

Accepted Manuscript

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PII: S0091-6749(16)31042-9

DOI: [10.1016/j.jaci.2016.09.009](https://doi.org/10.1016/j.jaci.2016.09.009)

Reference: YMAI 12361

To appear in: *Journal of Allergy and Clinical Immunology*

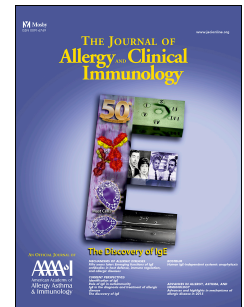
Received Date: 18 August 2016

Revised Date: 19 September 2016

Accepted Date: 21 September 2016

Please cite this article as: Mjösberg J, Spits H, Human Innate Lymphoid Cells, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.09.009.

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Human Innate Lymphoid Cells

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Abstract

Innate lymphoid cells (ILC) are increasingly acknowledged as important mediators of immune homeostasis and pathology. ILC act as early orchestrators on immunity, responding to epithelial-derived signals by expressing an array of cytokines and cell surface receptors, which shape subsequent immune responses. As such, ILC make up interesting therapeutic targets for several diseases. In allergy and asthma, group 2 ILC (ILC2) produce high amounts of IL-5 and IL-13, thereby contributing to type 2 mediated inflammation. ILC3 are implicated in intestinal homeostasis and psoriasis pathology through abundant IL-22 production, whereas ILC1 are accumulated in chronic inflammation of the gut (IBD) and lung (COPD) where they contribute to IFN- γ -mediated inflammation. Although the ontogeny of mouse ILC is slowly unraveling, the development of human ILC is far from understood. In addition, the growing complexity of the human ILC family in terms of previously unrecognized functional heterogeneity and plasticity has generated confusion within the field. Here we provide an updated view on the function and plasticity of human ILC in tissue homeostasis and disease.

Keywords

Innate lymphoid cells (ILC), mucosal immunity, inflammatory bowel disease, allergy, asthma, psoriasis, tumor immunity, GvHD.

Abbreviations

AD, atopic dermatitis; AML, acute myeloid leukemia; ART, antiretroviral therapy; BAL, bronchoalveolar lavage; BCL11b, B-Cell CLL/Lymphoma 11B; CCR, chemokine receptor; CD, cluster of differentiation; CHILP, common helper innate

lymphoid cell progenitor; CLA, cutaneous lymphocyte antigen; CLP, common lymphocyte progenitor; COPD, chronic obstructive pulmonary disorder; cNK cell, conventional natural killer cell; CRSwNP, chronic rhinosinusitis with nasal polyps; CCRTH2; chemoattractant receptor-homologous molecule expressed on Th2; EILP, early innate lymphoid progenitor; FALC, fat-associated lymphoid tissue; GALT, gut-associated lymphoid tissue; GATA3, GATA3 binding protein 3; GM-CSF, granulocyte-macrophage colony-stimulating factor; GVHD, graft-versus-host disease; HIV, human immunodeficiency virus; HSCT, hematopoietic stem cell transfer; IBD, inflammatory bowel disease; ICAM, Intracellular adhesion molecule; ieILC1, 10 intraepithelial ILC1; IFN, interferon; Id, inhibitor of DNA binding; IL, interleukin; ILC, innate lymphoid cells; ISC, intestinal stem cell; JAK, janus kinase; KLRG1, Killer cell lectin-like receptor subfamily G member 1; LMPP, lymphoid-primed multipotent progenitors; LN, lymph node; LP; lamina propria; LT_i, lymphoid tissue inducer; LTO, lymphoid tissue organizer; LXA4, lipoxin A4; MHC, major histocompatibility complex; Nfil3, Nuclear Factor, Interleukin 3 Regulated; NRP1, Neuropilin 1; PGE2, prostaglandin E2; PLZF, Promyelocytic Leukemia Zinc Finger; PP, Peyer's patch; PS, psoriasis; RA, retinoic acid; RAG, recombinase activating gene; ROR, retinoic acid-related orphan receptor; SCF, stem cell factor; SIV, simian immunodeficiency virus; Tbet, T-box protein expressed in T cells; TCF, T cell factor; 20 TL1A, TNF-like ligand 1A; TLR, Toll-like receptor; TNF, tumor necrosis factor; TOX, Thymocyte Selection Associated High Mobility Group Box; TSLP; thymic stromal lymphopoietin; VCAM, vascular cell adhesion molecule;

Introduction

Innate lymphoid cells (ILC) are transcriptional and functional parallels of the T cell system with the important difference that ILC lack clonally distributed antigen-specific receptors. Instead, ILC respond to stress-signals derived from mucosal stromal and myeloid cells, such as cytokines, cell-surface receptors and lipid mediators induced by microbes and allergens. ILC respond mainly by producing cytokines, which modulate the subsequent immune response. Hence, ILC function as translators of mucosal-damage signals and as early orchestrators of immunity. As such, ILC provide attractive therapy targets for a number of inflammatory disorders where ILC are implicated, including diseases affecting the lung (asthma), gut (inflammatory bowel disease and graft-versus host disease) and skin (atopic dermatitis and psoriasis). In this review, we will provide an update on the development, heterogeneity and functional plasticity of ILC, and the role these cells play in tissue-specific inflammation, with particular attention to data obtained from studies of humans. We will also discuss recent observations that support the idea of ILC as part of a multi-layered immune organization, where ILC and T cells, having overlapping functions, provide complementarity and robustness to the immune system.

Development, classification, heterogeneity and redundancy of ILC

ILC are divided into three subgroups based on their transcriptional requirements and cytokine profile (Figure 1, Table I-II). The subgrouping has been confused by the discovery of new ILC subsets/lineages but currently the following consensus applies; Group 1 ILC commonly require Tbet for development and produce IFN- γ as their main effector cytokine.¹ Group 1 ILC include conventional NK (cNK) cells and intraepithelial ILC1 (ieILC1),² which are both cytotoxic subsets that additionally

express the transcription factor Eomes, but that follow discrete developmental pathways.³ To the group 1 ILC also belong a subset of IFN- γ -producing cells, which develop along a completely separate lineage route and belong to the so called helper ILC.³ Commonly, helper ILC express the alpha-chain of the IL-7 receptor, CD127, and are typically non-cytotoxic. The IFN- γ producing helper ILC subset is referred to as ILC1 and can be found in both mouse^{3,4} and human intestine and lung where they participate in type 1 inflammation.^{5,6} Helper ILC additionally include the group 2 (ILC2) and group 3 ILC (ILC3) which depend on GATA3 and ROR γ t for their development, respectively.⁷⁻¹¹ Interestingly, ILC2^{6,12,13} and ILC3^{4,5} exposed to IL-12 lose expression of GATA3 and ROR γ t, respectively, and acquire features of ILC1, including Tbet and IFN- γ production. Currently, these cells are referred to as exILC2 and exILC3, respectively. The differentiation can be reversed by the addition of IL-4 to exILC2⁶ and IL-23/IL-1 β /retinoic acid to exILC3 cells.¹⁴ Thus ILC are highly plastic cells, which can change phenotype and function depending on their microenvironment, complicating their classification. In the mouse, fate-mapping experiments have revealed that there is a distinct population of ILC1, bona fide ILC1, which is not the result of ILC2 or ILC3 transdifferentiation.³ That distinction is impossible to make in humans. Hence, human ILC1 are a mix of exILC2, exILC3 and bona fide ILC1. Identification of markers that enables researchers to distinguish distinct plastic stages of ILC subsets cells is a major challenge.

ILC2 were identified in the mouse intestine¹⁵⁻¹⁷ and in human intestine,¹⁸ lung^{18,19} and skin^{20,21} where they produce type 2 cytokines IL-4, IL-5, IL-9 and IL-13⁸ and participate in allergic inflammation¹⁸. The third ILC subset, ILC3, produce cytokines typically produced by Th17/Th22 cells, namely IL-17 and IL-22. ILC3 are further subdivided into NKp44⁺ and NKp44⁻ ILC3 in humans where NKp44⁺ ILC3 produce

IL-22 whereas NKp44⁺, in particular those expressing HLA-DR, produce limited amounts of IL-17.^{22, 23} IL-22 producing ILC3 maintain gut barrier function in mice²⁴ and are likely also tissue protective in human gut^{5, 25} whereas IL-17 producing ILC were found to mediate colitis in mice²⁶ and to be accumulated in Crohn's disease patients.²⁷

In addition to helper ILC3, a subset of cells that phenotypically resemble NKp44⁺ ILC3 are identified in fetal mouse and humans as lymphoid tissue inducer cells (LTi) important for lymph node organogenesis.^{11, 28} In the mouse, LTis are derived from a precursor separate from that of the helper ILC and therefor considered to represent a
 10 separate lineage.³ Whether this is also the case in humans remains to be established

In recent years there has been a rapid advance in our understanding of the signals that govern ILC development, which has been nicely summarized elsewhere.^{29, 30} These studies were mostly carried out in transcription factor reporter mouse models^{3, 31-33 34} but data on human ILC ontogeny from a holistic perspective, i.e. not only dealing with the origin of individual ILC subsets, is now accumulating.³⁵⁻³⁸

In the mouse, ILC develop from the common lymphoid progenitor (CLP) in the bone marrow, which under the influence of the transcription factors TOX,³⁹ Nfil3⁴⁰ and TCF1³² develops into the so called early innate lymphoid progenitor (EILP) which has
 20 the potential to develop into all known ILC populations, including NK cells, LTi cells and helper ILC.³² Development of the NK cell precursor requires the action of Eomes and Nfil3.³³ Interestingly, Id2, which is necessary for all ILC subsets,^{28, 41} is not upregulated until the EILP stage, when it, along with GATA3,⁴² marks the commitment to the so called common helper ILC progenitor (CHILP).³ Once the CHILP has developed, it can give rise to LTi cells under the influence of ROR γ ³ or,

via a PLZF-expressing precursor³⁴, to all subsets of helper ILC under the influence of Tbet, GATA3/Bcl11b/RORa or ROR γ t, driving ILC1, ILC2 and ILC3 commitment, respectively.^{3, 9, 31, 43} Hence, although the transcriptional cues that are required for ILC lineage commitment are becoming known, further research is needed to identify the signals that induce these transcription factors. Recent work questioned whether ILC always develop from CLP as one study demonstrated that during the neonatal period ILC, in particular ILC2, were from CD127⁺ lymphoid-primed multipotent progenitors (LMPP) without passing the CLP stage.⁴⁴ Whether this means that fetal and adult ILC development follow distinct pathways is yet unclear.

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Human ILC have been generated in vitro from CD34⁺ progenitors in bone marrow, cord blood, peripheral blood³⁸ and thymus.⁴⁵ However, more committed precursors, only giving rise to ILC or NK cells seem to reside in tonsils and spleen but not in blood or bone marrow.^{35, 36} Presence of ILC precursors in secondary lymphoid tissues aligns with the observation that in the mouse, ILC are tissue resident,⁴⁶ indicating that they may originate from local precursors. Recently it was shown that all human ILC populations, including cytotoxic NK cells, can be in vitro derived from a common CD34⁺ innate lymphoid cell progenitor expressing ROR γ t, CD34, CD45RA, CD117, CD161, integrin β 7 and high levels of IL-1R1.³⁶ In vitro, this precursor did not give

20 rise to T cells but generated ILC1-3 as well as NK cells, hence representing an ILC-restricted precursor. In contrast, Montaldo et al found that in the tonsil, CD34⁺ROR γ t⁺CD117⁺CD45RA⁺ cells could only generate ILC3, and not NK cells.³⁵ It is unclear if the differentiation of ILC1 or ILC2 was investigated in that paper. There are several possible reasons for the discrepancies observed between the two studies. One could be that the progenitors studied were not identical (high³⁶ versus

high/low³⁵ expression of IL1R1). It is also possible that the different culture conditions (co culture with OP9-DL1³⁶ versus OP9³⁵) accounts for the discrepancy in results of the two studies. The finding that a ROR γ t expressing precursor develops into NK cells³⁶ goes against the finding in mice that NK cells show no past history of ROR γ t expression.⁴⁷ However, because ROR γ t⁻ precursors of NK cells have also been identified,³⁷ human NK cells may follow more than one route of development.

In summary, whereas the ontogeny of mouse ILC is now being elegantly dissected using genetically engineered mice, understanding the development of human ILC still represents a great challenge with so far several contradictory reports. Regardless of
10 the reasons, these contradictions need to be reconciled to fully understand the development of human ILC.

As will be discussed in this review, mouse models have demonstrated a critical role for ILC in chronic inflammatory diseases such as atopic dermatitis, psoriasis, inflammatory bowel disease and asthma. However, these studies are often confounded by the fact that they have been performed in immunodeficient mice, lacking adaptive lymphocytes. Considering the overlapping functions of ILC and T cells, this complicates the interpretation of the relative importance of these two major immune cell types. Additionally, it hampers a straightforward translation of data from mouse models to humans, also because the human immune system is not only fully immune
20 competent, but also antigen-primed, whereas mouse models often represent an immunologically naïve host, free of pathogens.

Recently, mouse studies have addressed the issue of the relative redundancy and complementarity of ILC versus T cells and revealed that ILC might be more redundant than previously thought.^{48, 49} Importantly, a very recent report indicates that also in humans, ILC might not be necessary for homeostasis when a competent T and

B cell system is present.⁵⁰ IL-2R γ c- and JAK3- deficient patients lack T cells and ILC because of the absence of functional IL-7R signaling required for development of these cells. After stem cell transplantation without myeloablation, these immunodeficient patients develop T cells but no ILC are detectable in peripheral blood. Long-term surviving patients did not experience major health problems as compared to aged-matched controls. One caveat of this study is that there was only a limited analysis of ILC in the tissues of these transplanted patients who remained healthy. This is important because mouse studies have clearly demonstrated that ILC are tissue resident and that there is only a limited influx of ILC into tissues.⁴⁶

10 Nonetheless it is possible that ILC are redundant for homeostasis and tissue repair when fully competent T cells are present including innate T cells such as NKT cells and MAIT cells. Another key question is obviously what the role of ILC is in disease. The patient group in the study with immune-deficient transplanted patients⁵⁰ was probably too small to answer the question whether the absence of ILC would influence the susceptibility for inflammatory diseases. Further studies are required to address this important question.

Effector functions and plasticity of ILC subsets in tissues

20 ILC in the formation of lymphoid organs – the function of LTi cells

Studies in the mouse have shown that LTi cells are essential for formation of some secondary lymphoid organs including peripheral and mesenteric lymphnodes (LN), Peyer's patches (PP), colonic patches and cryptopatches.⁵¹ However, a number of other lymphoid organs such as spleen-, nasal- and tear duct-associated lymphoid tissues and fat-associated lymphoid clusters (FALC) develop independently from

ROR γ ⁺ LTi cells.⁵¹ The mechanisms responsible for LTi-dependent LN and PP development have been investigated in mice and reviewed extensively elsewhere.^{51, 52}

An important trigger of LN formation is the induction of lymphotoxin (LT) α 1 β 2 on LTi cells by TRANCE, IL-7 and retinoic acid (RA) produced by mesenchymal cells. LT α 1 β 2 interacts with LT β receptor (LT β R) on lymphoid tissue organizer (LTO) cells which results in secretion of chemokines (CXCL13, CCL19 and CCL21) that attract T and B cells and expression of adhesion molecules (VCAM1, ICAM1 and MadCAM-1) on mesenchymal LTO cells, which promotes the formation of aggregates evolving into organized structures by yet unknown mechanisms.⁵¹ In the

10 mouse, LTi cells are enclosed within CCR6⁺ cells.^{28, 53} Two subsets of LTi cells have been identified that can be distinguished on the basis of CD4 expression, with CD4⁻ LTi cells being precursors of CD4⁺ LTi cells.^{54, 55}

Because LTi cells are dependent on ROR γ these cells are considered to be a member of group 3 ILC although in the mouse, LTi cells and ILC3 are derived from different precursors indicating that the developmental pathways of these cells are distinct.^{3, 31}

Our knowledge about LTi cells, LTi precursors and the mechanisms of LN formation in humans is limited. Cells that are very similar to mouse LTi cells have been found in human fetal lymph nodes although human LTi cells lack CD4.¹¹ Studies in patients

deficient in ROR γ , common γ chain (γ c) of the IL-2 receptor (IL-2R γ c) and Janus
20 kinase (JAK) 3, which is coupled to the IL-2R complex, lack LTi cells and also lymph nodes^{50, 56, 57} although in ROR γ deficient patients tonsils appear to be present⁵⁷

indicating that also in humans ROR γ ⁺ LTi cells are not required for establishment of all secondary lymph nodes. These observations also indicate that the requirement of IL-2R γ c and ROR γ for function and development of LTi cells is evolutionary conserved. The relationship between LTi cells and other ILC3 populations in humans

is presently unknown mainly because so far there were no reliable markers that distinguish between LT α cells and ILC3. Recently, however, neuropilin 1 (Nrp1) was found to be selectively expressed on mouse adult intestinal CCR6⁺ LT α cells⁵⁸ and recently we confirmed expression of Nrp1 on mouse fetal CD4⁺ LT α cells (*Shikhagaie et al., unpublished*). We also observed Nrp1 expression on a subpopulation of human ILC3 in fetal lymphnodes, which are able to induce VCAM-1 and ICAM-1 on mesenchymal stem cells, considered an in vitro surrogate for LT α activity. These observations suggest that Nrp1 is an evolutionary conserved marker for LT α cells, which will allow addressing the relationship of LT α cells and other ILC3 in humans.

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ILC in homeostasis, inflammation and infection in the intestine

The mouse intestine contains all ILC populations and these cells have been associated with innate immune responses against intestinal pathogens, including helminthes bacteria and viruses, containment of commensals and tissue (re) modeling (reviewed elsewhere⁵⁹).

The distribution of ILC in intestinal tissues of humans depends on the developmental status. Whereas ILC2 are clearly detectable in fetal intestine,¹⁸ their frequency in adult intestinal tissues is very low and ILC3 are present in both fetal and adult intestines.⁵

ILC1 are barely detectable in fetal intestine⁵ suggesting that in contrast to ILC2 and

20 ILC3, development of ILC1 requires microbiota or other postnatal signals. Fetal intestinal ILC2 and ILC3 express transcripts for IL-5 and IL-13 and IL-22, respectively,^{5, 18} indicating their activation but the nature of the activating agents and what the function is of ILC2 in the fetal intestine is unknown.

NKp44⁺ ILC3 is the most prevalent ILC population in the human gut under homeostatic conditions.⁵ Freshly isolated ILC3 from non-inflamed intestinal tissues

express IL-22 transcripts indicating that under homeostatic conditions IL-22 is produced, which may be important for containment of commensals and support renewal of epithelial cells by promoting growth of intestinal stem cells as described in mice (Figure 2a).^{60, 61} Upon inflammatory stimuli, dendritic cells and epithelial cells produce IL-1 β and IL-23 inducing production of elevated IL-22 but also IL-8, GM-CSF, IL-26, TNF, which act on neutrophils, dendritic cells and epithelial cells and the B cell factor Baff which acts on intestinal B cells to provide maximal protection against pathogens (reviewed elsewhere⁶²). For instance IL-22 is important in the mouse for a protective immune response against the attaching and effacing bacterium

10 *Citrobacter rodentium*.²⁴ Interestingly, it was shown that in the case of *C. rodentium*, ILC3 are critical for homeostasis in mice lacking adaptive immune cells, but that in immunocompetent mice, absence of ILC3 can be compensated for by the presence of T cells producing IL-22.^{48, 49} Hence, in this model, there is a high degree of complementarity and redundancy of ILC3 and T cells, ensuring the immune robustness of the host. The highly pathogenic *Escherichia coli* strain O157/H7 is an enterobacteria that causes attaching effacing lesions in humans and IL-22-producing ILC3 may function in a protective immune response against this *E. coli* strain, and based on the mouse studies,^{48, 49} this occurs most likely in conjunction with Th22 cells.^{63, 64} ILC3 may also be involved in induction of tolerance against commensals as

20 a proportion of ILC3 express class II MHC antigens and can present antigens to T cells.⁶⁵ However, because ILC3 lack costimulatory molecules, CD4⁺ T cells, which are specific for commensals, are killed rather than stimulated to proliferate.⁶⁶ Whether this occurs also in humans has yet to be determined but MHCII⁺ ILC3 were found in the intestine and were decreased in pediatric patients with Crohn's disease⁶⁶ in which tolerance towards intestinal commensal bacteria may be compromised.

The composition of ILC populations in inflamed intestinal tissues from humans change dramatically. NKp44⁺ ILC3, which produce IL-17, are rare in non-inflamed tissues but they have been found to be enriched in inflamed ileum and colon of Crohn's disease patients (Figure 2a)²⁷ and based on data in mouse models for colitis.²⁶ It was speculated that IL-17 produced by ILC3 could contribute to disease. However, this is contradicted by the observation that clinical trials with an anti-IL-17A antibody worsened disease⁶⁷ and that IL-17 has barrier protective effects, at least in mice.⁶⁸

There is evidence for involvement of ILC1 in human IBD (Figure 2a). Two populations of ILC1 are present in the human intestine; one in the lamina propria (LP ILC1) which expresses CD161 and CD127 but lacks markers for ILC3 and ILC2 such as cKit, CCR2 and NKp44.⁵ Another ILC population, expressing NKp44 and CD103 but lacking CD127 is located in the epithelium and therefore referred to as intraepithelial ILC1 (ieILC1).² As these ieILC1 also express NK markers such as CD56, CD94 and perforin and mediate cytotoxicity they are closely related to NK cells. Based on what we know of ILC1 in the mouse intestine, which mediates a protective response against the parasite *Toxoplasma gondii*,³ it is likely that these cells are involved in immunity against intracellular pathogens. In inflamed tissues of Crohn's disease patients, both LP ILC1 and ieILC1 populations are increased although the elevation of CD127⁺ LP ILC1 is much greater.^{2, 5, 14} The extent of increases of ILC1 in the ileum of Crohn's disease patients is associated with the severity of the disease.⁶⁹ In parallel, the proportion of NKp44⁺ ILC3 is strongly decreased.^{5, 70} Elevation of LP ILC1 at the cost of NKp44⁺ ILC3 is likely caused by transdifferentiation of ILC3 into ILC1 under influence of IL-12 produced by CD14⁺ DC which are also accumulated in inflamed tissues of Crohn's disease patients.⁵ Interestingly LP ILC1 can transdifferentiate back to ILC3 in response to IL-1 β , IL-23,

and retinoic acid which is produced by CD14⁺ DCs.¹⁴ The antibody Ustekinumab, which recognized the common p40 chain of IL-12 and IL-23 has shown beneficial effect in patients not responding to anti TNF although these effects were not persistent.⁷¹ The effect of Ustekinumab may be due to blocking IL-12. However, this antibody also neutralizes IL-23 which may promote tissue homeostasis which could be a reason why the effects of Ustekinumab are not durable. It would be of interest to test an antibody that exclusively targets IL-12 and not any of the other members of the IL-12 family (IL-23, IL-27 and IL-35) as a therapy in Crohn's disease.

10 ILC mediate inflammation in the respiratory system

It is well established that almost all ILC in the mouse lung under hemostatic conditions are ILC2. The proportion of other ILC subsets in the mouse lung is very low. In the mouse lung ILC2 contribute to the immune response against nematodes viruses and fungi⁷² and by secreting amphiregulin ILC2 contribute to tissue repair of damage evoked by pathogenic viruses.¹⁹ In the mouse, ILC2 express class II MHC antigens and present antigen to CD4⁺ T cells,⁷³ resulting in generation of Th2 cells and in maintenance of memory Th2 cells through licensing of dendritic cells.⁷⁴ A role of ILC2 in inflammatory respiratory diseases such as allergic asthma and diseases associated with viral infections has been firmly established in a variety of mouse models (reviewed elsewhere^{75, 76}).

The distribution of ILC subsets in the lung is different in humans as compared to mice. In fetal lung, NKp44⁺ ILC3 is the most frequent ILC subset; in adult lung the frequency of ILC2 is increased compared to fetal lung but this is still lower than of NKp44⁺ ILC3.⁶ However, the role of ILC subsets in normal immunity in the lung is

yet unknown and it is possible that ILC are redundant in the presence of a functional adaptive immune system.⁵⁰

On the other hand, ILC may play important roles in airway inflammation in humans (Figure 2b). Increased proportions of ILC2 producing IL-4, IL-5 and IL-13 are present in nasal polyps of patients suffering Chronic Rhinosinusitis with nasal polyps (CRSwNP) as compared to normal nasal mucosa.^{6, 18, 77} The allergic form of CRSwNP is characterized by high expression of TSLP and IL-4 in the polyps which are produced by epithelial cells and eosinophils, respectively.⁶ TSLP and IL-4 stimulate ILC2 to produce IL-5 that activate eosinophils that are abundantly present in nasal polyps of CRS patients creating a positive feed back loop that may contribute to the chronic type 2 inflammation in CRS.⁶ Based on these results one can envision that combinations of antibodies targeting IL-4, and IL-5 or their receptors have therapeutic potential in CRSwNP by disrupting this inflammatory feed back loop. Indeed an antibody against IL-5 Mepolizumab, a humanized anti-IL-5 mAb, emerged as a treatment option.⁷⁸ Reslizumab, another anti-IL-5 monoclonal antibody, improved disease in 50% of patients with CRSwNP, and nasal IL-5 levels correlated with the response.⁷⁹ It would be of interest to combine these anti IL-5 antibodies with those targeting the IL4R such as dupilumab as a therapy in CRSwNP.⁸⁰

Human ILC2 may also be involved in allergic asthma (Figure 2b) because higher proportions of these cells are present in airway tissues, including bronchioalveolar lavage (BAL) and sputum,⁸¹⁻⁸³ as well as PBMC of allergic individuals and these cells produce more IL-5 and IL-13 upon activation with IL-25 and IL-33.⁸⁴ Interestingly, recent studies from mouse models indicate that IL-27, as well as type I and II interferons, induced upon viral infection, inhibit ILC2 function.^{85, 86} Since asthmatics are known to generally produce less interferons upon viral airway infections, this

provides a mechanistic explanation for virus-induced asthma exacerbations. In addition to cytokines, lipid mediators such as prostaglandin D₂ (PGD₂) and lipoxin A₄ (LXA₄) regulate human ILC2 function in inflammatory conditions.⁸⁷ PGD₂ was shown to activate ILC2 cytokine secretion and migration,⁸⁸ whereas the pro-resolving molecule LXA₄ inhibits ILC2 function.⁸⁹

Recent studies have provided evidence for a role of ILC in Chronic Obstructive pulmonary disease (COPD) (Figure 2b), which is associated with long-term exposure to cigarette smoke causing progressive loss of lung function. Exposure of mice to cigarette smoke results in a strong type 1 response to influenza virus which was accompanied by a strong reduction of ILC2 and an increase of ILC1.⁹⁰ The changes in ratios of ILC1 and ILC2 were caused by IL-12 and IL-18, which induced transdifferentiation of ILC2 into ILC1. In humans with COPD, ILC2 were reduced and ILC1 were increased in lung tissues as well as in PBMC.^{6, 90} IL-12 induced in vitro transdifferentiation of human ILC2 into ILC1, which could be reversed by IL-4.⁶ Transdifferentiation of ILC2 could be initiated by IL-1 β which resulted in a downregulation of the ILC2 marker CCR6 and upregulation of the transcription factor Tbet, IFN- γ production and IL-12R β 2 expression. Mechanistically, ILC2 to ILC1 transdifferentiation was marked by induction of transcriptional accessibility of both the *IFNG* locus and the loci coding for IL-5 and IL-13.¹² Thus changes in the microenvironment in COPD caused by continuous exposure to cigarette smoke may result in type 1 responses to respiratory tract infections, which is at least in part mediated by ILC2-derived ILC1, worsening the symptoms of the disease. Whether ILC3 are associated with respiratory inflammatory diseases in humans is yet unknown. However, it was noteworthy that in the lungs of COPD patients, also increased numbers of NKp44⁺ ILC3 were present. IL-17A and IL-17F, which can be

produced by NKp44⁺ ILC3, are elevated in COPD⁹¹ and associate with the decline of lung function but if ILC3 are major producers of IL-17 in this setting remains to be determined.

ILC mediate tissue repair and inflammation in the skin

The skin forms a crucial barrier to protect against mechanical stress, pathogens and chemical insults. In terms of immune cells, the epidermis hosts primarily tissue resident cells. Conversely, the dermis, which underlies the epidermis, is richly populated by migrating immune cells.⁹² Interestingly, human ILC are only found in the dermis,^{20, 21, 93-95} indicating that they might be recruited there from the circulation. In support of that, a significant population of human circulating ILC expresses the cutaneous leukocyte antigen (CLA) and CCR10.^{95, 96} Given the relatively high abundance of ILC in the healthy skin (approximately 1% Lin⁻CD127⁺ of CD45⁺)⁹⁵ they might play a role in homeostasis and tissue repair. In mice, ILC2 were shown to control mast cell activation in the skin,⁹⁷ and human studies have revealed that ILC2 produce transcripts of the epidermal-growth factor-related molecule amphiregulin,²¹ possibly mediating skin tissue-repair as that described in mouse lungs.¹⁹ Indeed, recently repaired human skin contained significantly more ILC2 close to the wound margin as compared to the initial intact skin 10 days before wounding.⁹⁸

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However, in addition to mediating control of immune activation and tissue repair, ILC also seem to contribute to dysregulated skin immune responses such as those seen in atopic dermatitis (AD) and psoriasis (PS).

One of the strongest genetic risk factors for AD is loss-of-function mutations in the gene encoding filaggrin (*FLG*). *FLG* mutant humans show defective skin barrier

function, which includes reduced expression of the adhesion protein E-cadherin. Interestingly, E-cadherin seems to be an important inhibitor of skin ILC2, acting via KLRG1, expressed by dermal human ILC2, thereby suppressing ILC2 function (Figure 2c).²¹ Hence, *FLG* mutations might mediate its penetrance via enhanced activation of ILC2, which indeed was recently supported by the observation of an accumulation of ILC2 in suction blister skin of AD patients carrying *FLG* mutations as compared to *FLG* wild-types.⁹⁹

ILC2 are enriched in AD skin^{20, 21} and in HDM-allergic individuals, ILC2 are accumulated at the site of skin exposure 24 hours after HDM-injection,²¹ indicating that factors that promote ILC2 recruitment and/or expansion are triggered by HDM in these individuals (Figure 2c). Indeed, AD is associated with increased levels of IL-25, IL-33, TSLP and PGD₂, all which have been shown promote ILC2 recruitment and/or expansion, also in mice.^{20, 21, 88, 100} Furthermore, these factors, together with additional help of other signals, including the NKp30 ligand B7-H7 which is highly expressed in AD skin,¹⁰¹ will boost type 2 cytokine secretion by ILC2, ultimately leading to the inflammation characteristic to AD.

In PS patients, there is an accumulation of ILC3 expressing NKp44 in lesional and unlesional skin and blood as compared to healthy control subjects (Figure 2c).⁹³⁻⁹⁵ In addition, these cells were even shown to be enriched in the synovial fluid of PS patients suffering from arthritis as compared to patients with rheumatoid arthritis.¹⁰² In the skin, IL-23 and IL-1 β drives differentiation and IL-22 production from NKp44⁺ ILC3.⁹⁵ Interestingly, IL-23 can be blocked to efficiently ameliorate skin inflammation, suggesting that targeting ILC3 function might be a novel treatment strategy in PS. This is supported by a mouse study showing a causal link between

ILC3 activation and skin pathology.¹⁰³

ILC mediate protection against Graft-versus-Host Disease (GvHD)

Allogenic bone marrow transplantation (also known as allogenic hematopoietic stem cell transplantation; allo-HSCT) is the only available curative treatment for hematological malignancies, which do not respond to chemo- or radiotherapy. However, the treatment is associated with high morbidity and mortality. Allo-HSCT is preceded by conditioning chemo- and/or radiotherapy to marginalize the malignancy and the recipients own hematopoietic compartment. This causes significant tissue damage, in particular mucositis, which severely predisposes for graft-versus-host disease (GvHD), most often of the gut and skin, following allo-HSCT. Considering the emerging role for ILC in controlling tissue damage, we recently studied ILC in AML patients receiving pre-allo-HSCT conditioning chemoradiotherapy followed by allo-HSCT.⁹⁶ ILC were depleted by the pre-conditioning therapy and recovered slowly after HSCT. However, regardless of poor reconstitution of ILC, increased activation and tissue-homing potential of ILC, in particular of NKp44⁺ ILC3 homing to the skin and gut, capable of producing IL-22, was associated with protection against GvHD.⁹⁶ These data fit very well with a mouse model of intestinal GvHD where it was shown that ILC3-derived IL-22 protected mice against mucositis and GvHD following radiotherapy and HSCT. IL-22 acted on intestinal stem cells (ISCs), promoting the survival and subsequent restoration of the intestinal epithelium.⁶⁰

All together, ILC, in particular NKp44⁺ ILC3, might exert protection against tissue damage associated with conditioning therapy and allo-HSCT transfer in hematopoietic malignancies and should be considered as novel targets in the diagnosis, prevention

and treatment of GvHD.

ILC are depleted in HIV but accumulate in filarial infections

There is limited data on the potential role for ILC in infectious diseases in humans. It was reported already in 2010 that human CD127⁺ ILC express and respond to ligands of several TLRs, in particular TLR2, which triggered ILC cell proliferation and cytokine production.¹⁰⁴ Interestingly, mouse ILC do not respond to TLR2 engagement, possibly explaining why the role of TLRs, and other PRRs, in regulating ILC function has been so under-studied.

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We recently showed that ILC are depleted during acute HIV infection unless the patients were immediately treated with antiretroviral therapy (ART).¹⁰⁵ Upon ART treatment in the chronic phase of infection, as expected, viral loads were reduced and CD4⁺ counts were restored. However, ILC counts remained very low up to 2 years after initiation of successful ART. Hence, the ILC depletion caused by HIV infection seems to be irreversible. RNA-seq analysis revealed that ILC in early acutely HIV infected individuals were apoptotic, possibly explaining the ILC depletion. This observation is supported by findings in macaques, showing that ILC3 are depleted in GALT during SIV infection.¹⁰⁶ In that study, TLR2/4 ligands induced ILC apoptosis, indicating that the microbial translocation seen in HIV/SIV infection might contribute to ILC loss. Since human ILC do express functional TLR2,¹⁰⁴ this mechanism might contribute to the loss of ILC observed in HIV patients. Considering the tissue protective effects of ILC, ILC depletion could have important consequences for mucosal tissue integrity and immune dysfunction enhancing HIV propagation and susceptibility to opportunistic/co-infections such as tuberculosis.

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ILC loss upon infection is not a general phenomena. Filarial infection in humans instead led to an accumulation of ILC producing preferentially type 2 cytokines such as IL-5, -4 and -13, indicating that they might belong to the ILC2 lineage.¹⁰⁷ These data are interesting since they provide a first indication that ILC2 are involved in immune defense against parasitic intestinal worms in humans, as reported previously in mice.

In summary, the limited data that is available on the role for ILC in human infections indicate that ILC are indirectly targeted by HIV and filarial worm infections. The consequences and relative importance of ILC dysregulation in these infections remains to be elucidated

Tissue-dependent roles for ILC in tumor surveillance and rejection

NK cells have been known for years for their role in tumor rejection and adoptive transfer of NK cells have, at least semi-successfully, been clinically utilized for patients with hematological malignancies.¹⁰⁸ However, very little data exist on the role of more recently described ILC in human cancer. Carrega et al showed that NKp44⁺ ILC3 are enriched in human non-small cell lung tumors, particularly in tertiary lymphoid structures, and such structures are favorable for clinical outcome.¹⁰⁹ NKp44⁺ ILC3 respond to NKp44-ligation with enhanced TNF production.¹¹⁰ Indeed, in the lung tumor setting, interaction between NKp44⁺ ILC3 and tumor stroma promoted TNF production from tumor associated ILC3 in a partly NKp44-dependent manner. Hence, given the propensity of tumor-associated ILC3 to produce TNF and mediate organization of lymphoid structures, targeting ILC3 might be a promising

treatment strategy, at least in this type of cancer.

More data are available on the role for ILC in various mouse models of cancer. Intriguingly, they show contradicting results depending on tumor type. In melanoma, IL-12 driven, ROR γ t-dependent NKp46⁺ ILC promoted rejection of tumors.¹¹¹ In a mammary tumor model, a novel type of ILC1-like cell, termed type-1-like ILC (ILC1l) formed, together with TCR $\alpha\beta$ ⁺ and TCR $\gamma\delta$ ⁺ T cells, the major tissue-resident source of granzyme B, important for tumor rejection.¹¹² In a mouse model of colorectal cancer, ILC producing IL-22 was shown to drive colitis-induced tumor formation through activation of STAT3 signaling, cell proliferation and tumor growth.¹¹³ A similar tumor-promoting role for ILC was observed in cholangiocarcinoma where ILC2-derived IL-13 caused cholangiocyte hyperplasia and contributed to cholangiocarcinoma with liver metastasis.¹¹⁴

In summary, studies in mouse models, and limited data obtained from humans, show that depending on tumor setting, ILC might exert completely different roles. Although ILC can produce tumor-growth factors such as IL-22 in the colon and IL-13 in the gall bladder, the capacity of ILC to mediate formation of lymphoid structures and recruitment of leukocytes, as seen in tumors of the lung and skin, and produce cytotoxic molecules as seen in mammary glands, largely promotes tumor rejection. More studies of different ILC populations within human tumors are needed to elucidate the complex roles of ILC in cancer.

Conclusions

The parallel existence of innate and adaptive lymphocytes with overlapping functions

has probably evolved to ensure immune robustness.¹¹⁵ Although ILC may be redundant in the presence of an intact and functional adaptive immune system, there is enough evidence from mouse studies to support a role for ILC in a number of immune pathologies. In humans, ILC are dysregulated in several conditions of airway, skin and gut inflammation and therefore make up interesting targets for therapy. However, to fully utilize ILC as treatment targets for disease, we still need to learn much more about the ontogeny of human ILC, the functional heterogeneity and plasticity of these cells, their migratory capacities and how they are integrated in the complex network of immune cells and stroma during inflammation. Such research requires continued translational efforts combining results from studies of human patients and tissues with those from genetically engineered mice.

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Figure legends**Figure 1. Classification of human ILC**

ILC functionally resemble adaptive lymphocytes with the distinction that ILC lack antigen-specific receptors. Instead, ILC are activated by cytokines and cell-surface bound molecules to produce distinct sets of cytokines with important consequences for tissue homeostasis and disease. ILC display a high degree of plasticity and can transdifferentiate depending on inflammatory environment

10 **Figure 2.****a. Role of ILC in human intestinal homeostasis, inflammation and infection.**

ILC3 are important for intestinal homeostasis through production of IL-22 and inhibition of commensal-specific Th17 cells via MHCII expression. In pediatric IBD patients, ILC3 downregulate MHCII and might lose the ability to inhibit IL-17 production from T cells. There are also reports of increased numbers of IL-17 producing ILC3 in human IBD, ultimately increasing the total IL-17 production in IBD. These observations are paralleled by the finding of accumulated numbers of ieILC1 and ILC1 in the intestine of Crohn's disease patients. The enrichment of ILC1 could, at least in part, be explained by increased differentiation of ILC3 to exILC3

20 under the influence of IL-12 and IL-18 produced by conventional CD14⁺ DCs (cDC). Jointly, ieILC1, LP ILC1 and exILC3 contribute to increased IFN- γ production.

Generation of exILC3 is reversible, so that upon resolution of inflammation via the action of tolerogenic CD14⁻ DCs (tDC), ILC3 are generated and can contribute to tissue restoration. In human filarial infection, the frequency of circulating ILC2

increase which might be a reflection of their importance in clearing the intestinal parasite through a type 2 immune response.

b. Role of ILC in the human airway inflammation

Human ILC2 are accumulated in the airways of asthmatics where they are triggered by the cytokines IL-25, IL-33, TSLP, TL1A, the lipid mediator PGD₂ and the cell surface molecules MHCII and ICOS. This increases the production of IL-4, IL-5 and IL-13, causing type 2 mediated inflammation. The pro-resolving lipid LXA₄, IL-27 and type I and II interferons limit ILC2 responses and defects in the production of these molecules is associated with asthma. In human COPD lungs, the frequency of IFN- γ producing ILC increase, possibly as a result of exILC2 differentiation under the influence of IL-12 and IL-1 β .

c. Role of ILC in human skin homeostasis and inflammation

Human skin ILC2 express KLRG1, which is important for homeostatic inhibition of ILC2 function through interaction with E-cadherin. Indeed, reduced expression of E-cadherin, accumulation of ILC2 and increased cytokine production from ILC2 is associated with atopic dermatitis where ILC2 contribute to the type 2 mediated inflammation. In human psoriatic skin, lesional as well as non-lesional, there is an accumulation of ILC3, which, via production of IL-22, may contribute to immune pathology. The accumulation of IL-22 producing ILC3 may be the result of differentiation of NCR⁺ ILC3 under the influence of IL-23 and IL-1 β .

Table 1. Mouse & Human ILC phenotype

	Group 1 ILC						Group 2 ILC				Group 3 ILC							
	cNK		ieILC1		ILC1		ILC2		exILC2		exILC3		NCR+ ILC3		NCR- ILC3		LTI	
TFs	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>	<i>Hu</i>	<i>Ms</i>
Tbet	+	+	+	+	+	+	-	-	+	+	+	+	-	+	-	+	-	-
Eomes	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
GATA3	low	low	-	ND	low	low	+	+	low	low	low	low	low	low	low	low	low	low
RORgt	-	-	-	-	-	-	-/low	-	-/low	ND	-/low	-/low	+	+	+	+	+	+
SPs																		
CD127	-	-	-	-	+	+	+	+	+	ND	+	+	+	+	+	+	+	+
CD161	low		low		+		+		+		+		+		+		+	
CD117	-	-	-	ND	-	+	+/-	+/-	-	ND	-	-	+	+	+	-	+	+
Thy1		-		+		+		+		+		+		+		+		+
NK1.1		+		+		+		-		ND		+		dim		dim		-
NKG2A	+	+	+	+	-	-	-	-	ND	ND	-	-	-	-	-	-	-	-
NKp46	+	+	+	+	-	+	-	-	ND	ND	-	+	+	+	-	-	-	-
NKp44	+/-		+		-		-		-		-		+		-		-	
IL12RB1	+	+	+	+	+	+	ND	ND	+	+	+	+	+	ND	+	ND	ND	ND
CD25	+	+	ND	ND	-	ND	+	+	ND	+	-	-	+	+	+	+	+	+
CD122	+	+	+	+	ND	+	ND	low	ND	ND	+	+	-/low	-/low	-/low	-/low	-/low	-/low
CRTH2	-	-	ND	ND	-	ND	+	+	-	ND	-	ND	-	ND	-	ND	-	ND
ST2	-	-	ND	ND	-	ND	+	+	+	low	-	-	-	ND	ND	ND	ND	ND
IL17RB	-	-	ND	ND	-	ND	+	+	+	ND	-	-	-	-	ND	-	-	-
KLRG1	+	+	ND	ND	+	-	+	+	ND	ND	ND	-	ND	-	ND	-	ND	-
ICOS	-	low	ND	ND	+	ND	+	+	ND	ND	ND	ND	+	+	+	+	ND	ND
CCR6	-	-	-	ND	ND	ND	+	-	ND	ND	ND	-	+	-	+	-	+	+
IL23R	-	-	-	-	-	-	-	-	ND	ND	-	-	+	+	+	+	+	+
IL1R1	-	-	-	ND	ND	ND	+	ND	+	+	-	ND	+	+	+	+	+	+
MHCII	-	-	ND	ND	-	-	+	+	+	ND	-	-	+	-	ND	+	ND	+
CD4	-	-	ND	ND	+/-	ND	-	-	ND	ND	-	-	-	-	-	-	-	+/-

TFs: transcription factors

SPs: surface proteins

ND: Not determined

Dark fields: Expression or population lacking in mice or humans

* Albeit human ILC2 express ST2 mRNA, surface ST2 protein has been reported to be hard to detect.

** Human exILC2 and exILC3 refers to cell generated in vitro

Table II. Cytokines produced by stimulated human ILCs*

Cytokines	Group 1 ILC			Group 2 ILC		Group 3 ILC			
	cNK	ieILC1	ILC1	ILC2	exILC2**	exILC3**	NCR+ ILC3	NCR- ILC3	LTI***
TNF	+		+	+			+	+	+
IL-2	-		+	+			+	+	
IFN-g	+	+	+	-	+	+	-	-	
IL-4	-			+					
IL-5	-			+	+				
IL-6	+			+			-	-	
IL-8	+			+			-	-	+
IL-9	-			+					
IL-10	-			+					
IL-13	-		-	+	+	-	-	-	
IL-17A	-		-	-	-	-	-	+	+
IL-17F	-						-	+	
IL-22	-		-	-	-	-	+	-	+
GM-CSF	+		-	+			+	+	+

Light grey fields: not reported in the literature

* PMA/ionomycin or stimulations specific for each ILC subset:

NK cells: IL-15, IL-12 or K562 target cells,

ieILC1: IL-12

ILC1, exILC3 and exILC2: IL-2+IL-12+/- IL-18

ILC2: IL-2+IL-25+IL-33+TSLP

ILC3: IL-1b+IL-23

** Human exILC2 and exILC3 refers to cells generated in vitro

*** refers to fetal ILC3, Cupedo et al., 2009 (ref 11) and adult Nrp1+ ILC3 (Shikhagaie et al, unpublished)

Figure 1. Human ILC classification

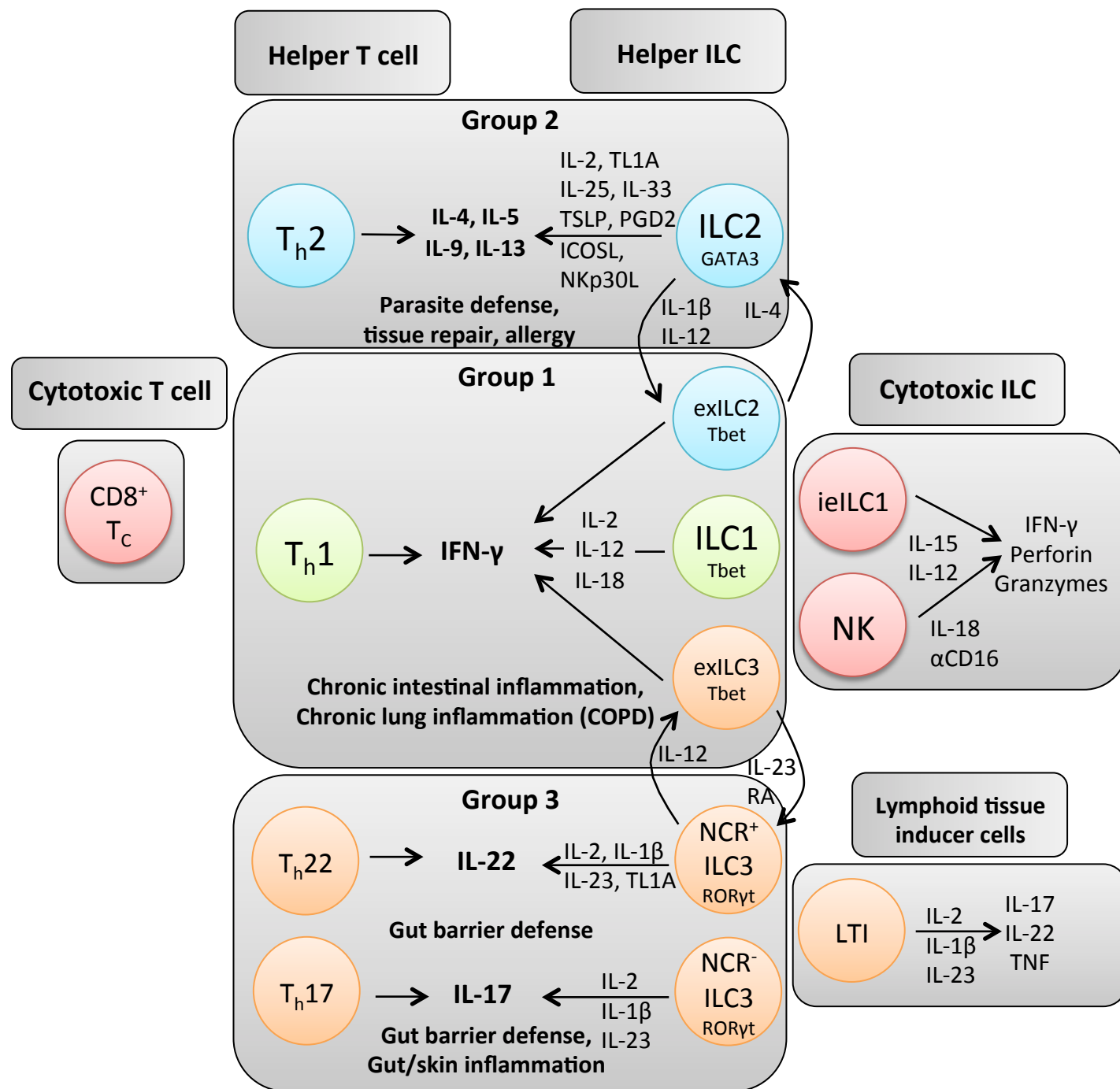


Figure 2a. ILC in human intestine

A.

Homeostasis/ Tissue restoration

Crohn's disease

Filarial infection

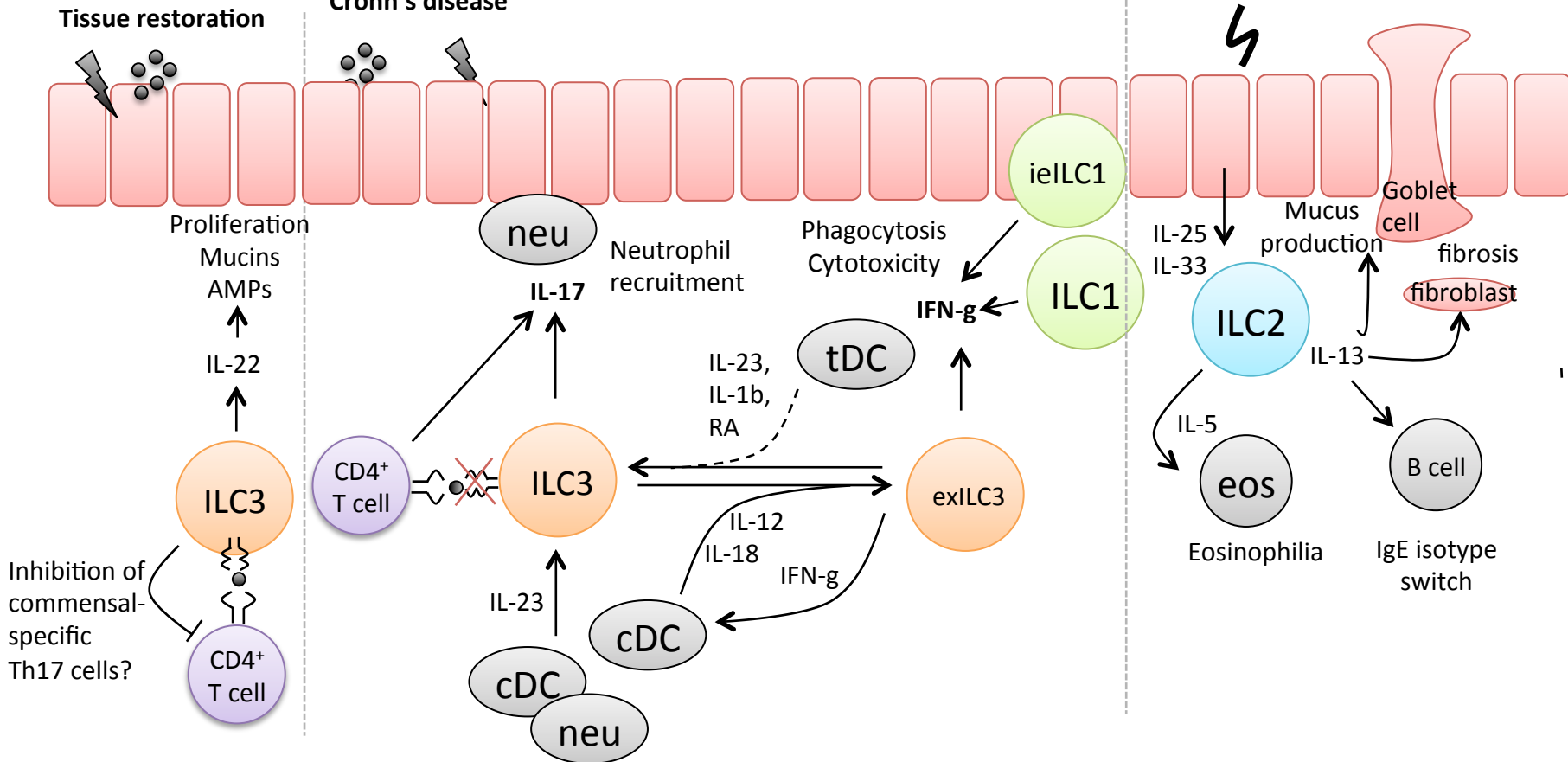


Figure 2b. ILC in human lung

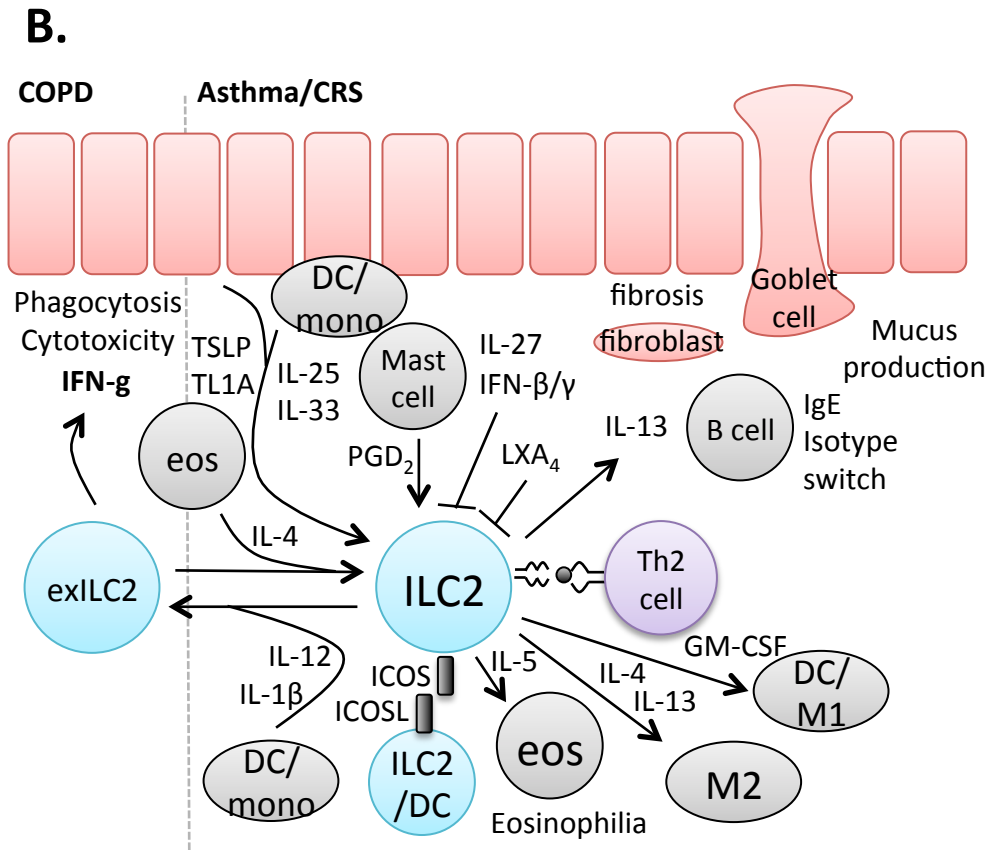


Figure 2c. ILC in human skin

