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Article type : Review

Characteristics of tissue–resident ILCs and their potential as therapeutic targets in mucosal and skin inflammatory diseases

Manuscript Acceptance Date: 12-Apr-2021

Short title:Tissue–resident ILCs as therapeutic targets

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ALL.14863

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Funding information

This work was supported by a grant from the National Center for Child Health and Development (#29-2 to H.M.);Grants-in-Aid for Scientific Research (B)(20H01622 to H.M. and 18H02847 to S.N.) anda Grant-in-Aid for Early-Career Scientist (19K17692 to K.O.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan; and a grant from the Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency (JPMJPR18H6 to S.N.)

KEYWORDS

Innate lymphoid cells, tissue–resident, heterogeneity, therapeutic target, prevention

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Abstract

Discovery of innate lymphoid cells (ILCs), which arenon–T, non–B lymphocytes that have no antigen–specific receptors, changed the classical concept of the mechanism of allergy, which had been explained mainly as antigen–specific acquired immunity based on IgE and Th2 cells. The discovery led to dramatic improvement in our understanding of the mechanism of non–IgE–mediated allergic inflammation. Numerous studies conducted in the past decade have elucidated the characteristics of eachILC subsetin various organs and tissues and theirontogeny.We now know that each ILC subset exhibits heterogeneity.Moreover, the functions and activating/suppressing factors of each ILC subset were found to differ among both organs and types of tissue. Therefore, in this review, we summarize our current knowledge of ILCs by focusing on the organ/tissue–specific features of each subset to understand their roles in various organs. We also discuss ILCs' involvement in human inflammatory diseases in various organs and potential therapeutic/preventive strategies that target ILCs.

1 INTRODUCTION

Discovery of innate lymphoid cells (ILCs), which are non–T, non–B lymphocytes that have no antigen–specific receptors,[1]changed the classical concept of the mechanism of allergy,which had been explained mainly as antigen–specific acquired immunitybased on IgE and Th2 cells. The discovery enabled us to logically explain the findings of genome–wide association studies (GWAS) and led to dramatic improvement inour understanding of the mechanism of non-IgE–mediated allergic inflammation. As a result, we are now able to better explainhow such allergic diseases as asthma and atopic dermatitisare exacerbated in an antigen–non–specific manner in reallife.ILCs are innate–type lymphocytes that lack antigen–specific receptors but produce cytokines in response to various stimuli at barrier surfaces.[2] ILCs were initially divided into three different subsets—ILC1, ILC2 and ILC3—based on their transcription factors and the cytokines

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they produced. That classification resembles the division of helper T cells into three subsets: Th1, Th2 and Th17 cells.[1] However, at present, ILCs are divided into five different subsets due to inclusion of developmentally diverse cell populations such as NK cells and lymphoid tissue-inducer (LTi) cells as separate subsets (Figure 1).[3] NK cells are characterized by eomesodermin (EOMES) expression, whereas ILC1s express T–box expressed on T cells (T–bet) but do not express EOMES.Numerous studies have been conducted in the past decade, dramatically improving our understanding of the detailed characteristics of each ILC subset, i.e., their developmental pathway,functions and activating/suppressing factors. On the other hand, it is becoming clear that, despite categorization of ILCs into five different subsets,[3]each subset comprises a heterogeneous cell population.[4-7]Moreover, the functions and activating/suppressing factors of each ILC subset were found to differ among both organs and types of tissue.

This review summarizesour current knowledge of ILCs by focusing on the organ/tissue–specific features of each subset in order to understand their roles in various organs. We also discuss ILCs' involvement in human inflammatory diseases in various organs and potential therapeutic/preventive strategies that target ILCs.

2SUBSETS OF ILCS AND THEIR PLASTICITY

Both NK cells and ILC1s produce type 1 (also known as Th1) cytokine, interferon (IFN)-γ, and are involved in immune responses against intracellular pathogens. NK cells and a minor population of ILC1sinspecific tissues, called intraepithelial ILC1s (ieILC1s), have a cytotoxic function via perforin and granzymes.NK cells are regulated by signals through various membrane–bound receptors, including killer–cell immunoglobulin–like receptors (KIRs) and natural cytotoxicity receptors (NCRs), in additionto cytokines.[8] However, the function of those receptors on ILC1s remains unclear.

ILC2s are characterized by expression ofGATA binding protein 3 (GATA-3) and production

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of type2 (also known as Th2) cytokines as well as amphiregulin (Areg) and IL-9;they are involved in the immune responses against helminths. ILC2s are regulated by various kinds of solublefactors such as cytokines,[9-30] neuropeptides/neurotransmitters[31-41] and lipid mediators.[42-47]They are also regulated via direct interaction of receptor–ligand pairs on ILCs and target cells (cell–cell contact) (Table 1).[48-52]

ILC3s express retinoic acid–related orphan receptor (RORγt) and produce such type3 (also known as Th17) cytokines as IL-17A and IL-22. They are responsible for the immune responses against extracellular pathogens. ILC3s are regulated by cytokines[53-58]and neuropeptides/neurotransmitters,[59,60]as well as cell–cell contact (Table 1).[61-63]ILC3s are generally divided into two distinct subtypes according to their expression of NCRs. NCR^{+}ILC3s preferentially express IL-22, whereas NCR^{-}ILC3s express both IL-17A and IL-22.[64]LTi cells also express RORγt and produce IL-17A and IL-22. Unlike ILC3s, LTi cells are involved in the formation of secondary lymphoid tissues during the fetal period.After birth, $CCR6^{+}$ILC3s inthe murine intestine[65] and neuropilin-1^{+}ILC3 in the human tonsil[66] show a similar phenotype to LTi cells, and they are considered to bethe adult counterparts of LTi cells.However, the characteristics of those cells in other organs remain unclear.

Resident IL-10–producing regulatory ILCs (initially named “ILCregs”) uniquely express inhibitor of DNA binding 3 (Id3)and resemble regulatory T cells (Tregs);[67]they were first identified in mouse intestine at steady state. Later studies found that IL-10–producingILCs were inducible, but not residential,[68]when type 2 inflammation was present, like in organs and tissues such as thelungin a murine asthma model[23,69,70]and the nasal tissue of patients withchronic rhinosinusitis with nasal polyp (CRSwNP).[23]Those cells were rarely detected in such organs/tissues at steady state. IL-10–producingILCs share some phenotypic features, like GATA-3 expression,with ILC2s, suggesting that they had undergone conversionfrom ILC2s.Indeed, such

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conversion was seen *in vitro* when human and mouse ILC2s were stimulated with retinoic acid (RA) (Figure 1).[23,69]Therefore, IL-10–producingILCsare not considered to be a distinct stable subset like Tregs, but rather a cell type converted from ILC2s. They arenow variously called ILCregs[23] or $ILC2_{10}$[69,71] or exhausted–like ILC2s[70,72]or IL-10–producingILCs.[73,74] Although they share some phenotypic features such as cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) expression,[23,69,70,73,74] further studies are needed to clarify whether they are the same cells. Similar to the findings for ILC2s, NK cells didnot produce IL-10 under homeostatic conditions, but started to produce it in the inflamed tissues of pathogen–infected mice.[75-77] These findings also support the concept that IL-10–producingILCs are derived from effector subsets.

ILCs are known to be highly plastic and change their function and phenotype depending onmicroenvironmental cues (Figure 1).[78] This phenomenon is called "plasticity", and it enables ILCs to respond promptly to microenvironmental changes without any need for differentiation or migration of new ILC subsets and/or progenitors adapted to a new microenvironment. These adaptations are obvious in the inflamed tissues of patients with inflammatory diseases that are biased to a certain specific immune response.[9,79-82] However, single-cell RNA sequencing (scRNA-seq) of tissue ILCs revealed that there are certain cell types in between two distinct subsets even at steady state.[6]

3ILC DIFFERENTIATION AND TISSUE RESIDENCY

ILC1, ILC2 and ILC3 emerge from common lymphoid progenitors (CLPs) after a series of differentiation stages into ILC precursors (ILCPs) in the fetal liver and postnatal bone marrow and spleen. ILCPs after birth also reside in peripheral tissues such as the lung,[57,83-85] intestine,[86] secondary lymphoid organs[85,87] and peripheral blood.[85] A recent study using a fate–mapping technique revealed that mosttissue ILC2s in adults arose largely during the neonatal–infancy period. Only a minority is replenished from hematopoietic stem cells during adulthood.[88]These

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findings suggest that tissue ILC2s are continuously replenished from local precursors residingin tissues during the neonatal–infancy period. Indeed, selective depletion of lung ILCPs in the neonatal period results in chronic marked reduction of lung ILC2s and ILC3s.[57] Parabiosis experiments demonstrated that matured ILC2s were tissue–resident and didnot migrate between organs during homeostasis.[22,89] In inflammatory states, ILC2s have been suggested to be mobilized from other organs into the inflamed tissues,[90-93]but the long-term capacity of these cells to adjust to different tissue environments remains unclear. Most studies have been conducted using ILC2s, and further studies are needed to improve our overall understanding of ILCs' tissue residency and migration.

Transcriptome and epigenome analysesfound each ILC subset to have unique open chromatin landscapes that specified stepwise development regardless of the organ.[94] However, all subsets of ILCs also had broadlyaccessible loci encoding lineage–defining transcription factors,[94] suggesting the existence of mechanisms that provide for phenotypic flexibility. In contrast, although ILC2s in the bronchoalveolar fluid and mesenteric lymphnodes have different phenotypes, their epigenetic featureswere highly similar.[95] These findings suggest that the phenotypic differences seen for each ILC subset in various organs may reflect the microenvironment rather than epigenetic modification.

4CHARACTERISTICS OF ILCS IN VARIOUS ORGANS AND TISSUES

4.1 Skin

The skin is comprised of epidermis, dermis and subcutaneous tissue. The composition of ILCs differsdepending on the skin layer: in mice, the predominant cell type isILC3s in the epidermis,ILC2sin the subcutaneous tissue, and both ILC2s and ILC3s in the dermis.[96]NK cells reside mainly in the dermis and rarely in the epidermisin both humans and mice (Figure 2A).[97]

IL-33, IL-25 and thymic stromal lymphopoietin (TSLP) are the major activators of

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ILC2s.However,the effects of those cytokines on ILC2s in tissues vary depending on the organ.The number of IL-5^{+}ILC2s in skin fromtriple–deficient (TKO) mice, which lack TSLP receptor (TSLPR), IL-25 and IL-33 receptor (ST2), was comparable to in wild–type (WT) mice at steady state, whereas their numbers in the lung, fat and gut from TKO mice were significantly reduced compared to WT mice.[29]These findings suggest thatthese three cytokines are not involved in IL-5–producingskin ILC2s at steadystate, unlike for ILC2s in other organs.In line with this, TSLPR, IL-17RB and ST2 are not expressed at the protein level in most healthy human skin ILC2s,[51] but they are expressed at the transcription level in mouse skin ILC2s.[96]Likewise, TSLP and IL-25 do not induce type2 cytokine production by healthy human skin ILC2s, whereas IL-33 does.[51]On the other hand, many skin ILC2s expressed IL-18R1, and IL-5^{+}ILC2s in the skin were significantly reduced in IL-18–deficient mice compared to WT mice,[29] suggesting the importance of IL-18 in IL-5–producing skin ILC2s at steady state.Recent scRNA–seq analysis showed that subcutaneous ILC2spredominantly expressed ST2, whereas dermal ILC2s expressed IL-18R1 (Figure 2A).[96] These findings suggest that thepredominant cytokines differ between subcutaneous ILC2s and dermal ILC2s.

In mice, ILC3sin both the epidermis and dermis expressed CCR6, and epidermal CCR6^{+}ILC3swere selectively reduced in the skin of CCR6–deficient mice.[96] These findings suggest the importance of CCR6 in localization of epidermal ILC3sand also as a possible therapeutic target. In humans, NCR^{-}ILC3s were the predominant population in skin from healthy individuals, while NCR^{+}ILC3swere a minor population (Figure 2A).[98]

4.2 Respiratory tract

The respiratory tract consists of the upper airways, includingthe nasal cavity and paranasal sinuses, and the lower airways, including bronchi,bronchioles and alveoli.In human lungs, NK cellsand CD127^{+}ILC1swere the predominant populations, followed by ILC3s. ILC2swere a minor

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population in human lungs, whereasthey were the predominant population in mouse lungs.[99]In human nasal tissue, ILC1s and ILC3swere the predominant populations, while ILC2swere a minor population (Figure 2B).[100]

Lung ILC2s and nasal ILC2s express ST2, and both produced type2 cytokines in response to IL-33 (Figure 2B).[101,102]TSLP alone did not induce production oftype2 cytokines, but it enhanced IL-33–induced cytokine production by lung ILC2s.[102]Of note, *in vitro*, TSLP inhibited corticosteroid–induced apoptosis of ILC2s through activationof signal transducer and activator of transcription 5 (STAT5). In addition, anti-TSLP treatment reduced the number of ILC2s in BALFs in the OVA/IL-33–induced corticosteroid–resistant asthma model and resulted in improvement of eosinophilic airway inflammation.[103]These findings suggest that the TSLP–ILC2 axis is involved in steroid resistance in eosinophilic airway inflammation.Lung ILC2s at steadystate expressed IL-17RB at a low level and hardly responded to IL-25 alone.[104]However, in the case of *Nippostrongylus brasiliensis* infection of primarily the intestines,IL-25–responsive killer cell lectin–like receptor G1 (KLRG-1)^{hi}IL-17RB^{hi}ILC2s (named inflammatory ILC2s; iILC2s) accumulatedin the lungsafter conversion from ILC2 precursors in the small intestine.[91,93,104]Airway ILC2s also expressed receptors for TNF superfamily (TNFS) cytokines. Briefly, mouse lung ILC2s expressedTNF receptor superfamily (TNFRSF) 25[30,105]and glucocorticoid–induced TNFR–related protein 18 (GITR/TNFRSF18).[20]Human nasal ILC2s expressed the receptor activator for NK-kB (RANK:TNFRSF11A).[19]Those ILC2s also produced type2 cytokines in response to their respective ligands (Table 1). IFN-α/β,[22,25] IFN-γ[22,25,26]and IL-27[22,25,27]suppressed activation of ILC2s. IL-27 receptor alpha was preferentially upregulated in mouse lung ILC2s compared to ILC2s in other organs,[29] suggesting the importance of IL-27 as a regulator of lung ILC2s. Mouse lung ILC2s expressed receptors for such neuropeptides/neurotransmitters as neuromedin U (NMU),[31,32]vasoactive intestinal peptide

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(VIP),[34,35]norepinephrine[36] and calcitonin gene-related peptide (CGRP).[37,38,40]VIP and NMU activated lung ILC2s to produce type 2 cytokines,[31,32,35]whereasnorepinephrine suppressed proliferation of lung ILC2s through beta-2 adrenergic receptor (β2-AR) (Table 1).[36] The effects of CGRP on lung ILC2s remain controversial.[37,38,40]Most of these studies investigated mouse lung ILC2s, and similar studies need to be conducted in regard to human lung and nasal ILC2s.In humans, a chemoattractant–receptor–homologous molecule expressed on Th2 cells (CRTh2), i.e., a receptor for prostaglandin D_2 (PGD_2), is used as a marker for ILC2s.[13] PGD_2inducedmigration[42,106]and cytokine production of ILC2s*in vitro*.[42,43]*In vivo*, $PGD_2$was crucial for migration of ILC2s to mouse lungs.[90]However,expression of CRTh2 on ILC2s differsdepending on the organ. Lung ILC2sin both humans and mice expressed less CRTh2 compared to those on peripheral blood (PB) ILC2s,[90]whereas CRTh2 expression was comparable on nasal andPB ILC2s (Figure 2B).[101] These findings suggest that lung ILC2s may be less responsive to PGD_2 than nasal ILC2s.

The characteristics of ILC3s in the respiratory tract have not been sufficiently studied. In mouse lungs, $CCR6^+$ILC3swere the predominant population among ILC3s and produced IL-17A and IL-22.[107]In high–fat–diet obese mice, IL-1β expanded $CCR6^+$ILC3s in the lungs, and thosecells inducedairway hyperreactivity,such as seen in asthma,through IL-17A.[58]In humans, NCR^-ILC3s were more abundant than NCR^+ILC3s in both lung[108] and nasal[100] tissues (Figure 2B).

4.3 Gastrointestinal tract

The gastrointestinal (GI) tract consists of the upper GI tract, including the esophagus, stomach, duodenum; and the lower GI tract, including the small intestine and colon.The roles, structure and microbiotaof each organ differ markedly. Therefore, the microenvironments of the organscan be presumed to be completely different.In humans, ILC1s, including $CD127^+$ILC1s and ieILC1s, arethe predominant population in the upper GI tract. In contrast, in the lower GI tract, ILC3s

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predominate.[109]ILC2sare a minor population throughout the GI tract.[109]In contrast, in mice, ILC2swere predominant in the stomach, whereas ILC3swere predominant in the lower GI tract (Figure 2C).[110]

Although human ILC2s from both the upper and lower GI tracts express CRTh2,[99]some of their other characteristics differ between the upper and lower GI tracts. Gastric ILC2s express high levels of ST2, whereas intestinal ILC2s predominantly express IL-17RB(Figure 2C) and a low level of ST2. Gastric ILC2s proliferatedin response to IL-33, but not IL-25,*in vitro*,[110] whereas small intestinal ILC2s secreted IL-13 in response to both IL-33 and IL-25.[111]IL-25,constitutively expressed by epithelial–lineage tuft cells in the intestines, sustained ILC2 homeostasis in the small intestine.[111-115]

Intestinal ILC2s are also regulated by food components and neuropeptides secreted in response to food ingestion.A vitamin Ametabolite, RA,acted as a negative regulator of ILC2 differentiation.[116]Like mouse lung ILC2s, mouse intestinal ILC2s expressed the receptor for VIP and increased after food ingestion, together with increased VIP.[34]In addition, mouse intestinal ILC2s expressed the receptor for NMUand were activated by NMU (Table 1).[32]

Intestinal ILC3s are regulated by the intestinal microbiota, either directly or indirectly.Indeed, the frequency of RORγt^{+}ILC3s as well as IL-22–producing ILC3sin the intestine was reduced in germ–free (GF) mice compared to conventional mice.[117]Metabolites such as short–chain fatty acids (SCFAs) are crucial factors for the microbiota.CCR6^{+}LTi–like cellsin the mouseileum and colon expresseda receptor for SCFAs, i.e.,free fatty acid receptor 2 (FFAR2: GPR43).Decreased frequencyand impaired function of ILC3s in GF mice were restored to some extent by feedingthem SCFAs.[117]Microbiota–induced cytokines are also crucial factors. Segmented filamentous bacteria (SFB) induced IL-23 production by myeloid cells, which in turn induced IL-22 production by CCR6^{+}LTi–like cells, NCR^{+}ILC3s and NCR^{-}ILC3s.[118,119]Microbiota–induced

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TNF–like cytokine 1A (TL1A) from CX3CR1^{+} mononuclear phagocytes, together with IL-23,activated CCR6^{+}LTi–like cells.[54,120]Microbiota also induced IL-1β production by intestinal macrophages, IL-1β in turn inducedgranulocyte–macrophage colony–stimulating factor (GM–CSF)production by ILC3s. ILC3–derived GM–CSF supported expansion of Tregs, which promote oral tolerance.[121]

Intestinal ILC3s are also regulated by food components and neuropeptidesthat are secreted in response to food ingestion (Table 1). RA supported the development of LTi–like cells,[122] as well as activation[116,123] and migration of intestinal ILC3s in mice.[124]Mouse intestinal ILC3swere localized in close proximity to VIP–expressing enteric neurons.[59,60]However, the effects of food ingestion and VIP on intestinal ILC3s remain unclear, since two reports presented conflicting results.[59,60]Recently, the circadian rhythm, by which organisms regulate physiological systems to adapt to environmental changes, was reported to also regulate intestinal ILC3s.[125-127]Briefly, deletion of the master clock gene *Arntl*,which encodes Bmal1, resulted in marked reduction of intestinal ILC3s.[127]In addition, deletion of a circadian related gene,*Nr1d1* (*Rev-Erba*), resulted in marked reduction and impairment of NCR^{+}ILC3s, whereas it increased IL-17 production by NCR^{-}ILC3.[126]Most studies have been conducted on the lower GI tract, so the characteristics of ILC3s in the upper GI remain unclear.

5AGE–RELATED FACTORS AFFECTING ILCS

Recent findings suggest that tissue ILCs are also affected by age. In mice, the number of ILC2s in the lungs peaked during the neonatal–infancy period after birth.[88,128,129]In addition, neonatal lung ILC2sspontaneously expressed IL-5 and IL-13 and produced more type2 cytokinesthan adult ILC2s did.[88,128]Mouse skin ILC2s and intestinal ILC2s also peaked during the neonatal–infancy period (Figure 3).[88] Interestingly, the number of lung ILC3s also peaked during the neonatal–

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infancy period in mice.[57]Moreover, human intestinal NK cells and CD127^{+}ILC1s showeda similar trend with age, and infant intestinal NK cells were functionally superior to adult NK cells.[130]These findings suggest that innate immunity, especially ILCs, is more developed during infancy than in adulthood, which compensates for the still–immature acquired immunity. Of note, as mentioned earlier,ILCs are tissue–resident,[22,88,131]and tissue ILC2s in adults comprise the population that arose largely during the neonatal–infancy period.[88]These findings also indicatethe importance of the neonatal–infancy period as a critical time–window for development oflife–long ILC2–dependent immunity (Figure 3).

Recently, the innate immune system, including ILC2s, has been shown to possess a kind of memory–like capacity called "trained immunity". This means long–term functional adaptability that enables them to respond quickly and strongly to secondary stimuli.[132]Briefly, allergen– (or stimuli–) experienced ILC2spersistedfor a long time atlocal tissue sites and responded potently upon re-exposure to the stimulus.[133]Interestingly, activation ("training") of lung ILC2s by endogenous IL-33 during theneonatal–infancy period was crucial for the function of adult lung ILC2s,[134]emphasizing the importance of that early period for development of life–long ILC2–dependent immunity. On the other hand,excessive activation (training) of ILC2s during the neonatal–infancy period may lead to hyperactivity of ILC2s later in life, which may result in allergic diseases. Indeed, human infants with severe respiratory syncytial virus (RSV) bronchiolitis had increased ILC2s and IL-33 in nasal aspirates compared with infants with mild RSV infection,[135] and the severe infection was shown to be a strong risk factor for allergic asthma at age 13.[136]

At present,the origin and lifespan oftissue ILCs other than ILC2s remain unclear.However, considering that trained immunity was also found in other subsets of ILCs,[137-139]exposure to various stimuli—including the microbiota, infection and other factors—during the neonatal–

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infancy period could be a crucial factor in the formation of life–long ILC–dependent tissue immunity.Moreover, excessive activation ("training") may lead to development of chronic inflammatory diseases later in life (Figure 3).

6INVOLVEMENT OF ILCS IN INFLAMMATORY DISEASE AND THERAPEUTIC APPROACHES TARGETING ILCS

6.1 Skin

6.1.1 Atopic dermatitis

Lesional skin of atopic dermatitis (AD) patients was compared to healthy skin, and both the frequencies of $ST2^+$ILC2s, IL-17RB^+ILC2s and $TSLPR^+$ILC2[51,140] and the expression of each of IL-33, IL-25 and TSLP[51,141-143] were increased. However, possibleinvolvement of an epithelial–derived cytokine/ILC2 axis in the pathophysiology of AD needsto be discussed further. In mice, topical application of a vitamin Danalogue (MC903)inducedAD–like skin inflammation in an ILC2–dependent manner.[140]Involvement of IL-33 in this model is controversial,[51,140,144]as is the effectiveness of anti-IL-33 monoclonal antibody (mAb) treatment forADand eczema in humans(Figure 4Aand Table E1).MC903–induced skin inflammation was attenuated in TSLPR–deficient mice compared to WT mice.[140]In that model, TSLP induced basophils to produce IL-4, which led to activation of skin ILC2s.[145]In humans, anti-TSLP mAb treatment ofADshowed numerical improvement over the placebo after 16 weeks of treatment but did not reach statistical significance (Figure 4A and Table E1).Based on receptor expressionby skin ILC2s, IL-18 could be a potential target, but no clinical study is in progress. mAbs targeting downstream of ILC2 activationshowed a substantial effect on AD (Table E1), suggesting that ILC2s may be involved in the pathophysiology of AD. In addition to ILC2s, recent work revealed the potential of NK cells as a therapeutic target for AD.[146]

6.1.2 Psoriasis

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The frequency of NCR$^+$ILC3swas increased in lesional skin,[98] non–lesional skin[147] and PB[98,147] of psoriasis patients compared to the controlsandcorrelated with disease severity.[98] In addition, the frequency of ILC2s was decreased in lesional skin of psoriasis patients, suggesting involvement of conversion of ILC2s into ILC3s in the pathophysiology.[82]Indeed, ILC3 activators and effector cytokines were increased in the lesional skin of psoriasis patients compared to healthy skin.[148]In mice, RORγt$^+$ILC3s were involved in toll–like receptor 7 (TLR7)agonist (Imiquimod)–induced psoriasis–like skin inflammation.[149]These findings point to involvement of NCR$^+$ILC3s in the pathophysiology of psoriasis.In humans, mAb treatments targeting IL-23p19,IL-12p40,IL-17A, IL-17A/IL17F andIL17RAwere highly effective for psoriasis patients (Figure 4B and Table E1).Based on the expression and role of skin ILC3s, CCR6 could also be a potential treatment target.

6.2 Respiratory tract

6.2.1 Asthma

A GWAS showed thatsingle nucleotide polymorphisms in IL-33 and ST2 as well as TSLP were associated with asthma development.The frequencies of ILC2s in the PB and bronchoalveolar lavage fluids (BALFs)of asthma patients were increased compared with control subjects and correlated negatively with lung function.[150,151]In addition, the levels of IL-33[151] and TSLP[152] in BALFswere increased in asthma patients. Of note,the TSLP level in asthma patients' BALF correlated with steroid resistance of ILC2s,[152]which supports earlier evidence of a role for TSLP in steroid resistance in mice.[103]Likewise, numerousmouse studies using various asthmamodels also showed involvement of ILC2s as well as IL-33 and TSLP in the pathophysiology of asthma.[153,154]In humans, three mAbs targeting downstream of ILC2 activation,i.e., anti-IL4Rα, anti-IL-5 and anti-IL-5Rα, showed a substantial effect on asthma (Table E1).In regard to upstream regulators, anti-IL-33 mAbs are currently undergoing clinical trials forpatients with moderate–

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severe asthma.Anti-TSLP mAb treatment significantly reduced asthma exacerbation in steroid–resistant severe asthma patients (Figure 5A and Table E1).Besides type2 inflammation, types1 and 3 may also be involved in the pathophysiology of asthma, especially the severe phenotype.[155]Expression of IL-17 in the lungs[156]wasincreased in severe asthma patients, suggesting involvement of ILC3s or newly–identified IL-17–producing ILC2s[157] in the pathophysiology of severe asthma.However, mAb treatments targeting type1 and type3 inflammation have so far not shown any clinical benefit in asthma patients(Figure 5A and Table E1).

6.2.2 CRSwNP

CRSwNP is characterized by eosinophil infiltration and type2 inflammation.The frequency of ILC2s in nasal polyps (NPs) is increased compared to healthy nasal tissues.[101,158-161]TSLP wasconsistently upregulated or increased in NPs of CRSwNP patients compared to controls,[162,163] suggesting that TSLP may be a potential therapeutic target (Figure 5B). In contrast, the levels of IL-33[160,162,164,165] and IL-25[160,162,164-166] in NPshave been variously reported as elevated or comparable to controls.RANK ligand (RANKL)[19]andlipidmediators such as PGD_2 and cysteinyl leukotrienes are also elevated in NPs of CRSwNP.[167,168]Therefore, these moleculesmay also be potential therapeutic targets.In humans, treatment withanti-IL4RαmAb reduced the size of NPs in CRSwNP patients.Anti-IL-33 mAbsare now in clinical trials for CRSwNP (Figure 5B and Table E1). Considering the evidence regarding ILC2s, anti-TSLP mAbs could potentially be clinicallybeneficial for CRSwNP, but no clinical trialsare currently in progress. Treatments targeting CRTh2arenow inphase 2 clinical trials (Figure5B and Table E1). A CRTh2–specific antagonist, fevipirant, did not show clinical benefit in asthma patients,[169]but it might be beneficial for CRSwNP in view of the higher expression of CRTh2 on nasal ILC2s.

6.2.3 Allergic rhinitis

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The frequency of ILC2s in PB of seasonal allergic rhinitis (AR) patients was increased inseason, but not out of season.[170] Local allergen challenge induced accumulation of ILC2s in the nasal tissue of AR patients, accompanied by increased levels ofIL-5, IL-13 and PGD_2 in their nasal lavage.[171]PGD_2 synthesized by activated mast cells upon allergen challenge may lead to migration of ILC2s to the nasal tissue in AR patients.The PB of AR patients who underwent allergen–specific immunotherapy (AIT) showed decreased ILC2s[73,170,172,173] and increasedIL-10–producing ILCs.[73,74]Moreover, the frequency of IL-10–producing ILCscorrelated with improvement in theclinical score.[73]Although the mechanism of how AIT induces IL-10–producing ILCs remains unclear, those cellsmay be involved in the improvement of symptoms seen in AR patients after AIT.Therefore, clarification of factors that induce IL-10–producing ILCs may lead to novel therapeutic strategies for AR.

6.2.4 Chronic obstructive pulmonary disease (COPD)

COPD is associated with long–term exposure to cigarette smoke and is most commonly characterized by type1 and type3 immunity, with increased numbers of neutrophils and macrophages.COPD patients showed increased frequencies of ILC1s and NCR$^-$ILC3s in the lungs[9,108]and ILC1s in the PB[80]compared to control subjects (Figure 5). In addition, the frequency of ILC1s in COPD patients' PBcorrelated negatively with lung function.[80] In parallel, the frequencies of ILC2s in COPD patients' PB[80]and lungs[9]were decreased. IL-12 induced conversion of ILC2s into ILC1s both *in vitro* and *in vivo* (Figure 1),[9,80]and that conversion may be involved in COPD's pathophysiology. However, in humans, anti-IL-1β and anti-IL-17A mAb treatments targeting type1 and type3 immunity showed no clinical benefit in COPD(Figure 5C and Table E1). Some COPD patients also show eosinophilic inflammation, and they are more likely to experience more–severe exacerbation. In addition, TSLP and IL-33 were upregulated (increased) in airway epithelial cells[174-176] and lungs[176] of COPD patients. Therefore, TSLP and IL-33are currently being

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targeted for treatment of COPD(Figure 5C and Table E1). In a murine cigarette–smoke–induced COPD model, IL-33 was upregulated in the lungs of the smoke–exposed mice. The smoke exposure silenced ILC2s by downregulating their expression of ST2, as a result of which the animals did not show typical type2 inflammation despite the upregulation of IL-33. However, ST2 expression was upregulated in macrophages, dendritic cells and NK cells,[176] suggesting that an alternative IL-33–mediatedpathway may be involved in the pathophysiology of COPD.

6.3 Gastrointestinal tract

6.3.1 Eosinophilic gastrointestinal diseases(EGIDs)

EGIDs, including eosinophilic esophagitis (EoE) and non-esophageal EGIDs, are diseases of the gastrointestinal tract that are accompanied by inappropriate accumulation of eosinophils in the tissues.The frequency of ILC2s in esophageal tissue of active EoE patients was significantly increased compared to controls and correlated positively withthenumber of infiltrated eosinophils.[177]In addition, expression ofboth IL-33[178]and TSLP[179]was upregulated in esophageal tissue of active EoE patients compared to controls, suggesting involvement of ILC2s in the pathophysiology (Figure 6A and Table E1).Further studies are needed to clarify ILC2s' possible involvement in non-esophageal EGIDs.However, such involvement is suggested by the finding that both IL-33 and TSLP levels in the colon were increased in infantile eosinophilic gastroenteritis patients compared to control subjects.[180]

6.3.2 Inflammatory bowel diseases(IBD)

IBDs, such as Crohn's disease (CD) and ulcerative colitis, are chronic inflammatory disorders ofthe gastrointestinal tract.A GWAS identified multiple potentially causal *IL23R*and*NOD2*variants for IBD, suggesting involvement of ILC1s and ILC3s in the pathophysiology through those signaling pathways.[181]IFN-γ–producing $CD127^+$ILC1sand ieILC1swere increased in the intestineof CD patients compared to the controls,[182,183] whereasIL-22–producing NCR^+ILC3s were

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decreased (Figure 6B).[184]Since human NCR$^+$ILC3s canundergo conversion to IFN-γ–producing CD127$^+$ILC1s (Figure 1),[79]an unbalanced shift from NCR$^+$ILC3s to ILC1s may occur in IBD patients.Interestingly, the decreased frequency of ILC3s in inflamed tissues of IBD patients accompanied altered expression of such circadian–related genes as *Nr1d1, Per3* and *Nfil3*.[127]Given that intestinal ILC3s are regulated by a circadian clock, disruption of that rhythm mightalso trigger the pathophysiology of IBD.IL-12 and IL-23 are the respective activators of ILC1s and ILC3s, and theymight be therapeutic targets for IBD. Anti-IL12p40mAbtreatment targeting both IL-12 and IL-23 was effective inCD patients (Figure 6B and TableE1).However, unlike in psoriasis, anti-IL-17A and anti-IL17RA mAb treatment did not show any clinical benefit in CD patients.

7CONCLUSION

As noted earlier, tissue–resident ILCs are heterogeneous, and their critical regulators may differ depending on the organ/tissue. Therefore, conventional upstream regulators may not always be effective when investigating ILCs as therapeutic targets. Considering the expression and expected function of receptors, some TNFRSF molecules and IL-27 in the airway, and IL-18 and CCR6 in the skin,may be potential targets. Development of more–effective therapies for inflammatory diseases will require further analysisto clarify the tissue–specific and disease–specificfeatures of ILCs, as well as biomarkers to stratifythese heterogeneous diseases. In addition, in view of the origin and lifespan of tissue–resident ILCs, the neonatal–infancy period may be a critical window for ILC–targeting preventive strategies for inflammatory diseases.

Acknowledgments

We thank Mr. Lawrence W. Stiver (Tokyo, Japan; Quality Translation Co., Ltd.; qualityt@gol.com) for editing our manuscript.

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REFERENCES

1. Spits H, Artis D, Colonna M, et al. Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol.* 2013;13(2):145-149.
2. Kortekaas Krohn I, Shikhagaie MM, Golebski K, et al. Emerging roles of innate lymphoid cells in inflammatory diseases: clinical implications. *Allergy.* 2017. 2018;73(4):837-850.
3. Vivier E, Artis D, Colonna M, et al. Innate Lymphoid Cells: 10 Years On. *Cell.* 2018;174(5):1054-1066.
4. Bjorklund AK, Forkel M, Picelli S, et al. The heterogeneity of human CD127(+) innate lymphoid cells revealed by single-cell RNA sequencing. *Nat Immunol.* 2016;17(4):451-460.
5. Gury-BenAri M, Thaiss CA, Serafini N, et al. The Spectrum and Regulatory Landscape of Intestinal Innate Lymphoid Cells Are Shaped by the Microbiome. *Cell.* 2016;166(5):1231-1246 e1213.
6. Cella M, Gamini R, Secca C, et al. Publisher Correction: Subsets of ILC3-ILC1-like cells generate a diversity spectrum of innate lymphoid cells in human mucosal tissues. *Nat Immunol.* 2019;20(10):1405.
7. Mazzurana L, Czarnewski P, Jonsson V, et al. Tissue-specific transcriptional imprinting and heterogeneity in human innate lymphoid cells revealed by full-length single-cell RNA-sequencing. *Cell Res.* 2021. doi: 10.1038/s41422-020-00445-x.
8. Riggan L, Freud AG, O'Sullivan TE. True Detective: Unraveling Group 1 Innate Lymphocyte Heterogeneity. *Trends Immunol.* 2019;40(10):909-921.
9. Bal SM, Bernink JH, Nagasawa M, et al. IL-1beta, IL-4 and IL-12 control the fate of group 2 innate lymphoid cells in human airway inflammation in the lungs. *Nat Immunol.* 2016;17(6):636-645.

This article is protected by copyright. All rights reserved

Accepted Article

10. Ohne Y, Silver JS, Thompson-Snipes L, et al. IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity. *Nat Immunol.* 2016;17(6):646-655.
11. Motomura Y, Morita H, Moro K, et al. Basophil-derived interleukin-4 controls the function of natural helper cells, a member of ILC2s, in lung inflammation. *Immunity.* 2014;40(5):758-771.
12. Turner JE, Morrison PJ, Wilhelm C, et al. IL-9-mediated survival of type 2 innate lymphoid cells promotes damage control in helminth-induced lung inflammation. *J Exp Med.* 2013;210(13):2951-2965.
13. Mjosberg JM, Trifari S, Crellin NK, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CRTH2 and CD161. *Nat Immunol.* 2011;12(11):1055-1062.
14. Mjosberg J, Bernink J, Golebski K, et al. The transcription factor GATA3 is essential for the function of human type 2 innate lymphoid cells. *Immunity.* 2012;37(4):649-659.
15. Hurrell BP, Galle-Treger L, Jahani PS, et al. TNFR2 Signaling Enhances ILC2 Survival, Function, and Induction of Airway Hyperreactivity. *Cell Rep.* 2019;29(13):4509-4524 e4505.
16. Fonseca W, Rasky AJ, Ptaschinski C, et al. Group 2 innate lymphoid cells (ILC2) are regulated by stem cell factor during chronic asthmatic disease. *Mucosal Immunol.* 2019;12(2):445-456.
17. Patel DF, Peiro T, Bruno N, et al. Neutrophils restrain allergic airway inflammation by limiting ILC2 function and monocyte-dendritic cell antigen presentation. *Sci Immunol.* 2019;4(41).
18. Loh Z, Simpson J, Ullah A, et al. HMGB1 amplifies ILC2-induced type-2 inflammation and airway smooth muscle remodelling. *PLoS Pathog.* 2020;16(7):e1008651.

This article is protected by copyright. All rights reserved

Accepted Article

19. Ogasawara N, Poposki JA, Klingler AI, et al. Role of RANK-L as a potential inducer of ILC2-mediated type 2 inflammation in chronic rhinosinusitis with nasal polyps. *Mucosal Immunol.* 2020;13(1):86-95.
20. Nagashima H, Okuyama Y, Fujita T, et al. GITR cosignal in ILC2s controls allergic lung inflammation. *J Allergy Clin Immunol.* 2018;141(5):1939-1943 e1938.
21. Morita H, Arae K, Unno H, et al. An Interleukin-33-Mast Cell-Interleukin-2 Axis Suppresses Papain-Induced Allergic Inflammation by Promoting Regulatory T Cell Numbers. *Immunity.* 2015;43(1):175-186.
22. Moro K, Kabata H, Tanabe M, et al. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. *Nat Immunol.* 2016;17(1):76-86.
23. Morita H, Kubo T, Ruckert B, et al. Induction of human regulatory innate lymphoid cells from group 2 innate lymphoid cells by retinoic acid. *J Allergy Clin Immunol.* 2019;143(6):2190-2201 e2199.
24. Ogasawara N, Poposki JA, Klingler AI, et al. IL-10, TGF-beta, and glucocorticoid prevent the production of type 2 cytokines in human group 2 innate lymphoid cells. *J Allergy Clin Immunol.* 2018;141(3):1147-1151 e1148.
25. Duerr CU, McCarthy CD, Mindt BC, et al. Type I interferon restricts type 2 immunopathology through the regulation of group 2 innate lymphoid cells. *Nat Immunol.* 2016;17(1):65-75.
26. Molofsky AB, Van Gool F, Liang HE, et al. Interleukin-33 and Interferon-gamma Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation. *Immunity.* 2015;43(1):161-174.
27. McHedlidze T, Kindermann M, Neves AT, Voehringer D, Neurath MF, Wirtz S. IL-27

This article is protected by copyright. All rights reserved

suppresses type 2 immune responses in vivo via direct effects on group 2 innate lymphoid cells. *Mucosal Immunol.* 2016;9(6):1384-1394.

28. Shamji MH, Layhadi JA, Achkova D, et al. Role of IL-35 in sublingual allergen immunotherapy. *J Allergy Clin Immunol.* 2019;143(3):1131-1142 e1134.
29. Ricardo-Gonzalez RR, Van Dyken SJ, Schneider C, et al. Tissue signals imprint ILC2 identity with anticipatory function. *Nat Immunol.* 2018;19(10):1093-1099.
30. Machida K, Aw M, Salter BMA, et al. The Role of the TL1A/DR3 Axis in the Activation of Group 2 Innate Lymphoid Cells in Subjects with Eosinophilic Asthma. *Am J Respir Crit Care Med.* 2020;202(8):1105-1114.
31. Wallrapp A, Riesenfeld SJ, Burkett PR, et al. The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature.* 2017;549(7672):351-356.
32. Klose CSN, Mahlakoiv T, Moeller JB, et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature.* 2017;549(7671):282-286.
33. Cardoso V, Chesne J, Ribeiro H, et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature.* 2017;549(7671):277-281.
34. Nussbaum JC, Van Dyken SJ, von Moltke J, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature.* 2013;502(7470):245-248.
35. Talbot S, Abdulnour RE, Burkett PR, et al. Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. *Neuron.* 2015;87(2):341-354.
36. Moriyama S, Brestoff JR, Flamar AL, et al. beta2-adrenergic receptor-mediated negative regulation of group 2 innate lymphoid cell responses. *Science.* 2018;359(6379):1056-1061.
37. Sui P, Wiesner DL, Xu J, et al. Pulmonary neuroendocrine cells amplify allergic asthma responses. *Science.* 2018;360(6393).
38. Wallrapp A, Burkett PR, Riesenfeld SJ, et al. Calcitonin Gene-Related Peptide Negatively

This article is protected by copyright. All rights reserved

Accepted Article

Regulates Alarmin-Driven Type 2 Innate Lymphoid Cell Responses. *Immunity.* 2019;51(4):709-723 e706.

39. Xu H, Ding J, Porter CBM, et al. Transcriptional Atlas of Intestinal Immune Cells Reveals that Neuropeptide alpha-CGRP Modulates Group 2 Innate Lymphoid Cell Responses. *Immunity.* 2019;51(4):696-708 e699.
40. Nagashima H, Mahlakoiv T, Shih HY, et al. Neuropeptide CGRP Limits Group 2 Innate Lymphoid Cell Responses and Constrains Type 2 Inflammation. *Immunity.* 2019;51(4):682-695 e686.
41. Galle-Treger L, Suzuki Y, Patel N, et al. Nicotinic acetylcholine receptor agonist attenuates ILC2-dependent airway hyperreactivity. *Nat Commun.* 2016;7:13202.
42. Xue L, Salimi M, Panse I, et al. Prostaglandin D2 activates group 2 innate lymphoid cells through chemoattractant receptor-homologous molecule expressed on TH2 cells. *J Allergy Clin Immunol.* 2014;133(4):1184-1194.
43. Barnig C, Cernadas M, Dutile S, et al. Lipoxin A4 regulates natural killer cell and type 2 innate lymphoid cell activation in asthma. *Sci Transl Med.* 2013;5(174):174ra126.
44. von Moltke J, O'Leary CE, Barrett NA, Kanaoka Y, Austen KF, Locksley RM. Leukotrienes provide an NFAT-dependent signal that synergizes with IL-33 to activate ILC2s. *J Exp Med.* 2017;214(1):27-37.
45. Doherty TA, Khorram N, Lund S, Mehta AK, Croft M, Broide DH. Lung type 2 innate lymphoid cells express cysteinyl leukotriene receptor 1, which regulates TH2 cytokine production. *J Allergy Clin Immunol.* 2013;132(1):205-213.
46. Zhou W, Toki S, Zhang J, et al. Prostaglandin I2 Signaling and Inhibition of Group 2 Innate Lymphoid Cell Responses. *Am J Respir Crit Care Med.* 2016;193(1):31-42.
47. Maric J, Ravindran A, Mazzurana L, et al. Prostaglandin E2 suppresses human group 2

This article is protected by copyright. All rights reserved

innate lymphoid cell function. *J Allergy Clin Immunol.* 2018;141(5):1761-1773 e1766.

48. Maazi H, Patel N, Sankaranarayanan I, et al. ICOS:ICOS-ligand interaction is required for type 2 innate lymphoid cell function, homeostasis, and induction of airway hyperreactivity. *Immunity.* 2015;42(3):538-551.
49. Yu Y, Tsang JC, Wang C, et al. Single-cell RNA-seq identifies a PD-1hi ILC progenitor and defines its development pathway. *Nature.* 2016;539(7627):102-106.
50. Salimi M, Xue L, Jolin H, et al. Group 2 Innate Lymphoid Cells Express Functional NKp30 Receptor Inducing Type 2 Cytokine Production. *J Immunol.* 2016;196(1):45-54.
51. Salimi M, Barlow JL, Saunders SP, et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med.* 2013;210(13):2939-2950.
52. Lei AH, Xiao Q, Liu GY, et al. ICAM-1 controls development and function of ILC2. *J Exp Med.* 2018;215(8):2157-2174.
53. Buonocore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature.* 2010;464(7293):1371-1375.
54. Castellanos JG, Woo V, Viladomiu M, et al. Microbiota-Induced TNF-like Ligand 1A Drives Group 3 Innate Lymphoid Cell-Mediated Barrier Protection and Intestinal T Cell Activation during Colitis. *Immunity.* 2018;49(6):1077-1089 e1075.
55. Giacomin PR, Moy RH, Noti M, et al. Epithelial-intrinsic IKKalpha expression regulates group 3 innate lymphoid cell responses and antibacterial immunity. *J Exp Med.* 2015;212(10):1513-1528.
56. Bando JK, Gilfillan S, Song C, et al. The Tumor Necrosis Factor Superfamily Member RANKL Suppresses Effector Cytokine Production in Group 3 Innate Lymphoid Cells. *Immunity.* 2018;48(6):1208-1219 e1204.
57. Oherle K, Acker E, Bonfield M, et al. Insulin-like Growth Factor 1 Supports a Pulmonary

This article is protected by copyright. All rights reserved

Accepted Article

Niche that Promotes Type 3 Innate Lymphoid Cell Development in Newborn Lungs. *Immunity.* 2020;52(2):275-294 e279.

58. Kim HY, Lee HJ, Chang YJ, et al. Interleukin-17-producing innate lymphoid cells and the NLRP3 inflammasome facilitate obesity-associated airway hyperreactivity. *Nat Med.* 2014;20(1):54-61.
59. Talbot J, Hahn P, Kroehling L, Nguyen H, Li D, Littman DR. Feeding-dependent VIP neuron-ILC3 circuit regulates the intestinal barrier. *Nature.* 2020;579(7800):575-580.
60. Seillet C, Luong K, Tellier J, et al. The neuropeptide VIP confers anticipatory mucosal immunity by regulating ILC3 activity. *Nat Immunol.* 2020;21(2):168-177.
61. Glatzer T, Killig M, Meisig J, et al. RORgammat(+) innate lymphoid cells acquire a proinflammatory program upon engagement of the activating receptor NKp44. *Immunity.* 2013;38(6):1223-1235.
62. Seo GY, Shui JW, Takahashi D, et al. LIGHT-HVEM Signaling in Innate Lymphoid Cell Subsets Protects Against Enteric Bacterial Infection. *Cell Host Microbe.* 2018;24(2):249-260 e244.
63. Vacca P, Pesce S, Greppi M, et al. PD-1 is expressed by and regulates human group 3 innate lymphoid cells in human decidua. *Mucosal Immunol.* 2019;12(3):624-631.
64. Wang Q, Colonna M. Keeping time in group 3 innate lymphoid cells. *Nat Rev Immunol.* 2020;20(12):720-726.
65. Wang W, Li Y, Hao J, et al. The Interaction between Lymphoid Tissue Inducer-Like Cells and T Cells in the Mesenteric Lymph Node Restrains Intestinal Humoral Immunity. *Cell Rep.* 2020;32(3):107936.
66. Shikhagaie MM, Bjorklund AK, Mjosberg J, et al. Neuropilin-1 Is Expressed on Lymphoid Tissue Residing LTi-like Group 3 Innate Lymphoid Cells and Associated with Ectopic

This article is protected by copyright. All rights reserved

Accepted Article

Lymphoid Aggregates. *Cell Rep.* 2017;18(7):1761-1773.

67. Wang S, Xia P, Chen Y, et al. Regulatory Innate Lymphoid Cells Control Innate Intestinal Inflammation. *Cell.* 2017;171(1):201-216 e218.
68. Bando JK, Gilfillan S, Di Luccia B, et al. ILC2s are the predominant source of intestinal ILC-derived IL-10. *J Exp Med.* 2020;217(2).
69. Seehus CR, Kadavallore A, Torre B, et al. Alternative activation generates IL-10 producing type 2 innate lymphoid cells. *Nat Commun.* 2017;8(1):1900.
70. Miyamoto C, Kojo S, Yamashita M, et al. Runx/Cbfbeta complexes protect group 2 innate lymphoid cells from exhausted-like hyporesponsiveness during allergic airway inflammation. *Nat Commun.* 2019;10(1):447.
71. Howard E, Lewis G, Galle-Treger L, et al. IL-10 production by ILC2s requires Blimp-1 and cMaf, modulates cellular metabolism, and ameliorates airway hyperreactivity. *J Allergy Clin Immunol.* 2020. doi: 10.1016/j.jaci.2020.08.024.
72. Ebihara T, Taniuchi I. Exhausted-like Group 2 Innate Lymphoid Cells in Chronic Allergic Inflammation. *Trends Immunol.* 2019;40(12):1095-1104.
73. Boonpiyathad T, Tantilipikorn P, Ruxrungtham K, et al. IL-10-producing innate lymphoid cells increased in house-dust-mite allergic rhinitis patients following immunotherapy. *J Allergy Clin Immunol.* 2020. doi: 10.1016/j.jaci.2020.10.029.
74. Golebski K, Layhadi JA, Sahiner U, et al. Induction of IL-10-producing type 2 innate lymphoid cells by allergen immunotherapy is associated with clinical response. *Immunity.* 2021. doi: 10.1016/j.immuni.2020.12.013.
75. De Maria A, Fogli M, Mazza S, et al. Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. *Eur J Immunol.* 2007;37(2):445-455.

This article is protected by copyright. All rights reserved

76. Clark SE, Schmidt RL, Aguilera ER, Lenz LL. IL-10-producing NK cells exacerbate sublethal Streptococcus pneumoniae infection in the lung. *Transl Res.* 2020;226:70-82.
77. Perona-Wright G, Mohrs K, Szaba FM, et al. Systemic but not local infections elicit immunosuppressive IL-10 production by natural killer cells. *Cell Host Microbe.* 2009;6(6):503-512.
78. Bal SM, Golebski K, Spits H. Plasticity of innate lymphoid cell subsets. *Nat Rev Immunol.* 2020;20(9):552-565.
79. Bernink JH, Krabbendam L, Germar K, et al. Interleukin-12 and -23 Control Plasticity of CD127(+) Group 1 and Group 3 Innate Lymphoid Cells in the Intestinal Lamina Propria. *Immunity.* 2015;43(1):146-160.
80. Silver JS, Kearley J, Copenhaver AM, et al. Inflammatory triggers associated with exacerbations of COPD orchestrate plasticity of group 2 innate lymphoid cells in the lungs. *Nat Immunol.* 2016;17(6):626-635.
81. Lim AI, Menegatti S, Bustamante J, et al. IL-12 drives functional plasticity of human group 2 innate lymphoid cells. *J Exp Med.* 2016;213(4):569-583.
82. Bernink JH, Ohne Y, Teunissen MBM, et al. c-Kit-positive ILC2s exhibit an ILC3-like signature that may contribute to IL-17-mediated pathologies. *Nat Immunol.* 2019;20(8):992-1003.
83. Harly C, Cam M, Kaye J, Bhandoola A. Development and differentiation of early innate lymphoid progenitors. *J Exp Med.* 2018;215(1):249-262.
84. Zeis P, Lian M, Fan X, et al. In Situ Maturation and Tissue Adaptation of Type 2 Innate Lymphoid Cell Progenitors. *Immunity.* 2020;53(4):775-792 e779.
85. Lim AI, Li Y, Lopez-Lastra S, et al. Systemic Human ILC Precursors Provide a Substrate for Tissue ILC Differentiation. *Cell.* 2017;168(6):1086-1100 e1010.

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

86. Ebihara T, Song C, Ryu SH, et al. Runx3 specifies lineage commitment of innate lymphoid cells. *Nat Immunol.* 2015;16(11):1124-1133.

87. Scoville SD, Mundy-Bosse BL, Zhang MH, et al. A Progenitor Cell Expressing Transcription Factor RORgammat Generates All Human Innate Lymphoid Cell Subsets. *Immunity.* 2016;44(5):1140-1150.

88. Schneider C, Lee J, Koga S, et al. Tissue-Resident Group 2 Innate Lymphoid Cells Differentiate by Layered Ontogeny and In Situ Perinatal Priming. *Immunity.* 2019;50(6):1425-1438 e1425.

89. Gasteiger G, Fan X, Dikiy S, Lee SY, Rudensky AY. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science.* 2015;350(6263):981-985.

90. Wojno ED, Monticelli LA, Tran SV, et al. The prostaglandin D(2) receptor CRTH2 regulates accumulation of group 2 innate lymphoid cells in the inflamed lung. *Mucosal Immunol.* 2015;8(6):1313-1323.

91. Huang Y, Mao K, Chen X, et al. S1P-dependent interorgan trafficking of group 2 innate lymphoid cells supports host defense. *Science.* 2018;359(6371):114-119.

92. Winkler C, Hochdorfer T, Israelsson E, et al. Activation of group 2 innate lymphoid cells after allergen challenge in asthmatic patients. *J Allergy Clin Immunol.* 2019;144(1):61-69.e7.

93. Ricardo-Gonzalez RR, Schneider C, Liao C, Lee J, Liang HE, Locksley RM. Tissue-specific pathways extrude activated ILC2s to disseminate type 2 immunity. *J Exp Med.* 2020;217(4):e20191172.

94. Shih HY, Sciume G, Mikami Y, et al. Developmental Acquisition of Regulomes Underlies Innate Lymphoid Cell Functionality. *Cell.* 2016;165(5):1120-1133.

95. Stadhouders R, Li BWS, de Bruijn MJW, et al. Epigenome analysis links gene regulatory

This article is protected by copyright. All rights reserved

elements in group 2 innate lymphocytes to asthma susceptibility. *J Allergy Clin Immunol.* 2018;142(6):1793-1807.

96. Kobayashi T, Voisin B, Kim DY, et al. Homeostatic Control of Sebaceous Glands by Innate Lymphoid Cells Regulates Commensal Bacteria Equilibrium. *Cell.* 2019;176(5):982-997 e916.

97. Luci C, Reynders A, Ivanov, II, et al. Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. *Nat Immunol.* 2009;10(1):75-82.

98. Teunissen MB, Munneke JM, Bernink JH, et al. Composition of innate lymphoid cell subsets in the human skin: enrichment of NCR(+) ILC3 in lesional skin and blood of psoriasis patients. *J Invest Dermatol.* 2014;134(9):2351-2360.

99. Yudanin NA, Schmitz F, Flamar AL, et al. Spatial and Temporal Mapping of Human Innate Lymphoid Cells Reveals Elements of Tissue Specificity. *Immunity.* 2019;50(2):505-519 e504.

100. Golebski K, Ros XR, Nagasawa M, et al. IL-1beta, IL-23, and TGF-beta drive plasticity of human ILC2s towards IL-17-producing ILCs in nasal inflammation. *Nat Commun.* 2019;10(1):2162.

101. Poposki JA, Klingler AI, Tan BK, et al. Group 2 innate lymphoid cells are elevated and activated in chronic rhinosinusitis with nasal polyps. *Immun Inflamm Dis.* 2017;5(3):233-243.

102. Toki S, Goleniewska K, Zhang J, et al. TSLP and IL-33 reciprocally promote each other's lung protein expression and ILC2 receptor expression to enhance innate type-2 airway inflammation. *Allergy.* 2020;75(7):1606-1617.

103. Kabata H, Moro K, Fukunaga K, et al. Thymic stromal lymphopoietin induces

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Accepted Article

corticosteroid resistance in natural helper cells during airway inflammation. *Nat Commun.* 2013;4:2675.

104. Huang Y, Guo L, Qiu J, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells. *Nat Immunol.* 2015;16(2):161-169.
105. Yu X, Pappu R, Ramirez-Carrozzi V, et al. TNF superfamily member TL1A elicits type 2 innate lymphoid cells at mucosal barriers. *Mucosal Immunol.* 2014;7(3):730-740.
106. Chang JE, Doherty TA, Baum R, Broide D. Prostaglandin D2 regulates human type 2 innate lymphoid cell chemotaxis. *J Allergy Clin Immunol.* 2014;133(3):899-901 e893.
107. Van Maele L, Carnoy C, Cayet D, et al. Activation of Type 3 innate lymphoid cells and interleukin 22 secretion in the lungs during Streptococcus pneumoniae infection. *J Infect Dis.* 2014;210(3):493-503.
108. De Grove KC, Provoost S, Verhamme FM, et al. Characterization and Quantification of Innate Lymphoid Cell Subsets in Human Lung. *PLoS One.* 2016;11(1):e0145961.
109. Kramer B, Goeser F, Lutz P, et al. Compartment-specific distribution of human intestinal innate lymphoid cells is altered in HIV patients under effective therapy. *PLoS Pathog.* 2017;13(5):e1006373.
110. Satoh-Takayama N, Kato T, Motomura Y, et al. Bacteria-Induced Group 2 Innate Lymphoid Cells in the Stomach Provide Immune Protection through Induction of IgA. *Immunity.* 2020;52(4):635-649 e634.
111. von Moltke J, Ji M, Liang HE, Locksley RM. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature.* 2016;529(7585):221-225.
112. Gerbe F, Sidot E, Smyth DJ, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature.* 2016;529(7585):226-230.

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113. Howitt MR, Lavoie S, Michaud M, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science.* 2016;351(6279):1329-1333.
114. Nadjsombati MS, McGinty JW, Lyons-Cohen MR, et al. Detection of Succinate by Intestinal Tuft Cells Triggers a Type 2 Innate Immune Circuit. *Immunity.* 2018;49(1):33-41 e37.
115. Schneider C, O'Leary CE, von Moltke J, et al. A Metabolite-Triggered Tuft Cell-ILC2 Circuit Drives Small Intestinal Remodeling. *Cell.* 2018;174(2):271-284 e214.
116. Spencer SP, Wilhelm C, Yang Q, et al. Adaptation of innate lymphoid cells to a micronutrient deficiency promotes type 2 barrier immunity. *Science.* 2014;343(6169):432-437.
117. Chun E, Lavoie S, Fonseca-Pereira D, et al. Metabolite-Sensing Receptor Ffar2 Regulates Colonic Group 3 Innate Lymphoid Cells and Gut Immunity. *Immunity.* 2019;51(5):871-884 e876.
118. Sano T, Huang W, Hall JA, et al. An IL-23R/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses. *Cell.* 2015;163(2):381-393.
119. Atarashi K, Tanoue T, Ando M, et al. Th17 Cell Induction by Adhesion of Microbes to Intestinal Epithelial Cells. *Cell.* 2015;163(2):367-380.
120. Longman RS, Diehl GE, Victorio DA, et al. CX(3)CR1(+) mononuclear phagocytes support colitis-associated innate lymphoid cell production of IL-22. *J Exp Med.* 2014;211(8):1571-1583.
121. Mortha A, Chudnovskiy A, Hashimoto D, et al. Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis. *Science.* 2014;343(6178):1249288.
122. van de Pavert SA, Ferreira M, Domingues RG, et al. Maternal retinoids control type 3

This article is protected by copyright. All rights reserved

Accepted Article

innate lymphoid cells and set the offspring immunity. *Nature.* 2014;508(7494):123-127.

123. Goverse G, Labao-Almeida C, Ferreira M, et al. Vitamin A Controls the Presence of RORgamma+ Innate Lymphoid Cells and Lymphoid Tissue in the Small Intestine. *J Immunol.* 2016;196(12):5148-5155.

124. Kim MH, Taparowsky EJ, Kim CH. Retinoic Acid Differentially Regulates the Migration of Innate Lymphoid Cell Subsets to the Gut. *Immunity.* 2015;43(1):107-119.

125. Godinho-Silva C, Domingues RG, Rendas M, et al. Light-entrained and brain-tuned circadian circuits regulate ILC3s and gut homeostasis. *Nature.* 2019;574(7777):254-258.

126. Wang Q, Robinette ML, Billon C, et al. Circadian rhythm-dependent and circadian rhythm-independent impacts of the molecular clock on type 3 innate lymphoid cells. *Sci Immunol.* 2019;4(40):eaay7501.

127. Teng F, Goc J, Zhou L, et al. A circadian clock is essential for homeostasis of group 3 innate lymphoid cells in the gut. *Sci Immunol.* 2019;4(40):eaax1215..

128. Steer CA, Martinez-Gonzalez I, Ghaedi M, Allinger P, Matha L, Takei F. Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. *J Allergy Clin Immunol.* 2017;140(2):593-595.e3..

129. de Kleer IM, Kool M, de Bruijn MJ, et al. Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung. *Immunity.* 2016;45(6):1285-1298.

130. Sagebiel AF, Steinert F, Lunemann S, et al. Tissue-resident Eomes(+) NK cells are the major innate lymphoid cell population in human infant intestine. *Nat Commun.* 2019;10(1):975.

131. Bando JK, Liang HE, Locksley RM. Identification and distribution of developing innate lymphoid cells in the fetal mouse intestine. *Nat Immunol.* 2015;16(2):153-160.

This article is protected by copyright. All rights reserved

Accepted Article

132. Mulder WJM, Ochando J, Joosten LAB, Fayad ZA, Netea MG. Therapeutic targeting of trained immunity. *Nat Rev Drug Discov.* 2019;18(7):553-566.

133. Martinez-Gonzalez I, Matha L, Steer CA, Ghaedi M, Poon GF, Takei F. Allergen-Experienced Group 2 Innate Lymphoid Cells Acquire Memory-like Properties and Enhance Allergic Lung Inflammation. *Immunity.* 2016;45(1):198-208.

134. Steer CA, Matha L, Shim H, Takei F. Lung group 2 innate lymphoid cells are trained by endogenous IL-33 in the neonatal period. *JCI Insight.* 2020;5(14).

135. Vu LD, Siefker D, Jones TL, et al. Elevated Levels of Type 2 Respiratory Innate Lymphoid Cells in Human Infants with Severe Respiratory Syncytial Virus Bronchiolitis. *Am J Respir Crit Care Med.* 2019;200(11):1414-1423.

136. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med.* 2005;171(2):137-141.

137. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nat Immunol.* 2006;7(5):507-516.

138. Paust S, Gill HS, Wang BZ, et al. Critical role for the chemokine receptor CXCR6 in NK cell-mediated antigen-specific memory of haptens and viruses. *Nat Immunol.* 2010;11(12):1127-1135.

139. Wang X, Peng H, Cong J, et al. Memory formation and long-term maintenance of IL-7Ralpha(+) ILC1s via a lymph node-liver axis. *Nat Commun.* 2018;9(1):4854.

140. Kim BS, Siracusa MC, Saenz SA, et al. TSLP elicits IL-33-independent innate lymphoid cell responses to promote skin inflammation. *Sci Transl Med.* 2013;5(170):170ra116.

141. Alysandratos KD, Angelidou A, Vasiadi M, et al. Increased affected skin gene expression and serum levels of thymic stromal lymphopoietin in atopic dermatitis. *Ann Allergy*

This article is protected by copyright. All rights reserved

Accepted Article

Accepted Article

Asthma Immunol. 2010;105(5):403-404.

142. Savinko T, Matikainen S, Saarialho-Kere U, et al. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol.* 2012;132(5):1392-1400.

143. Nakamura N, Tamagawa-Mineoka R, Yasuike R, et al. Stratum corneum interleukin-33 expressions correlate with the degree of lichenification and pruritus in atopic dermatitis lesions. *Clin Immunol.* 2019;201:1-3.

144. Pietka W, Sundnes O, Hammarstrom C, Zucknick M, Khnykin D, Haraldsen G. Lack of interleukin-33 and its receptor does not prevent calcipotriol-induced atopic dermatitis-like inflammation in mice. *Sci Rep.* 2020;10(1):6451.

145. Kim BS, Wang K, Siracusa MC, et al. Basophils promote innate lymphoid cell responses in inflamed skin. *J Immunol.* 2014;193(7):3717-3725.

146. Mack MR, Brestoff JR, Berrien-Elliott MM, et al. Blood natural killer cell deficiency reveals an immunotherapy strategy for atopic dermatitis. *Sci Transl Med.* 2020;12(532):eaay1005.

147. Villanova F, Flutter B, Tosi I, et al. Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J Invest Dermatol.* 2014;134(4):984-991.

148. von Stebut E, Boehncke WH, Ghoreschi K, et al. IL-17A in Psoriasis and Beyond: Cardiovascular and Metabolic Implications. *Front Immunol.* 2019;10:3096.

149. Pantelyushin S, Haak S, Ingold B, et al. Rorgammat+ innate lymphocytes and gammadelta T cells initiate psoriasiform plaque formation in mice. *J Clin Invest.* 2012;122(6):2252-2256.

150. Liu T, Wu J, Zhao J, et al. Type 2 innate lymphoid cells: A novel biomarker of

This article is protected by copyright. All rights reserved

eosinophilic airway inflammation in patients with mild to moderate asthma. *Respir Med.* 2015;109(11):1391-1396.

151. Christianson CA, Goplen NP, Zafar I, et al. Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33. *J Allergy Clin Immunol.* 2015;136(1):59-68 e14.
152. Liu S, Verma M, Michalec L, et al. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. *J Allergy Clin Immunol.* 2018;141(1):257-268 e256.
153. Morita H, Moro K, Koyasu S. Innate lymphoid cells in allergic and nonallergic inflammation. *J Allergy Clin Immunol.* 2016;138(5):1253-1264.
154. Orimo K, Saito H, Matsumoto K, Morita H. Innate Lymphoid Cells in the Airways: Their Functions and Regulators. *Allergy Asthma Immunol Res.* 2020;12(3):381-398.
155. McDowell PJ, Heaney LG. Different endotypes and phenotypes drive the heterogeneity in severe asthma. *Allergy.* 2020;75(2):302-310.
156. Al-Ramli W, Prefontaine D, Chouiali F, et al. T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol.* 2009;123(5):1185-1187.
157. Cai T, Qiu J, Ji Y, et al. IL-17-producing ST2(+) group 2 innate lymphoid cells play a pathogenic role in lung inflammation. *J Allergy Clin Immunol.* 2019;143(1):229-244 e229.
158. Shaw JL, Fakhri S, Citardi MJ, et al. IL-33-responsive innate lymphoid cells are an important source of IL-13 in chronic rhinosinusitis with nasal polyps. *Am J Respir Crit Care Med.* 2013;188(4):432-439.
159. Walford HH, Lund SJ, Baum RE, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin Immunol.* 2014;155(1):126-135.

This article is protected by copyright. All rights reserved

Accepted Article

160. Miljkovic D, Bassiouni A, Cooksley C, et al. Association between group 2 innate lymphoid cells enrichment, nasal polyps and allergy in chronic rhinosinusitis. *Allergy.* 2014;69(9):1154-1161.

161. Tojima I, Kouzaki H, Shimizu S, et al. Group 2 innate lymphoid cells are increased in nasal polyps in patients with eosinophilic chronic rhinosinusitis. *Clin Immunol.* 2016;170:1-8.

162. Ogasawara N, Klingler AI, Tan BK, et al. Epithelial activators of type 2 inflammation: Elevation of thymic stromal lymphopoietin, but not IL-25 or IL-33, in chronic rhinosinusitis with nasal polyps in Chicago, Illinois. *Allergy.* 2018;73(11):2251-2254.

163. Nagarkar DR, Poposki JA, Tan BK, et al. Thymic stromal lymphopoietin activity is increased in nasal polyps of patients with chronic rhinosinusitis. *J Allergy Clin Immunol.* 2013;132(3):593-600 e512.

164. Liao B, Cao PP, Zeng M, et al. Interaction of thymic stromal lymphopoietin, IL-33, and their receptors in epithelial cells in eosinophilic chronic rhinosinusitis with nasal polyps. *Allergy.* 2015;70(9):1169-1180.

165. Lam M, Hull L, Imrie A, et al. Interleukin-25 and interleukin-33 as mediators of eosinophilic inflammation in chronic rhinosinusitis. *Am J Rhinol Allergy.* 2015;29(3):175-181.

166. Hong HY, Chen FH, Sun YQ, et al. Local IL-25 contributes to Th2-biased inflammatory profiles in nasal polyps. *Allergy.* 2018;73(2):459-469.

167. Yoshimura T, Yoshikawa M, Otori N, Haruna S, Moriyama H. Correlation between the prostaglandin D(2)/E(2) ratio in nasal polyps and the recalcitrant pathophysiology of chronic rhinosinusitis associated with bronchial asthma. *Allergol Int.* 2008;57(4):429-436.

168. Perez-Novo CA, Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Prostaglandin,

This article is protected by copyright. All rights reserved

Accepted Article

leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. *J Allergy Clin Immunol.* 2005;115(6):1189-1196.

169. Brightling CE, Gaga M, Inoue H, et al. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. *Lancet Respir Med.* 2020;9(1):43-56.
170. Lao-Araya M, Steveling E, Scadding GW, Durham SR, Shamji MH. Seasonal increases in peripheral innate lymphoid type 2 cells are inhibited by subcutaneous grass pollen immunotherapy. *J Allergy Clin Immunol.* 2014;134(5):1193-1195 e1194.
171. Dhariwal J, Cameron A, Trujillo-Torralbo MB, et al. Mucosal Type 2 Innate Lymphoid Cells Are a Key Component of the Allergic Response to Aeroallergens. *Am J Respir Crit Care Med.* 2017;195(12):1586-1596.
172. Mitthamsiri W, Pradubpongsa P, Sangasapaviliya A, Boonpiyathad T. Decreased CRTH2 Expression and Response to Allergen Re-stimulation on Innate Lymphoid Cells in Patients With Allergen-Specific Immunotherapy. *Allergy Asthma Immunol Res.* 2018;10(6):662-674.
173. Eljaszewicz A, Ruchti F, Radzikowska U, et al. Trained immunity and tolerance in innate lymphoid cells, monocytes, and dendritic cells during allergen-specific immunotherapy. *J Allergy Clin Immunol.* 2020. doi: 10.1016/j.jaci.2020.08.042.
174. Ying S, O'Connor B, Ratoff J, et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J Immunol.* 2008;181(4):2790-2798.
175. Xia J, Zhao J, Shang J, et al. Increased IL-33 expression in chronic obstructive pulmonary disease. *Am J Physiol Lung Cell Mol Physiol.* 2015;308(7):L619-627.
176. Kearley J, Silver JS, Sanden C, et al. Cigarette smoke silences innate lymphoid cell

This article is protected by copyright. All rights reserved

Accepted Article

function and facilitates an exacerbated type I interleukin-33-dependent response to infection. *Immunity.* 2015;42(3):566-579.

177. Doherty TA, Baum R, Newbury RO, et al. Group 2 innate lymphocytes (ILC2) are enriched in active eosinophilic esophagitis. *J Allergy Clin Immunol.* 2015;136(3):792-794 e793.

178. Travers J, Rochman M, Caldwell JM, Besse JA, Miracle CE, Rothenberg ME. IL-33 is induced in undifferentiated, non-dividing esophageal epithelial cells in eosinophilic esophagitis. *Sci Rep.* 2017;7(1):17563.

179. Noti M, Wojno ED, Kim BS, et al. Thymic stromal lymphopoietin-elicited basophil responses promote eosinophilic esophagitis. *Nat Med.* 2013;19(8):1005-1013.

180. Shoda T, Matsuda A, Arai K, et al. Sera of patients with infantile eosinophilic gastroenteritis showed a specific increase in both thymic stromal lymphopoietin and IL-33 levels. *J Allergy Clin Immunol.* 2016;138(1):299-303.

181. Moschen AR, Tilg H, Raine T. IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. *Nat Rev Gastroenterol Hepatol.* 2019;16(3):185-196.

182. Bernink JH, Peters CP, Munneke M, et al. Human type 1 innate lymphoid cells accumulate in inflamed mucosal tissues. *Nat Immunol.* 2013;14(3):221-229.

183. Fuchs A, Vermi W, Lee JS, et al. Intraepithelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-gamma-producing cells. *Immunity.* 2013;38(4):769-781.

184. Takayama T, Kamada N, Chinen H, et al. Imbalance of NKp44(+)NKp46(-) and NKp44(-)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn's disease. *Gastroenterology.* 2010;139(3):882-892, 892 e881-883.

185. Vashist N, Trittel S, Ebensen T, Chambers BJ, Guzman CA, Riese P. Influenza-Activated

This article is protected by copyright. All rights reserved

ILC1s Contribute to Antiviral Immunity Partially Influenced by Differential GITR Expression. *Front Immunol.* 2018;9:505.

186. Nabekura T, Riggan L, Hildreth AD, O'Sullivan TE, Shibuya A. Type 1 Innate Lymphoid Cells Protect Mice from Acute Liver Injury via Interferon-gamma Secretion for Upregulating Bcl-xL Expression in Hepatocytes. *Immunity.* 2020;52(1):96-108 e109.
187. Bonne-Annee S, Bush MC, Nutman TB. Differential Modulation of Human Innate Lymphoid Cell (ILC) Subsets by IL-10 and TGF-beta. *Sci Rep.* 2019;9(1):14305.
188. Ruiter B, Patil SU, Shreffler WG. Vitamins A and D have antagonistic effects on expression of effector cytokines and gut-homing integrin in human innate lymphoid cells. *Clin Exp Allergy.* 2015;45(7):1214-1225.
189. Bartemes K, Chen CC, Iijima K, Drake L, Kita H. IL-33-Responsive Group 2 Innate Lymphoid Cells Are Regulated by Female Sex Hormones in the Uterus. *J Immunol.* 2018;200(1):229-236.
190. Gour N, Smole U, Yong HM, et al. C3a is required for ILC2 function in allergic airway inflammation. *Mucosal Immunol.* 2018;11(6):1653-1662.
191. Knipfer L, Schulz-Kuhnt A, Kindermann M, et al. A CCL1/CCR8-dependent feed-forward mechanism drives ILC2 functions in type 2-mediated inflammation. *J Exp Med.* 2019;216(12):2763-2777.
192. Ishii T, Muroi M, Horiguchi K, Tanamoto KI, Nagase T, Yamashita N. Activation through toll-like receptor 2 on group 2 innate lymphoid cells can induce asthmatic characteristics. *Clin Exp Allergy.* 2019;49(12):1624-1632.
193. Laffont S, Blanquart E, Savignac M, et al. Androgen signaling negatively controls group 2 innate lymphoid cells. *J Exp Med.* 2017;214(6):1581-1592.
194. Li S, Bostick JW, Ye J, et al. Aryl Hydrocarbon Receptor Signaling Cell Intrinsically

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Accepted Article

Inhibits Intestinal Group 2 Innate Lymphoid Cell Function. *Immunity.* 2018;49(5):915-928 e915.

195. Thio CL, Chi PY, Lai AC, Chang YJ. Regulation of type 2 innate lymphoid cell-dependent airway hyperreactivity by butyrate. *J Allergy Clin Immunol.* 2018;142(6):1867-1883.e12.

196. Victor AR, Nalin AP, Dong W, et al. IL-18 Drives ILC3 Proliferation and Promotes IL-22 Production via NF-kappaB. *J Immunol.* 2017;199(7):2333-2342.

197. Duffin R, O'Connor RA, Crittenden S, et al. Prostaglandin E(2) constrains systemic inflammation through an innate lymphoid cell-IL-22 axis. *Science.* 2016;351(6279):1333-1338.

198. Dalli J, Colas RA, Arnardottir H, Serhan CN. Vagal Regulation of Group 3 Innate Lymphoid Cells and the Immunoresolvent PCTR1 Controls Infection Resolution. *Immunity.* 2017;46(1):92-105.

199. Kiss EA, Vonarbourg C, Kopfmann S, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. *Science.* 2011;334(6062):1561-1565.

200. Konya V, Czarnewski P, Forkel M, et al. Vitamin D downregulates the IL-23 receptor pathway in human mucosal group 3 innate lymphoid cells. *J Allergy Clin Immunol.* 2018;141(1):279-292.

Figure Legends

FIGURE 1. Subsets of innate lymphoid cells and their plasticity

Innate lymphoid cells are currently divided into five different subsets, ILC1s, ILC2s, ILC3s, NK cells and lymphoid tissue–inducer (LTi) cells,based on their transcription factors, cytokines produced by the cells and the developmental pathway. ILCs are highly plastic, able to change their

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function and phenotype in response to microenvironmental cues such as cytokines. IL-10–producing ILCs are not considered to be a distinct, stable subset like Tregs, but rather a cell type that is converted from effector subsets.

FIGURE 2. Distribution of ILCs in the skin, respiratory tract and gastrointestinal tract

A, Distribution of ILCs in the skin. $CCR6^{+}$ILC3s reside in the epidermis and dermis. ILC2s reside in the dermis and subcutaneous tissue. Dermal ILC2s express IL-18R1, whereas subcutaneous ILC2s express ST2. NK cells reside in the dermis, whereas ILC1sare rarely detected in the skin. B, Distribution of ILCs in therespiratory tract.NK cells, ILC1s and ILC3s are the predominant populations in the human lung and nasal tissues, while ILC2sare a minor population. Human nasal ILC2s express CRTh2, whereas human lung ILC2s express them less. Unlike in man, ST2–expressing ILC2sarepredominant in mouse lungs. C, Distribution of ILCs in the gastrointestinal (GI) tract. In man, ILC1sare predominant in the upper GI tract, while ILC3sarepredominant in the lower GI tract. ILC2sare a minor population throughout the human GI tract. In contrast, ILC2sare the predominant population in the mouse stomach. Gastric ILC2s express ST2, whereas intestinal ILC2s preferentially express IL-17RB.

FIGURE 3. Ontogeny of tissue–resident ILC2s, and the potential preventive target–period

The number of ILC2s is at a peak in various tissues, including the lung, skin and intestine, during the neonatal–infancy period. ILC2s are tissue–resident cells that are continuously replenished from local precursors. Tissue ILC2s in adultscomprise the population that arises largely during the neonatal–infancy period, and only a minority is replenished from hematopoietic stem cells during adulthood. Considering ILCs' memory–like capacity—called "trained immunity"—the neonatal–infancy period may be a critical time–period during which life–long ILC2–dependent immunity develops in tissues. That would also mean that this could be a critical time–period for application of ILC–targeting preventive strategiesfor inflammatory diseases,including allergic diseases.

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FIGURE 4. Therapeutic targets for ILCs in atopic dermatitis and psoriasis

A, Therapeutic targets for ILC2s in atopic dermatitis (AD).Since ILC2s are increased and activated in the skin of AD patients, various known activators of ILC2s are current targets of therapy. B, Therapeutic targets for ILCs in psoriasis.Since ILC3s are increased and activated in the skin of psoriasis patients, various known activators of ILC3s are current targets of therapy.Treatments that have shown efficacy are shown in boxes labeled with a green tab. The treatment shown in thered–labeledbox was ineffective. The efficacy of the treatment shown in the yellow–labeled box remains controversial.

FIGURE 5. Therapeutic targets for ILCs in asthma, CRSwNP and COPD

A, Therapeutic targets for ILCs in asthma. Since ILC2s are increased and activated in the lungs of asthma patients, various known activators of ILC2s are current targets of therapy. Severe cases are thought to involve IL-17–producing ILC3s, in addition to ILC2s. However, to date, treatments targeting ILC3s have not shown clinical benefit. B, Therapeutic targets for ILC2s in chronic rhinosinusitis with nasal polyps (CRSwNP). Since ILC2s are increased and activated in the nasal tissues of CRSwNP patients, various known activators of ILC2s are current targets of therapy. C, Therapeutic targets for ILCs in chronic obstructive pulmonary disease (COPD). Since ILC1s are increased and activated in the lungs of COPD patients, anactivator of ILC1s has been the target of therapy.ILC2s are also thought to be involved in some COPD cases. Therefore,treatments targeting ILC2s are currently undergoing clinical trials. The treatment shown in the green–labeled box has shown efficacy. Treatments thatwere ineffective are shown in red–labeled boxes. Treatments still undergoing clinical trial are shown in green–labeled boxes.HMGB1: high mobility group box 1;RAGE: receptor for advanced glycation end–products;PD-L1: programmed death–ligand 1; PD-1: programmed cell death 1; DR3: death receptor 3.

FIGURE6. Therapeutic targets for ILCs in EoE and IBD

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A, Therapeutic targets for ILC2s in eosinophilic esophagitis (EoE). Since ILC2s are increased in the tissues of EoE patients, various known activators of ILC2s are current targets of therapy. B, Therapeutic targets for ILCs in inflammatory bowel disease (IBD). Since ILC1s and ILC3s are increased in the intestinal tissues of IBD patients, various known activators of ILC1s and ILC3s are current targets of therapy. Treatments that have shown efficacy are shown in green–labeledboxes. The treatment shown in the red–labeled box was ineffective. Treatments still under clinical trial are shown in green–labeled boxes. AhR: aryl hydrocarbon receptor.

TABLE1. Factors known to activate or suppress ILCs

ILC subset	Function	Classification	Molecules
ILC1	Activation	Cytokine	IL-12,[183] IL-15,[183] IL-18[185]
		Vitamin	Retinoic acid[124]
		Others	ATP[186]
	Suppression	Cytokine	TGF-β[187]
ILC2	Activation	Cytokine	IL-1β,[9,10] IL-2, IL-4,[11] IL-7. IL-9,[12] IL-18,[29] IL-25,[13] IL-33,[13] TSLP,[14] TNF-α,[15] SCF,[16] G-CSF,[17] HMGB1,[18] TL1A,[30] RANKL,[19] GITRL[20]
		Lipid	LTB_4,[44] LTC_4,[44] LTD_4,[44,45] LTE_4,[44] PGD_2[42,43]
		Neurotransmitter	NMU,[31-33] VIP,[34,35] CGRP[37]
		Vitamin	Retinoic acid[188]
		Cell–cell interaction	ICOSL (ILC2s),[48] B7-H6 (tumor cells, monocytes and neutrophils),[50] ICAM-1 (T cells, B cells, epithelial cells and endothelial cells)[52]
		Sex hormone	Estrogen[189]
		Others	C3a,[190] CCL1,[191] TLR1/2/6 agonist[192]
	Suppression	Cytokine	IL-10,[21,23,24] IL-27,[22,25,27] TGF-β,[24] IFN-β,[22,25] IFN-γ,[22,25,26] IL-35[28]
		Lipid	PGE_2,[47] PGI_2,[46] LXA_4[43]
		Neurotransmitter	CGRP,[38-40]norepinephrine,[36]acetylcholine[41]
		Vitamin	Vitamin D[188]

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		Cell–cell interaction	PD-L1,[49] E-cadherin (epithelial cells)[51]
		Sex hormone	Androgen[193]
		Others	AhR ligand,[194]butyrate[195]
ILC3	Activation	Cytokine	IL-1β,[58] IL-2, IL-7, IL-18,[196] IL-23,[53] TL1A,[54] IGF-1[57]
		Lipid	PGE_2[197]
		Neurotransmitter	VIP,[59,60] acetylcholine[198]
		Vitamin	Retinoic acid[124]
		Cell–cell interaction	NKp44 ligand,[61] LIGHT(T cells, NK cells and dendritic cells)[62]
		Others	AhR ligand,[194,199]SCFA (FFAR2 agonist)[117]
	Suppression	Cytokine	TSLP,[55] RANKL,[56]
		Vitamin	Vitamin D[200]
		Cell–cell interaction	PD-L1[63]

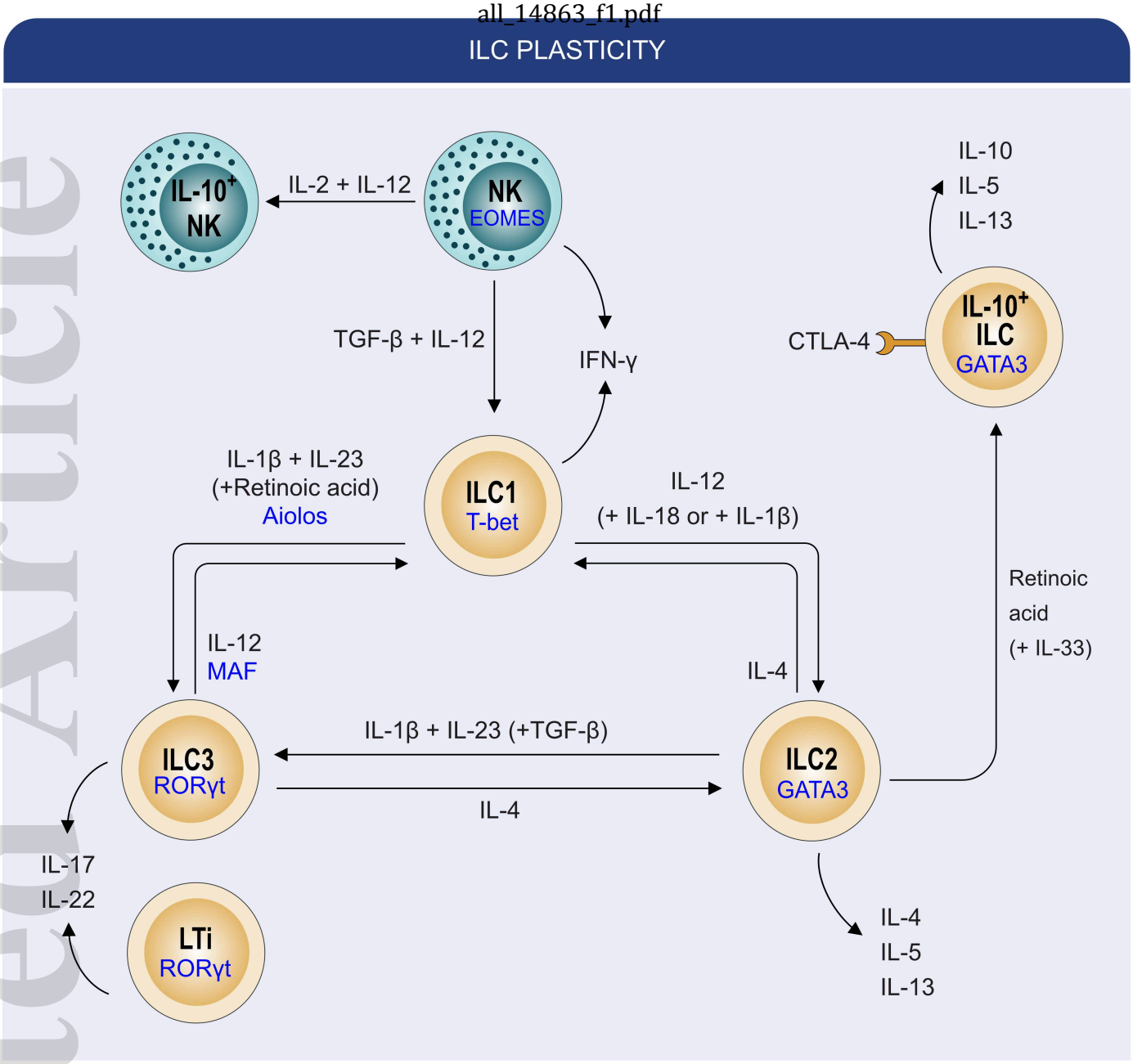
ATP: adenosine triphosphate; AhR: aryl hydrocarbon receptor; TNFSF14: tumor necrosis factor superfamily member 14; SCFA: short–chain fatty acid.

Cells known to interact with each ILC subset are shown in parentheses.

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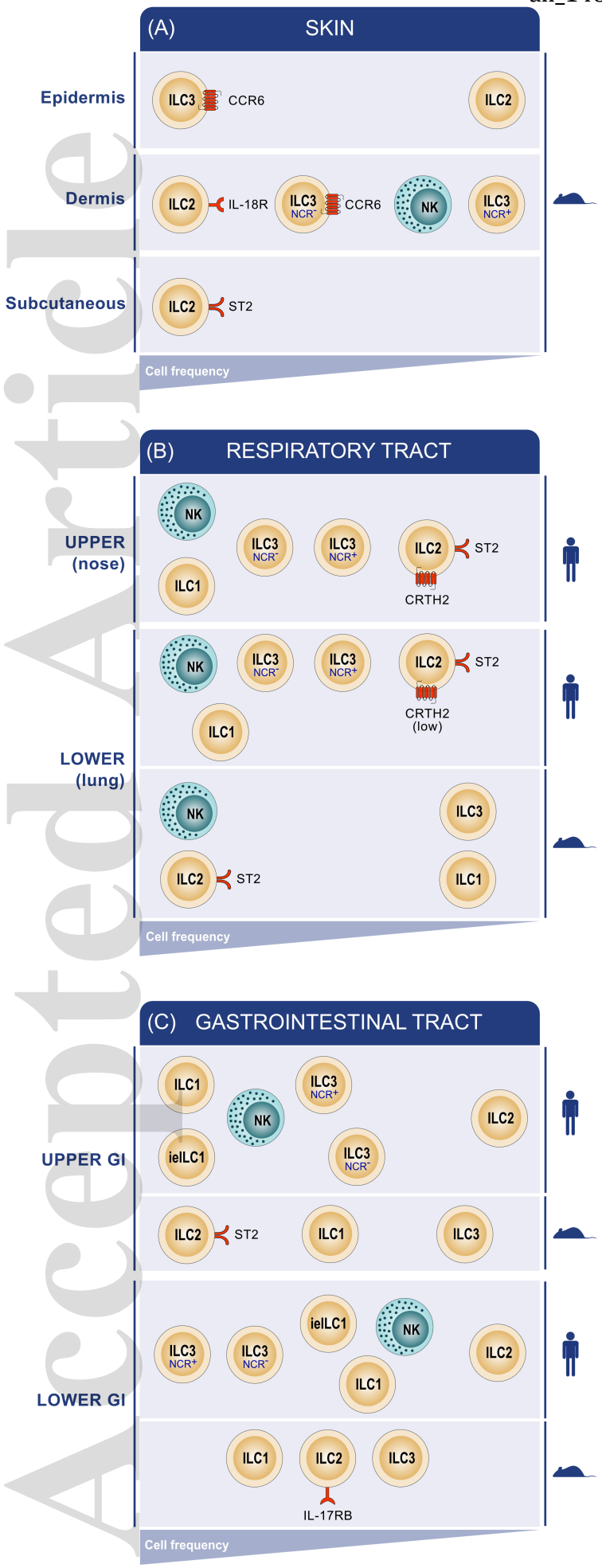
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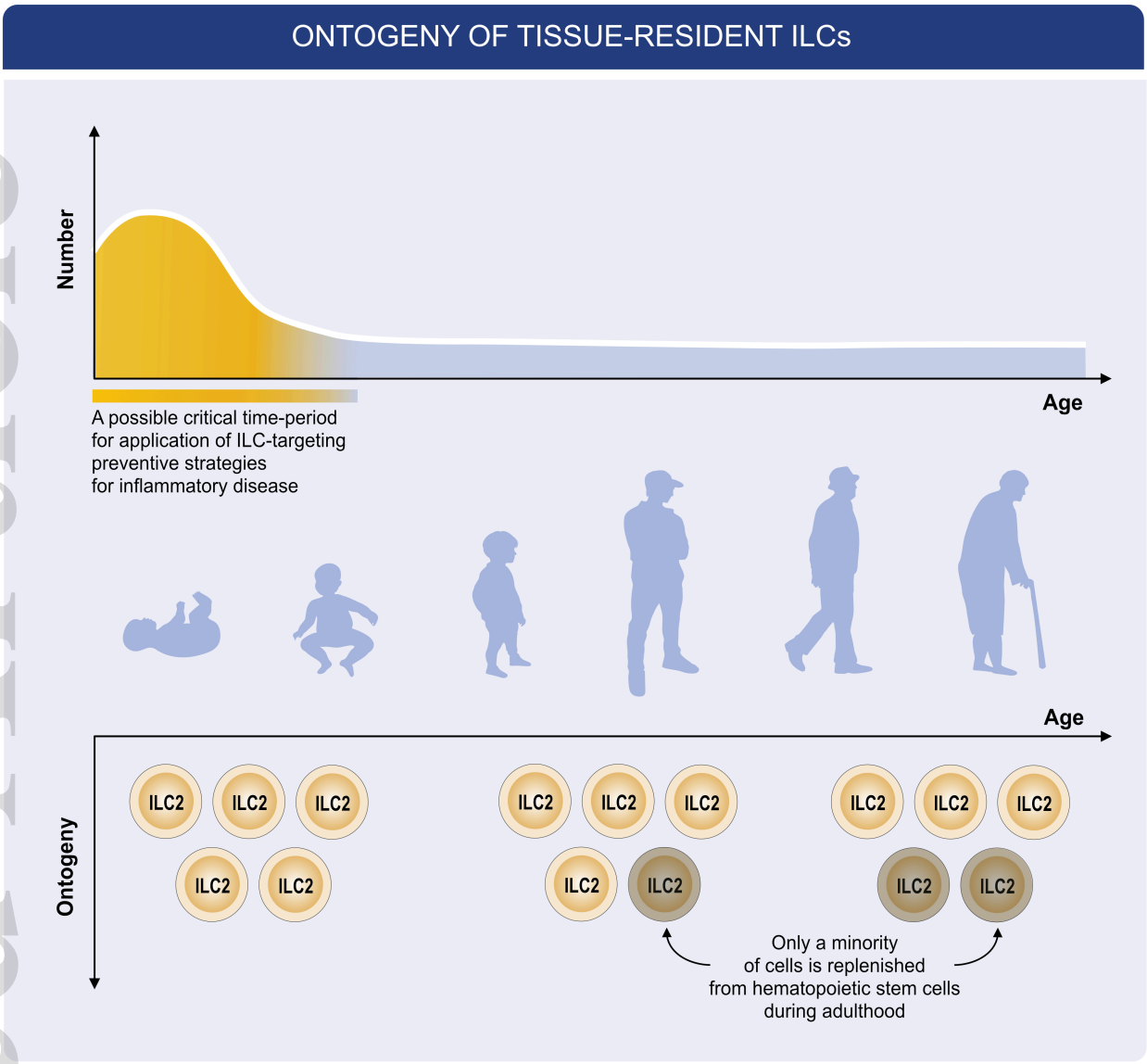
Figure 1_Orimo K et al.



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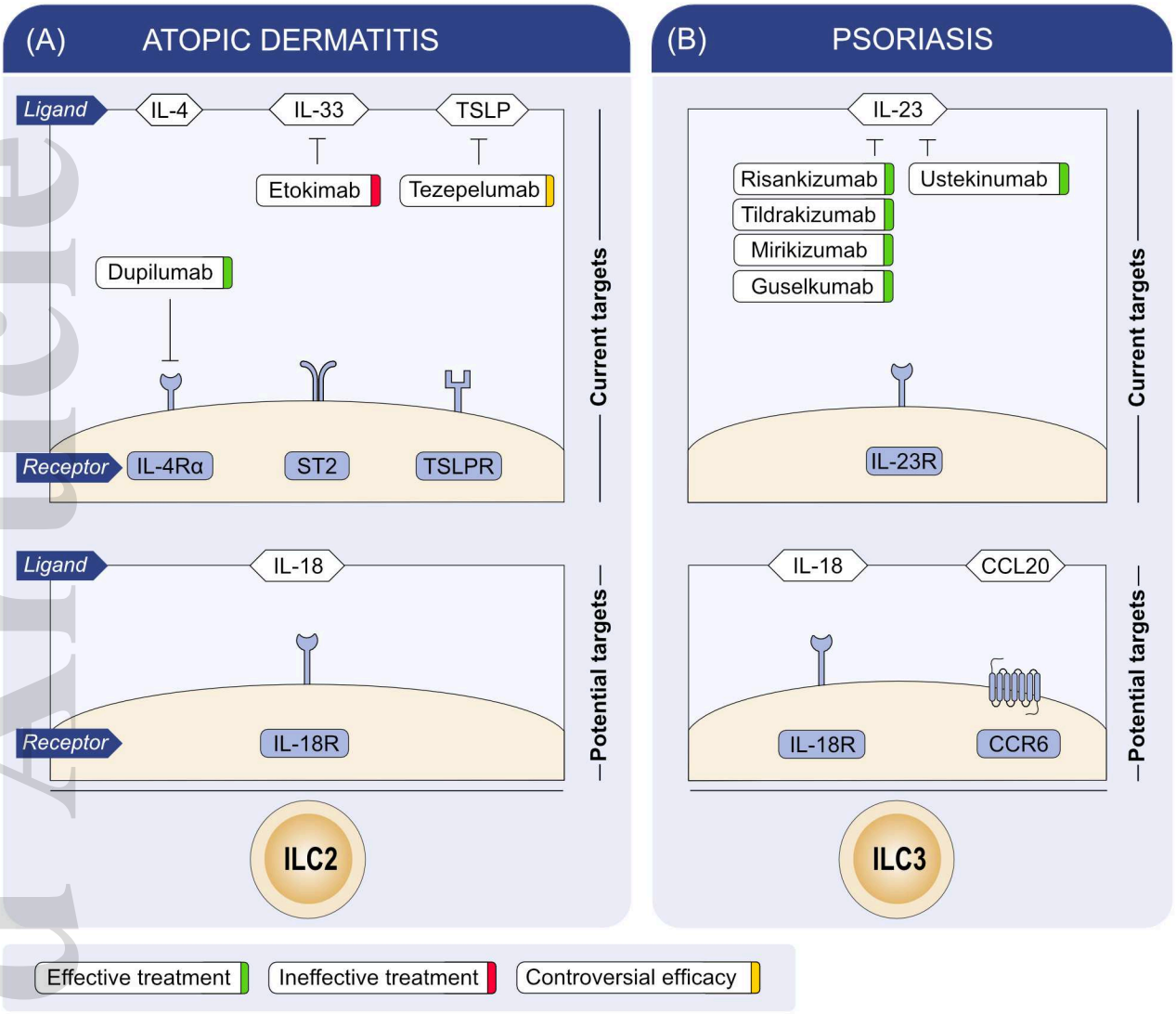
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Figure 3_Orimo K et al.

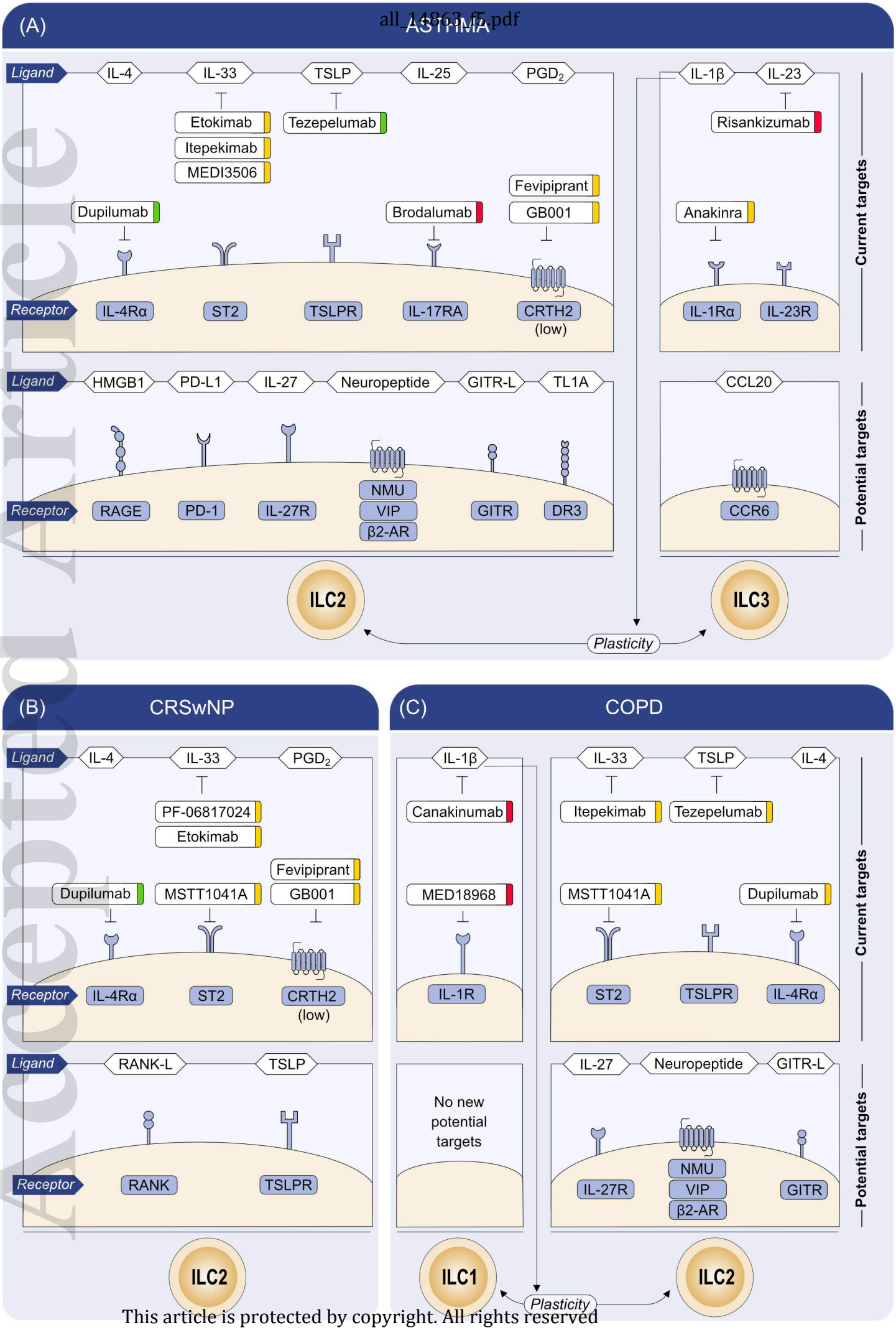


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Figure 4_Orimo K et al.

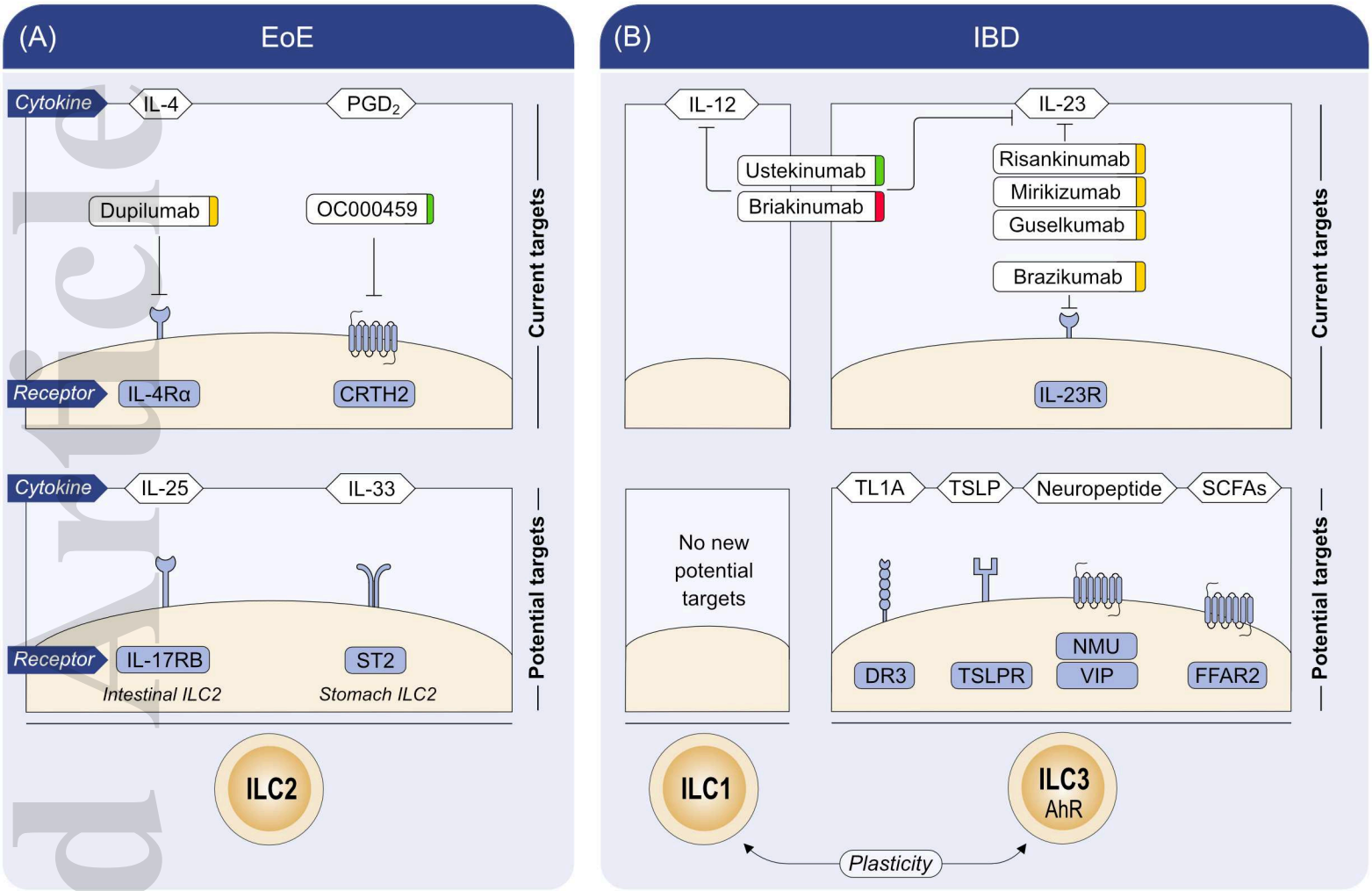
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Effective treatment | Ineffective treatment | Undergoing clinical trial

Figure 5_Orimo K et al.



Effective treatment | Ineffective treatment | Undergoing clinical trials

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Table E1. Clinical trials of therapies targeting upstream regulators and downstream effector cytokines of ILCs

Disease	Targeted ILC subset	Up/Dn	Targeted molecule	Drug	Phase	Efficacy	Ref
AD	ILC2	Up	IL-33	Etokimab	2	Ineffective	NCT03533751
			TSLP	Tezepelumab	2		[1], NCT03809663
		Up/Dn	IL-4Rα	Dupilumab	3	Effective	[2,3,4]
		Dn	IL-13	Tralokinumab, Lebrikizumab	2	Effective	[5,6]
				Tralokinumab	3	Effective	[7]
				Lebrikizumab	3		NCT04392154, NCT04626297, NCT04250350, NCT04178967, NCT04146363, NCT04250337
	ILC3	Up	IL-12/23p40	Ustekinumab	2	Ineffective	[8]
		Dn	IL-22	Fezakinumab	2		[9]
Psoriasis	ILC3	Up	IL-23p19	Risankizumab, Tildrakizumab, Mirikizumab, Guselkumab	[10], 3	Effective	[10, 11] NCT03482011 [12]
			IL-12/23p40	Ustekinumab	3	Effective	[13]
		Dn	IL-17A	Secukinumab, Ixekizumab	3	Effective	[14, 15, 16]

			IL-17A/IL-17F	Bimekizumab	3	Effective	NCT03370133
			IL-17RA	Brodalumab	3	Effective	[17]
Asthma	ILC1/2/3	Up	IL-1R1	Anakinra	1-2		NCT04035109
	ILC2	Up	IL-33	Etokimab, MEDI 3506, Itepekimab	2		NCT03469934, NCT04570657, NCT03387852
			ST2	MSTT1041A	2		NCT02918019
			TSLP	Tezepelumab	2	Effective	[18]
			TSLP	Tezepelumab	3		NCT03406078 NCT03706079 NCT03347279 NCT03927157
			CRTh2	Fevipiprant	3	Ineffective	[19]
			CRTh2	GB001	2	Ineffective	[20]
		Up/Dn	IL-4Rα	Dupilumab	3	Effective	[21]
		Dn	IL-5	Mepolizumab, Reslizumab	3	Effective	[22,23,24]
			IL-5Rα	Benralizumab	3	Effective	[25,26]
			IL-13	Lebrikizumab	3		[27]
			IL-13	Tralokinumab	3	Ineffective	[28]
	ILC3	Up	IL-23p19	Risankizumab	2	Ineffective	NCT02443298
		Dn	IL-17RA	Brodalumab	2	Ineffective	[29]
COPD	ILC1/2/3	Up	IL-1β	Canakinumab	2	Ineffective	NCT00581945
			IL-1R1	MED18968	2	Ineffective	[30]
	ILC2	Up	IL-33	Itepekimab	2		NCT03546907

			ST2	MSTT1041A	2		NCT03615040
			TSLP	Tezepelumab	2		NCT04039113
		Up/Dn	IL-4Rα	Dupilumab	3		NCT03930732, NCT04456673
		Dn	IL-5	Mepolizumab	3	Effective	[31]
			IL-5Rα	Benralizumab	3		NCT04053634
	ILC3	Dn	IL-17A	CNTO 6785	2	Ineffective	[32]
CRSwNP	ILC2	Up	IL-33	Etokimab	2		NCT03614923
			IL-33	PF-06817024	1		NCT02743871
			CRTh2	GB001	2		NCT03956862
			CRTh2	Fevipiprant	3		NCT03681093
		Up/Dn	IL-4Rα	Dupilumab	3	Effective	[33]
		Dn	IL-5	Mepolizumab	3		NCT04607005
			IL-5Rα	Benralizumab	3		NCT04157335, NCT03401229
EoE	ILC2	Up	CRTh2	OC000459	2	Effective	[34]
		Up/Dn	IL-4Rα	Dupilumab	3		NCT04394351, NCT03633617
		Dn	IL-5	Mepolizumab, Reslizumab	2		[35,36]
			IL-5Rα	Benralizumab	3		NCT04543409
EGE	ILC2	Up/Dn	IL-4Rα	Dupilumab	2		NCT03678545
		Dn	IL-5Rα	Benralizumab	2		NCT03473977
CD	ILC1/2/3	Up	IL-18	GSK1070806	1-2		NCT03681067
	ILC1/3	Up	IL-12/23p40	Ustekinumab	3	Effective	[37]

			IL-12/23p40	Briakinumab	2	Ineffective	[38,39]
	ILC3	Up	IL-23p19	Risankizumab	2	Effective	[40]
			IL-23p19	Risankizumab, Brazikumab, Mirikizumab, Guselkumab	3		NCT03105128, NCT03105102, NCT04524611, NCT03104413, NCT04232553, NCT03926130, NCT04397263, NCT03466411, NCT03759288
		Dn	IL-17A	Secukinumab	2	Ineffective	[41]
			IL-17RA	Brodalumab	2	Ineffective	[42]
UC	ILC1/ILC3	Up	IL-12p40	Ustekinumab	3	Effective	[43]
		Up	IL-23p19	Mirikizumab	2	Effective	[44]
			IL-23p19	Risankizumab, Mirikizumab, Guselkumab	3		NCT03398148, NCT03398135, NCT03519945, NCT03518086, NCT03524092, NCT04033445

Up: upstream; Dn: downstream.

1. Simpson EL, Parnes JR, She D, et al. Tezepelumab, an anti-thymic stromal lymphopoietin monoclonal antibody, in the treatment of moderate to severe atopic dermatitis: A randomized phase 2a clinical trial. *J Am Acad Dermatol.* 2019;80(4):1013-1021.
2. Simpson EL, Bieber T, Guttman-Yassky E, et al. Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med.* 2016;375(24):2335-2348.
3. Beck LA, Thaçi D, Hamilton JD, et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med.* 2014;371(2):130-139.
4. Paller AS, Siegfried EC, Thaci D, et al. Efficacy and safety of dupilumab with concomitant topical corticosteroids in children 6 to 11 years old with severe atopic dermatitis: A randomized, double-blinded, placebo-controlled phase 3 trial. *J Am Acad Dermatol.* 2020;83(5):1282-1293.
5. Guttman-Yassky E, Blauvelt A, Eichenfield LF, et al. Efficacy and Safety of Lebrikizumab, a High-Affinity Interleukin 13 Inhibitor, in Adults With Moderate to Severe Atopic Dermatitis: A Phase 2b Randomized Clinical Trial. *JAMA Dermatol.* 2020;156(4):411-420.
6. Wollenberg A, Howell MD, Guttman-Yassky E, et al. Treatment of atopic dermatitis with tralokinumab, an anti-IL-13 mAb. *J Allergy Clin Immunol.* 2019;143(1):135-141.
7. Wollenberg A, Blauvelt A, Guttman-Yassky E, et al. Tralokinumab for moderate-to-severe atopic dermatitis: results from two 52-week, randomized, double-blind, multicentre, placebo-controlled phase III trials (ECZTRA 1 and ECZTRA 2). *Br J Dermatol.* 2020.
8. Khattri S, Brunner PM, Garcet S, et al. Efficacy and safety of ustekinumab treatment in adults with moderate-to-severe atopic dermatitis. *Exp Dermatol.* 2017;26(1):28-35.
9. Guttman-Yassky E, Brunner PM, Neumann AU, et al. Efficacy and safety of fezakinumab (an IL-22 monoclonal antibody) in adults with moderate-to-severe atopic dermatitis inadequately controlled by conventional treatments: A randomized, double-blind, phase 2a trial. *J Am Acad Dermatol.* 2018;78(5):872-881.e876.
10. Gordon KB, Strober B, Lebwohl M, et al. Efficacy and safety of risankizumab in moderate-to-severe plaque psoriasis (UltIMMa-1 and UltIMMa-2): results from two double-blind, randomised, placebo-controlled and ustekinumab-controlled phase 3 trials. *Lancet.* 2018;392(10148):650-661.
11. Reich K, Warren RB, Iversen L, et al. Long-term efficacy and safety of tildrakizumab for moderate-to-severe psoriasis: pooled analyses of two randomized phase III clinical trials (reSURFACE 1 and reSURFACE 2) through 148 weeks. *Br J Dermatol.* 2020;182(3):605-617.
12. Mease PJ, Rahman P, Gottlieb AB, et al. Guselkumab in biologic-naive patients with active psoriatic arthritis (DISCOVER-2): a double-blind, randomised, placebo-controlled phase 3 trial. *Lancet.* 2020;395(10230):1126-1136.
13. Scanlon JV, Exter BP, Steinberg M, Jarvis CI. Ustekinumab: treatment of adult

moderate-to-severe chronic plaque psoriasis. *Ann Pharmacother.* 2009;43(9):1456-1465.

14. Langley RG, Elewski BE, Lebwohl M, et al. Secukinumab in plaque psoriasis--results of two phase 3 trials. *N Engl J Med.* 2014;371(4):326-338.
15. Thaci D, Blauvelt A, Reich K, et al. Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate to severe plaque psoriasis: CLEAR, a randomized controlled trial. *J Am Acad Dermatol.* 2015;73(3):400-409.
16. Gordon KB, Blauvelt A, Papp KA, et al. Phase 3 Trials of Ixekizumab in Moderate-to-Severe Plaque Psoriasis. *N Engl J Med.* 2016;375(4):345-356.
17. Lebwohl M, Strober B, Menter A, et al. Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis. *N Engl J Med.* 2015;373(14):1318-1328.
18. Corren J, Parnes JR, Wang L, et al. Tezepelumab in Adults with Uncontrolled Asthma. *N Engl J Med.* 2017;377(10):936-946.
19. Brightling CE, Gaga M, Inoue H, et al. Effectiveness of fevipiprant in reducing exacerbations in patients with severe asthma (LUSTER-1 and LUSTER-2): two phase 3 randomised controlled trials. *Lancet Respir Med.* 2020.
20. Asano K, Sagara H, Ichinose M, et al. A Phase 2a Study of DP(2) Antagonist GB001 for Asthma. *J Allergy Clin Immunol Pract.* 2020;8(4):1275-1283.e1271.
21. Castro M, Corren J, Pavord ID, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *N Engl J Med.* 2018;378(26):2486-2496.
22. Chupp GL, Bradford ES, Albers FC, et al. Efficacy of mepolizumab add-on therapy on health-related quality of life and markers of asthma control in severe eosinophilic asthma (MUSCA): a randomised, double-blind, placebo-controlled, parallel-group, multicentre, phase 3b trial. *Lancet Respir Med.* 2017;5(5):390-400.
23. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med.* 2014;371(13):1198-1207.
24. Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. *Lancet Respir Med.* 2015;3(5):355-366.
25. Bleecker ER, FitzGerald JM, Chanez P, et al. Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting β(2)-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet.* 2016;388(10056):2115-2127.
26. FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet.* 2016;388(10056):2128-2141.

27. Hanania NA, Korenblat P, Chapman KR, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med.* 2016;4(10):781-796.
28. Busse WW, Brusselle GG, Korn S, et al. Tralokinumab did not demonstrate oral corticosteroid-sparing effects in severe asthma. *Eur Respir J.* 2019;53(2).
29. Busse WW, Holgate S, Kerwin E, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med.* 2013;188(11):1294-1302.
30. Calverley PMA, Sethi S, Dawson M, et al. A randomised, placebo-controlled trial of anti-interleukin-1 receptor 1 monoclonal antibody MEDI8968 in chronic obstructive pulmonary disease. *Respir Res.* 2017;18(1):153.
31. Pavord ID, Chanez P, Criner GJ, et al. Mepolizumab for Eosinophilic Chronic Obstructive Pulmonary Disease. *N Engl J Med.* 2017;377(17):1613-1629.
32. Eich A, Urban V, Jutel M, et al. A Randomized, Placebo-Controlled Phase 2 Trial of CNTO 6785 in Chronic Obstructive Pulmonary Disease. *Copd.* 2017;14(5):476-483.
33. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *Lancet.* 2019;394(10209):1638-1650.
34. Straumann A, Hoesli S, Bussmann C, et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy.* 2013;68(3):375-385.
35. Straumann A, Conus S, Grzonka P, et al. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut.* 2010;59(1):21-30.
36. Spergel JM, Rothenberg ME, Collins MH, et al. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol.* 2012;129(2):456-463, 463.e451-453.
37. Feagan BG, Sandborn WJ, Gasink C, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. *N Engl J Med.* 2016;375(20):1946-1960.
38. Mannon PJ, Fuss IJ, Mayer L, et al. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med.* 2004;351(20):2069-2079.
39. Panaccione R, Sandborn WJ, Gordon GL, et al. Briakinumab for treatment of Crohn's disease: results of a randomized trial. *Inflamm Bowel Dis.* 2015;21(6):1329-1340.
40. Feagan BG, Sandborn WJ, D'Haens G, et al. Induction therapy with the selective interleukin-23 inhibitor risankizumab in patients with moderate-to-severe Crohn's disease: a randomised, double-blind, placebo-controlled phase 2 study. *Lancet.*

2017;389(10080):1699-1709.

41. Hueber W, Sands BE, Lewitzky S, et al. Secukinumab, a human anti-IL-17A monoclonal antibody, for moderate to severe Crohn's disease: unexpected results of a randomised, double-blind placebo-controlled trial. *Gut.* 2012;61(12):1693-1700.
42. Targan SR, Feagan B, Vermeire S, et al. A Randomized, Double-Blind, Placebo-Controlled Phase 2 Study of Brodalumab in Patients With Moderate-to-Severe Crohn's Disease. *Am J Gastroenterol.* 2016;111(11):1599-1607.
43. Sands BE, Sandborn WJ, Panaccione R, et al. Ustekinumab as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med.* 2019;381(13):1201-1214.
44. Sandborn WJ, Ferrante M, Bhandari BR, et al. Efficacy and Safety of Mirikizumab in a Randomized Phase 2 Study of Patients With Ulcerative Colitis. *Gastroenterology.* 2020;158(3):537-549 e510.