2 signature. It has been recognized that type 2 cytokines are critical for pulmonary recruitment of type 2 effector cells, such as eosinophils (IL-5 and GM-CSF), mast cells (IL-9), and IgE-producing B cells (IL-4 and IL-13), and cytokines that directly affect target tissue
(e.g. IL-13 induced fibrosis). It is now recognized that not only Th2 cells but also ILC2 are the cellular source of type 2 cytokines in the lung. In humans, IL-5 and IL-13 producing innate cells that resemble ILC2 have been found in the sputum of asthma patients, and in the lung parenchyma and broncho-alveolar lavage (BAL) fluids of lung transplantation patients and patients with idiopathic pulmonary fibrosis. We identified CD34- CRTH2+ ILC2 in nasal polyp tissue of patients suffering from chronic rhinosinusitis (CRS), a typical type 2 inflammatory disease characterized by eosinophilia and high IgE levels. Interestingly, TSLP activates human ILC2 by directly upregulating GATA3 via STAT5 resulting in the production of high amounts of type 2 cytokines. This observation is highly relevant in the context of chronic rhinosinusitis and also asthma because TSLP protein expression was significantly increased in epithelial cells derived from nasal polyps of CRS patients and in the airway epithelium and lamina propria of asthmatic patients, particularly in patients with severe asthma. TSLP immunostaining in both compartments correlated with the severity of airflow obstruction. This study also provided evidence that majority of leukocytes expressing IL-13 were ILC2. Taken together, these data indicate that human ILC2 are involved in lung inflammation and pathology. This conclusion is confirmed by numerous studies in mouse models of type 2 inflammatory diseases, in particular of allergic asthma (reviewed by Walker and colleagues).

Little is known about the role of other ILC subsets in the lung. Besides ILC2 we have also detected ILC1 and ILC3 in the human lung but their function is unknown. In a mouse model of lung inflammation induced by ovalbumin, IL-22 producing ILC3
reduced airway inflammation by lowering the production of pro-inflammatory cytokines such as IL-33.\textsuperscript{81}

**ILC in the skin**

Several ILC subsets have been characterized in the skin of healthy wild type mice and humans (Figure 3C). Kim et al were the first to identify an ILC subset (ILC2) in the human skin, which expressed ST2, a component of the IL-33 receptor, but not CRTH2.\textsuperscript{17} The presence of ILC2 in healthy skin was confirmed by us\textsuperscript{60} and another groups but in these studies the dermal ILC2 were found to express CRTH2.\textsuperscript{82} We also detected ILC1 and NCR\textsuperscript{-} ILC3 but not NCR\textsuperscript{+} ILC3 in the human skin. A proportion of circulating ILC1, CRTH2\textsuperscript{+} ILC2 and NKp44\textsuperscript{+} ILC3 expressed the skin homing markers CLA and CCR10, suggesting that the dermal ILC populations are derived from circulating ILC that migrate to the skin.\textsuperscript{60} In the mouse skin, dermal ILC2 express CD103 and are the major cellular source of IL-13 under homeostatic conditions.\textsuperscript{10} Both human and mouse dermal ILC2 produce IL-4 when activated with IL-33\textsuperscript{83} and TSLP,\textsuperscript{88} respectively, whereas mouse lung ILC2 do not produce IL-4. It is unclear whether this reflects a differential signaling in the lung and skin or whether there is a distinct IL-4-producing ILC2 subset in the skin. Interestingly, mouse dermal ILC2 most strongly interact with mast cells, and ILC2-produced IL-13 may moderate mast cell responses.\textsuperscript{88} Whether ILC2 also interact with mast cells in human skin remains to be determined. Mast cells are able to produce Prostaglandin D\textsubscript{2} (PGD\textsubscript{2}), which is the ligand for CRTH2. PGD\textsubscript{2} strongly enhanced production of IL-13 by human CRTH2\textsuperscript{+} ILC2, an effect mimicked by supernatant-activated human
mast cells. In addition PGD$_2$ induced migration of ILC2. Interestingly, CRTH2 antagonists strongly inhibited PGD$_2$-mediated migration and cytokine production by ILC2. This data suggests an attenuating role of mast cells through its product PGD$_2$ and raise the possibility that small molecular CRTH2 antagonists modify the function of human ILC2 in vivo.

In the diseased skin of human atopic dermatitis patients increased numbers of ILC2 were observed compared to healthy controls, suggesting that ILC2 play a role in atopic dermatitis. Interestingly, interaction of ILC2-expressed Killer Lectin-like Receptor G1 (KRLG1) with E-cadherin widely expressed on keratinocytes and Langerhans cells suppressed IL-33-induced production of IL-5 and IL-13 by dermal ILC2. This is suggestive for an involvement of ILC2 in atopic dermatitis because interrupted E-cadherin signaling may be a key factor in the development of atopic dermatitis. Consistently, also in a mouse model of atopic dermatitis, increased numbers of ILC2 were found. These ILC2 induced skin inflammation when stimulated with IL-2 or with the dermatitis causing vitamin D analog calcipotriol in RAG2 deficient mice. In addition transgenic overexpression of IL-33 in keratinocytes results in an atopic dermatitis-like syndrome which correlated strong infiltration of ILC2.

The skin also contains ILC3, that in mice have been shown to interact with fibroblasts via the production of IL-22 to mediate wound healing. Dermal ILC3 may also mediate pathology. Psoriasis is an autoimmune disease of the skin that is driven by IL-17A, IL-17F and IL-22. Topical exposure of mouse skin to the TLR7 agonist imiquod causes skin inflammation that bears some similarity to psoriasis.
and therefore has been used as an experimental model for psoriatic skin disease. In this model, IL-17A, F and IL-22 contribute to disease and these cytokines were shown to be produced by γδ-T cells and RORγt+ ILC3. In patients with psoriasis, an accumulation of NCR+ ILC3 was observed in affected skin, suggesting that these IL-22 producing innate cells may be involved in the pathology of psoriasis. Interestingly, we and others observed not only significantly elevated numbers of NKp44+ ILC3 in the diseased skin of psoriasis patients but also in the peripheral blood whereas these cells are hardly present in peripheral blood of healthy individuals. Interestingly, a favorable response to treatment of psoriasis with anti TNF antibody adalimumab in one patient was associated with a significant reduction of NKp44+ ILC3 in the peripheral blood. Future studies are needed to determine whether the number of NKp44+ ILC3 can indeed be used as a biomarker for psoriasis.

**ILC and cancer**

The correlation between chronic inflammatory responses and an increased susceptibility to develop cancer has since long been recognized. With the accumulating evidence that ILC play pivotal roles in autoimmunity and inflammation as discussed in the foregoing paragraphs, it can be postulated that ILC may also be involved in the development of malignancies. In colo-rectal carcinoma (CRC) patients, IL-22 was found to be highly expressed by tumor infiltrating lymphocytes, which turned out to comprise of IL-22 producing CD3+ and CD3- lymphocytes. Moreover, IL-22 production in cancerous tissue was significantly
higher than in non-tumor tissue sections of the same patients.\textsuperscript{88} Using an established mouse model of microbe induced, inflammatory bowel disease-associated CRC it was found that IL-22 producing ILC drove induction and maintenance of CRC.\textsuperscript{88} Thus, CRC did not develop in mice that were depleted of IL-22 producing ILC3, and treatment with IL-22 blocking agents did protect against the development of CRC in these mice. Interestingly, neutralization of ILC3 derived IL-17 in the colon of mice did lead to a reduction in inflammation, but did not prevent CRC, suggesting that the oncogenic effect of ILC may be specifically attributable to IL-22. Another study confirmed the association of IL-22 with the occurrence of CRC in a mouse model.\textsuperscript{89} Also in other malignancies such as cutaneous T cell lymphoma\textsuperscript{90} and hepatic carcinoma\textsuperscript{91} IL-22 has been shown to play a key role in humans. In these malignancies, IL-22 is produced by T cells but also by non-T cells and it will be of interest to determine whether ILC3 are that cellular source.

**Hematopoietic stem cell transplantation**

Considering the involvement of ILC in inflammatory responses and tissue repair, and given the critical effects of chemotherapy, radiotherapy and allo-immune responses on epithelial barriers and mucosal tissues, it can be postulated that ILC are important modulators of pre- and post- allogeneic hematopoietic stem cell transplantation (HSCT) immunity.

In a mouse model of acute graft versus host disease (GvHD) IL-22, that is produced by IL-23-responsive, radiotherapy-resistant recipient ILC3, protects intestinal stem cells against the detrimental effects of GvHD since in the absence of ILC3, GvHD
severity was significantly increased. The same group showed that IL-22 producing ILC3 are essential in the recovery of thymic epithelial cells after radiation-induced damage, suggesting that ILC3, which should be radio-resistant, may be important in post-HSCT T lymphocyte reconstitution in these mice. We have longitudinally studied ILC recovery in a group of acute myeloid leukemia (AML) patients, following induction chemotherapy and after allogeneic HSCT. Reconstituting ILC were activated and expressed tissue homing molecules, and after allogeneic HSCT, were of donor origin. Interestingly, patients with high proportions of CD69+ gut-homing ILC2, NCR- ILC3 and NCR+ ILC had less mucositis and GvHD. In addition, following induction chemotherapy and after allogeneic HSCT a large number of NKp44+ ILC3 appeared in the circulation, which was associated with an absence of GvHD (Munneke et al, revised manuscript submitted to Blood).

The post-HSCT period is characterized by a significant susceptibility to develop opportunistic infections. This is generally attributed to the absence of a full T cell repertoire in particular during the first 1-2 years after SCT, and the frequent use of immunosuppressants including steroids, to prevent or treat GvHD. However, recent data suggest that also here, ILC may be involved since it was shown in a mouse model that RORyt dependent, IL-23 responsive IL-17 producing ILC are imperative for the clearance of fungal infections such as Candida albicans. In another paper it was shown that IL-22 is important in clearance of Aspergillus fumigatus infection, however, the source of IL-22 production in this mouse model was not specified.
Hematologic malignancies arising from ILCs or ILC progenitors

ILC may transform into malignant cells. To identify malignancies that are derived from ILC progenitors, a more profound understanding of the developmental pathways of ILC is needed. However, it can be speculated from data available in literature that ILC progenitor malignancies do exist. About 4% of all acute leukemias are of ambiguous lineage (WHO 2008), including acute undifferentiated leukemias that do not express any lineage specific antigens, and mixed phenotype leukemias co-expressing antigens of myeloid and lymphoid lineages. A proportion of the acute undifferentiated leukemias (AUL) are now thought to represent leukemias of NK cell progenitors. As certain human NK cell progenitor subsets such as stage III NK cell progenitor cells recently have been shown to include ILC,\textsuperscript{53-55} it is tempting to postulate that AUL may include ILC lineage-derived leukemias. Several series of NK cell precursor malignancies such as myeloid/NK cell precursor acute leukemia and blastic NK cell lymphoma/leukemia have been described in the past decades. Leukemic blasts in the earliest of these series were characterized by a lymphoblastic morphology and co-expression of lymphoid markers CD7 and CD56 and myeloid markers such as CD33 and CD34.\textsuperscript{96,97} More recently, the healthy counterparts of myeloid/NK cell precursor acute leukemia were proposed to be stage 1 pro-NK cell progenitors and stage 2 pre-NK cell progenitors. In particular stage 2 pre-NK cells are characterized by a CD34\textsuperscript{+} CD33\textsuperscript{+} CD117\textsuperscript{+} phenotype with a variable expression of CD161 and CD56\textsuperscript{98} and could therefore very well include ILC. Precursor NK lymphoblastic lymphomas/leukemias are derived from CD34\textsuperscript{-} CD33\textsuperscript{+} CD117\textsuperscript{+} CD161\textsuperscript{+} CD56\textsuperscript{+} or CD56\textsuperscript{-} stage III progenitor NK cells,\textsuperscript{98} cells that include
More extensive immunophenotyping, that includes CD161 and the IL7 receptor CD127, in combination with analyses of transcription factor expression, is needed to further characterize these rare type leukemias and to determine to what extent they include malignantly transformed ILC progenitors.

The same holds true for difficult to categorize, NK cell like lymphomas that may be derived from more mature ILC subsets. For example, refractory celiac disease is characterized by the presence of lymphocytes with an unusual phenotype that have a tendency to develop into lymphomas. It was recently observed that the non-malignant counterparts of these aberrant lymphocytes include lineage–CD127+CD7+CD56+ or CD56–lymphocytes that express CD122, the IL-2/IL-15Rβ subunit which are very similar to CD127low ILC1 described by Fuchs et al,28 and it was suggested that under the influence of chronic stimulation with IL-15 these cells may undergo malignant transformation.100

Concluding remarks

Over the past 6 years ILC subsets have been discovered that play important roles in innate immunity, homeostasis of a variety of cell types and tissue (re) modeling. ILC show a remarkable similarity with T helper cell subsets, which has aided the rapid identification of networks of transcription factors that drive development and function of these cells. Understandably the knowledge on the mechanisms underlying ILC function and development in humans lags behind that of mice but the ILC system is conserved in mice and man which helps translation of fundamental findings in ILC biology in animal model systems to that of humans.
Experiments in mice have laid the groundwork for our understanding of the developmental pathways but these pathways have yet to be fully deciphered in humans.

Studies in mouse models of inflammatory diseases indicate that ILC can be involved in inflammatory diseases. Moreover changes in composition of ILC have been observed in inflamed tissues in a number of inflammatory diseases in humans. In depth comparisons of the characteristic of ILC in diseased tissues with those in healthy tissues will help to further delineate their possible roles in disease and to determine whether targeting ILC will help to prevent or treat these diseases. ILC have also been associated with cancer in mouse models. Elucidating their role in human cancer will be a challenge for the future. It seems obvious that ILC and their precursors can undergo malignant transformation. It would be highly interesting to see whether some of the yet undefined leukemias and lymphomas are in fact derived from (pre) ILC.
Acknowledgements

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Authorship

Contribution: H.S. and M.D.H. wrote the manuscript and designed the figures.

Conflict of Interest Disclosure

The authors declare no competing financial interests.
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Table 1: Phenotype of human ILC

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Table 1: Phenotype of human ILC. ILC are categorized into CD127- NK cells, CD127- ILC1 and CD127+ ILC1, ILC2 and ILC3, the latter including LTi, NCR- ILC and NCR+ ILC3. +/- denotes expression that is upregulated by ILC after activation or by a non-activated subset of ILC.
Table 2: Signature cytokines of human ILC

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Table 2: Cytokine production of human ILC. ILC are categorized into CD127- NK cells, CD127- ILC1 and CD127+ ILC1, ILC2 and ILC3, the latter including LTi, NCR- ILC and NCR+ ILC3. +/- denotes cytokine expression by a subset of ILC after activation for example with PMA/ionomycin.
Figure legends

**Figure 1:** Morphology and phenotype of peripheral blood ILC. (A) May-Grünwald-Giemsa staining (100x), after cyto spin, of human lineage- CD127+ CRTH2+ ILC2 that were sort-purified from the peripheral blood. (B) Phenotype and gating strategy for ILC1, ILC2 and NCR- and NCR+ ILC3 derived from tonsil (upper panels) and peripheral blood (lower panels) of healthy humans. The lineage cocktail contains markers for T cells (CD3, TCR), B cells (CD19), NK cells (CD94), myeloid and plasmacytoid dendritic cells (CD1a, CD11c, CD123, BDCA2), monocytes and macrophages (CD14), mast cells (Fcer1) and stem cells (CD34). In the peripheral blood, NCR+ ILC3 are virtually absent in healthy individuals.

**Figure 2:** The developmental relationship of ILC. The NK cell progenitor (pre-NK) and the ILC progenitor (pre-ILC) evolve from the common lymphoid progenitor (CLP), but the phenotype and developmental requirements of the pre-ILC have not been defined in humans (dotted lines). ILC3 and ILC2 develop from pre-ILC under the influence of the transcription factors Rorγt and GATA3, respectively. CD127+ ILC1 may derive from the pre-ILC, or may be developmentally separated as part of the NK branch together with conventional NK cells (cNK) and CD127low ILC1. Insert: ILC have plasticity, as Rorγt+ NCR- ILC3 can differentiate in vitro into ILC1 and into NCR+ ILC3; the latter, in turn, can be induced into a NKp44- cKit- CRTH2- ILC1, and vice versa, depending on specific activation signals. Whether these ILC1 are similar to the NKp44- cKit- CRTH2- ILC1 that can be found in human tissues and blood
remains to be determined. During these processes these cells downregulate Rorγt and upregulate Tbet.

Figure 3: ILC in gut, lung and skin. (A) In the healthy situation, ILC3 produce IL-22 to maintain the epithelial barrier, generate antimicrobial products such as RegIIIβ, RegIIIγ and β-defensins, and suppress the reactivity of commensal bacteria-specific T cells (left panel). Crohn’s disease is characterized by an accumulation of IFNγ producing ILC1 (middle panel). During intestinal inflammation, ILC3 produce IL-22 to maintain epithelial barrier homeostasis. Colorectal carcinoma develops when this auto-regenerative function is not switched off in time (right panel). (B) Airway hypersensitivity is characterized by stromal production of TSLP and IL-33 that induces IL-5 and IL-13 production by ILC2 and subsequent recruitment and activation of eosinophils and mast cells (left panel). Upon viral airway infection however, ILC2 are induced to produce amphiregullin that is involved in airway epithelium repair and maintenance and thereby function as tissue protective cells (right panel). (C) In the healthy skin, ILC2 maintain close interactions with mast cells, suppressing their pro-inflammatory function, while ILC3 are involved in wound repair (left panel). Atopic dermatitis is an ILC2 mediated disease (middle panel) while in psoriasis, ILC3 are accumulated (right panel).
Figure 3A

Gut: healthy situation
- RegIIIβ
- RegIIIγ
- B-defensins
- MHCII
- CD4
- IFNγ
- IL22
- IL23
- CCL20
- BAFF
- B

Gut: Crohn’s
- ILC1
- IL17
- IFNγ
- IL22

Gut: cancer
- ILC3
**Lung: airway hypersensitivity**

- Ova
- Papain
- House dust mite

**Lung: virus**

- Areg

Cells and cytokines involved:
- Eo (Eosinophil)
- IL5
- IL9
- IL13
- ILC2
- CRTH2
- TSLP
- IL33
- Mast cell
- ILC3

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Healthy skin & wound healing  
Skin: atopic dermatitis  
Skin: psoriasis
Human innate lymphoid cells

Mette D. Hazenberg and Hergen Spits

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