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Innate lymphoid cells as regulators of immunity, inflammation and tissue homeostasis

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Research over the last 7 years has led to the formal identification of innate lymphoid cells (ILCs), increased the understanding of their tissue distribution and has established essential functions of ILCs in diverse physiological processes. These include resistance to pathogens, the regulation of autoimmune inflammation, tissue remodeling, cancer and metabolic homeostasis. Notably, many ILC functions appear to be regulated by mechanisms distinct from those of other innate and adaptive immune cells. In this Review, we focus on how group 2 ILC (ILC2) and group 3 ILC (ILC3) responses are regulated and how these cells interact with other immune and non-immune cells to mediate their functions. We highlight experimental evidence from mouse models and patient-based studies that have elucidated the effects of ILCs on the maintenance of tissue homeostasis and the consequences for health and disease.

Innate lymphoid cells (ILCs) are the most recently identified cell types to be added to the complex cellular map of the immune system. While they were first identified at barrier surfaces, it is now clear that ILCs populate almost every tissue thus far examined. As part of this Focus on ILCs, this Review will discuss recent advances in the understanding of how ILC responses are regulated and how they contribute to inflammation and tissue homeostasis in the context of host defense, autoimmunity, cancer and metabolic disease. While another Review in this issue by Spits and colleagues will discuss natural killer (NK) cell and ILC1 biology[1], this Review will focus on the biology of ILC2s and ILC3s. We will describe the tissue distribution of the ILC subsets, how their responses are regulated and their roles in physiological and patho-physiological processes.

The ILC family

Although ILCs fulfill important functions in various tissues, including the intestine, lungs, skin, liver, adipose tissue and mesenteric lymph nodes, their tissue distribution is remarkable for lymphocytes. ILCs are clearly underrepresented in lymphoid tissues, but parenchymal tissues, especially mucosal surfaces, show substantial enrichment for ILCs. At the mucosa of the intestine and lungs, ILCs seem to be particularly important regulators of epithelial barriers. The complex interplay among immune cells, epithelial cells, the microbiota and metabolites in the intestine has been an area of intense research efforts. The intestinal barrier is colonized by the microbiota and serves as the primary entry port for many pathogens. The maintenance and re-establishment of barrier integrity are essential for the resolution of inflammation after infection or inflammatory responses. Because of their strategic location, ILCs are among the first immune cells to react to pathogens by the induction of responses to infection and shaping of the adaptive immune response. The localization of these evolutionarily ancient cells in non-lymphoid tissue suggests that in addition to mounting pro-inflammatory responses, ILCs regulate tissue development, integrity and homeostasis.

Unlike adaptive lymphocytes, ILCs do not express rearranged antigen receptors that recognize 'non-self' structures, but they do exhibit a functional diversity similar to that of T cells. With the exception of Foxp3$^+$ regulatory T cells (T_{reg} cells), innate counterparts for each T cell subset, such as cytotoxic ILCs for CD8$^+$ T cells, and non-cytotoxic ILCs for the T_H1, T_H2, and T_H17 subsets of helper T cells, have been identified and extensively discussed in other reviews[2–4]. ILCs express and developmentally depend on key transcription factors that control a cytokine profile comparable to that of their corresponding adaptive counterparts[2,5]. Conventional NK (cNK) cells are considered the innate counterpart of CD8$^+$ T cells because both are equipped with similar functions such as cytotoxicity and interferon-γ (IFN-γ) production and both express the transcription factors Eomes and T-bet. T_H1 cells and their innate counterparts, ILC1s, express T-bet and produce IFN-γ to fight intracellular pathogens[2,6]. GATA-3^{hi} ILC2s, like T_H2 cells, secrete interleukin 5 (IL-5), IL-13 and the epidermal-growth-factor-like molecule amphiregulin to control helminth infection[7–11]. RORγt$^+$ ILC3s correspond to T_H17 cells and are heterogeneous in mice and humans. In humans, expression of the activating receptor NKp44 defines two subpopulations among CCR6$^+$c-Kit$^+$ ILC3s. In mice, expression of the chemokine receptor CCR6 distinguishes CCR6$^+$ ILC3s and CCR6$^-$ ILC3s, which both produce IL-22 (refs. 12–14). The CCR6$^+$ ILC3 population comprises lymphoid-tissue-inducer cells (LTi) cells and LTi-like cells, which can be either CD4$^+$ or CD4$^-$. In addition to secreting IL-22, CCR6$^+$ ILC3s secrete IL-17, a cytokine crucial for resistance to fungal infection[15–17]. During development, CCR6$^+$ ILC3s are essential for the formation of lymphoid

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organs, whereas in the adult mouse, they are mainly clustered in aggregates together with stromal cells, dendritic cells (DCs) and B cells in cryptopatches, isolated lymphoid follicles or mature isolated lymphoid follicles[15]. In contrast, CCR6$^-$ ILC3s, ILC1s and ILC2s are scattered throughout the intestine. The majority of CCR6$^-$ ILC3s express T-bet and NKp46 and are therefore commonly referred to as 'natural-cytotoxicity-receptor-positive' (NCR$^+$) ILC3s. Upregulation of T-bet expression in ILC3s induces downregulation of the transcription factor RORγt and adaptation to a phenotype similar to that of ILC1s, characterized by the ability to produce IFN-γ and promote tissue inflammation[13,14,18–22] (**Fig. 1**).

Trafficking and tissue residency of ILCs

The functionality of ILCs is dependent on their microenvironment. ILC1s, ILC2s and ILC3s are localized mainly in mucosa-associated tissues, whereas cNK cells are 'preferentially' localized in secondary lymphoid organs. All ILCs develop from precursors in the bone marrow. cNK cells express the selectin CD62L, which allows them

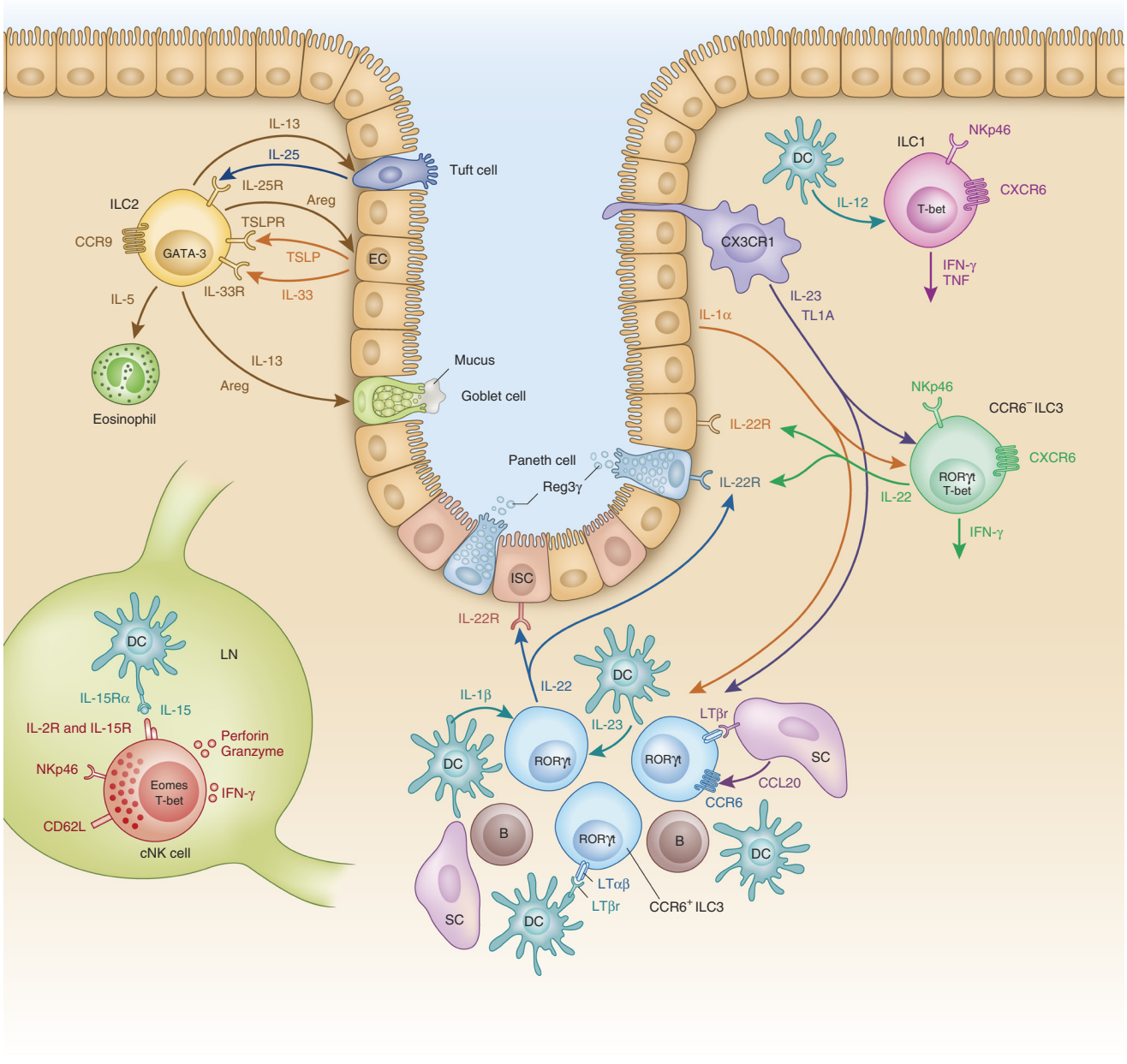

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Figure 1 The distribution of ILCs at the mucosa of the intestine. Non-cytotoxic ILCs (such as ILC1s, ILC2s and CCR6$^-$ ILC3s) are scattered in the lamina propria, whereas CCR6$^+$ ILC3s reside in organized intestinal follicles, and cNK cells reside in lymphoid organs. In lymphoid organs, DCs prime cNK cells via trans-presentation of IL-15. After being activated, cNK cells secrete perforin, granzymes and IFN-γ. ILC1s produce IFN-γ and TNF and are activated by myeloid-cell-derived IL-12. ILC2s receive activating signals such as IL-33 and TSLP from epithelial cells and myeloid cells and IL-25 from tuft cells. ILC2s secrete IL-5 to recruit eosinophils and IL-13 to stimulate mucus production by goblet cells. ILC3s are activated by IL-23, IL-1α and IL-1β derived from myeloid cells and epithelial cells. CCR6$^+$ ILC3s reside in intestinal follicles, where they interact with DCs, B cells and stromal cells. IL-22 produced by ILC3s stimulates ISC and paneth cells. Areg, amphiregulin; TSLPR, receptor for TSLP; EC, epithelial cell; ISC, intestinal stem cell; SC, stromal cell; B, B cell; LN, lymph node.

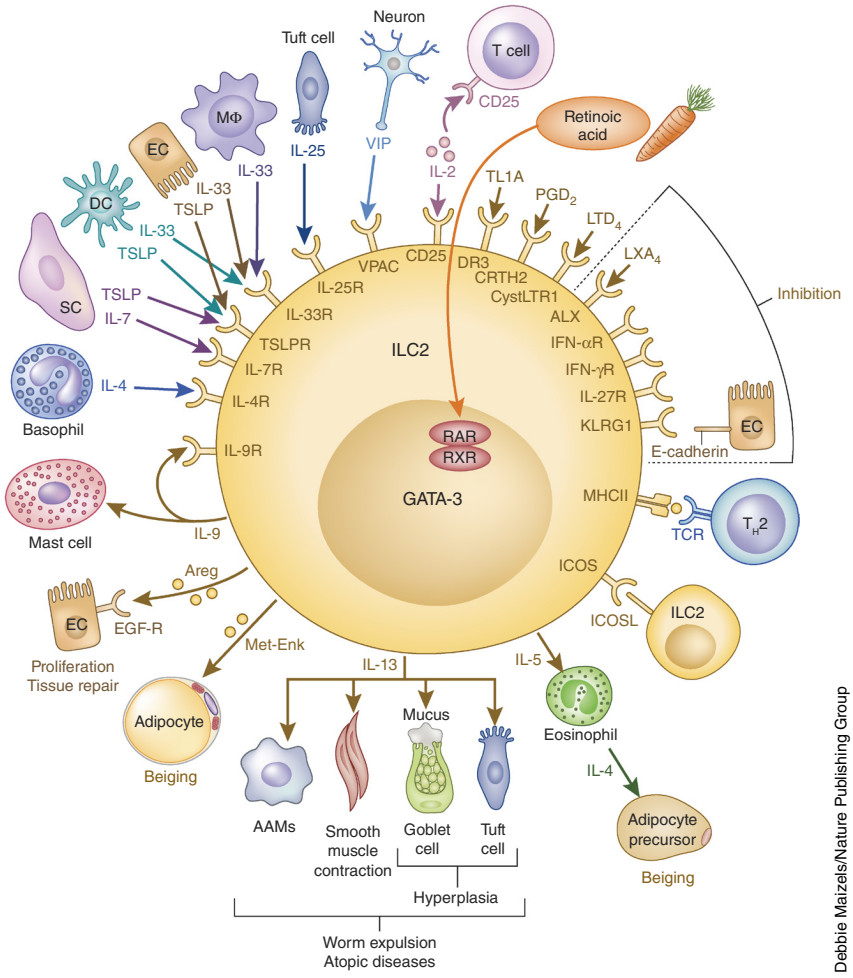

Figure 2 Integration of ILC2s in the type 2 immune response. ILC2s integrate multiple signals (mainly soluble factors) by expressing an array of activatory and inhibitory receptors[146]. ILC2s are activated by alarmins (IL-33, IL-25 and TSLP), other cytokines (IL-2, IL-4, IL-7 and IL-9) and lipid mediators (PGD_2, LTD_4 and LXA_4). Inhibitory signals via receptors for IL-27, IFN-γ and IFN-α are integrated, as is cell-to-cell interaction (KLRG1 and ICOS). Effector molecules include IL-5, IL-13, methionine-enkephalin (Met-Enk) and amphiregulin. MΦ, macrophage; EGF-R, receptor for epithelial growth factor; VPAC, receptor for VIP; TL1A, TNF-family cytokine; DR3, member of the TNF-receptor superfamily; CysLTR1, G-protein-coupled receptor for cysteinyl leukotrienes; ALX, G-protein-coupled receptor for the arachidonic acid metabolite LXA_4; MHCII, MHC class II; TCR, T cell antigen receptor; AAMs, alternatively activated macrophages.

to migrate from the blood via high endothelial venules to lymphoid organs, where they need constant priming by DCs to mount an immune response[6,23]. In contrast, barrier surfaces show enrichment for other ILC populations, with lower numbers being found in most lymphoid tissues at steady state. Consistent with that, ILC precursors express the integrin $\alpha_4\beta_7$, the interaction partner of the adhesion molecule MadCAM-1, expressed by high endothelial venules of mucosal lymphoid tissue such as Peyer's patches, and the chemokine receptor CXCR6, for migration to the intestine[6,24]. Studies of parabiosis have demonstrated that ILC1s, ILC2s and ILC3s rarely undergo replenishment from the bone marrow at steady state or during disruption of homeostasis[25–27]. In contrast, cNK cells and adaptive lymphocytes undergo constant replacement from hematogenous sources. The data obtained in parabiosis experiments suggest that non-cytotoxic ILCs populate their niches early in ontogeny and remain in their environment throughout life. The local proliferation of tissue-resident progenitor cells supports the self-renewal of ILCs in tissues[28]. It has been reported that retinoic acid induces upregulation of the integrin $\alpha_4\beta_7$ and the chemokine receptor CCR9 and downregulation of the chemokine receptor CCR7 on ILC1s and ILC3s to facilitate homing of these cells to the gut[29]. Conversely, ILC2 precursors in the bone marrow express CCR9, which allows them to migrate directly to the intestine[7,29]. Therefore, $\alpha_4\beta_7$, CCR9, CCR6 and CXCR6 direct the homing of ILCs to tissues, presumably in a certain time window during embryogenesis, but this area requires further investigation. In adults, ILCs could use afferent lymph vessels to migrate from the lamina propria to the mesenteric lymph nodes, where $CCR6^+$ ILC3s interact with T cells to delete auto-reactive T cells[30].

Non-cytotoxic ILCs are, to a large degree, sessile immune cells that share hallmarks with tissue-resident lymphocytes, as has been reviewed[31]. These include localization to non-lymphoid tissue, enrichment at barrier surfaces, self-renewal and rapid responses to environmental stimuli. In addition to mounting local immune responses, ILCs might influence systemic immunity by regulating adaptive responses, as described in detail in a related Perspective by Colonna and Bando in this special ILC Focus issue of *Nature Immunology*[32].

Initiation of ILC responses

Mouse ILCs lack pattern-recognition receptors, which are broadly expressed by other immune cells for the detection of pathogen-associated molecular patterns[33]. Instead, mouse ILCs react to pathogens indirectly by sensing myeloid-cell- or epithelial-cell-derived cytokines, alarmins and inflammatory mediators, such as IL-12, for ILC1s[6]; IL-33, IL-25, TSLP, IL-2, IL-4, IL-7, TL1A, prostaglandin D_2 and leukotriene D_4, for ILC2s[10,34–39]; and IL-23, IL-7, TL1A, prostaglandin E_2, IL-1α and IL-1β, for ILC3s[40–44] (**Figs. 2** and **3**). IL-23 alone is sufficient to activate the production of IL-22 and IL-17 by ILC3s. However, additional cytokines such as IL-1β and IL-7 stimulate the proliferation and survival of ILC3s[14,45,46].

Although IL-25 and IL-33 elicit strong ILC2 responses *in vivo* and both contribute to the expulsion of *Nippostrongylus brasiliensis*, their precise role in the activation of ILC2s is incompletely understood[9,10]. IL-33 alone induces strong activation of ILC2s *in vitro*, whereas IL-25 stimulates ILC2s only moderately[7,9]. IL-2, IL-7 and TSLP alone are insufficient for the activation of ILC2s *in vitro* but boost activation in combination and enhance the effect of IL-33 (refs. 8,47). *In vivo*, IL-25 elicits multipotent progenitor type 2 responses[48] and the expansion of a subset of ILC2s called 'inflammatory ILC2s'[49]. Inflammatory ILC2s are characterized by high expression of the maturation marker KLRG1 and the IL-25 receptor. In addition to mediating anti-helminth immunity, inflammatory ILC2s express RORγt and produce IL-17 to combat *Candida albicans* infection[49]. However, further investigation is needed for precise segregation of

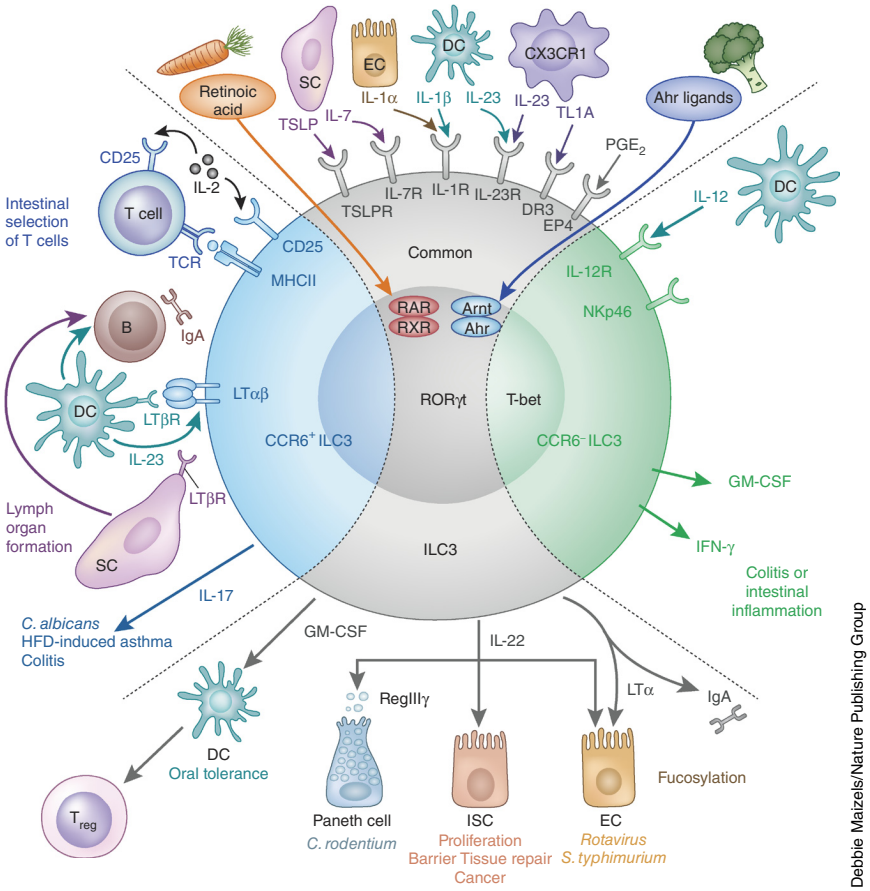

Figure 3 ILC3 responses at epithelial barriers. CCR6$^+$ ILC3s (left) and CCR6$^-$ ILC3s (right) have overlapping and distinct functions. Both receive activating signals such as IL-23, TSLP, TL1A, IL-1α and IL-1β (top), which overlap to a large degree, and secrete IL-22 (bottom). Ahr ligands and retinoic acid are sensed by intranuclear receptors. EP4, PGE_2 receptor; Arnt, nuclear translocator of Ahr; RegIIIγ, antimicrobial peptide; HFD, high-fat diet; IgA, immunoglobulin A; T_{reg}, T_{reg} cell.

the lineage relationship and plasticity among ILC2s and ILC3s.

The activation of cNK cells or T cells is mediated to a large degree by immunoreceptors that recognize ligands not expressed by healthy cells. In contrast, the importance of immunoreceptors for the activation of non-cytotoxic ILCs is less well established. For example, NK cells detect either a lack of ligands such as major histocompatibility complex (MHC) class I ('missing self') or the presence of ligands ('induced self') that are normally not expressed by target cells[50]. The balance of stimulatory signals and inhibitory signals determines if an NK cell is activated or not[23]. Deficiency in NK cell receptors such as NKG2D or NKp46 results in altered NK cell function[51,52]. Although NKp46 and NKG2D are also expressed by ILC1s and NCR$^+$ ILC3s and cross-linking of NCRs triggers the release of proinflammatory cytokines, including TNF, from ILC3s, a non-redundant function in a disease model remains to be demonstrated[6,53,54]. Of note, inhibitory receptors of the Ly49 family, which are essential for the normal development and function of cNK cells, do not appear to be expressed by other ILC subsets[6].

The co-stimulatory molecule ICOS and KLRG1 are among the few receptors on ILC2s that interact with membrane-bound ligands. ICOS and its ligand ICOSL are co-expressed on ILC2s; this interaction promotes the proliferation of ILC2s and might constitute a self-amplifying mechanism[55,56]. In contrast, the interaction of KLRG1 and E-cadherin has been shown to inhibit human ILC2s[57]. However, a crucial function remains to be established *in vivo*, because KLRG1 is dispensable for NK cells and for the development and function of T cells[58].

It should be noted that in some cases, the expression of pattern-recognition receptors and NCRs diverges in mouse ILCs versus human ILCs. For example, in contrast to mouse ILCs, human ILC3s express Toll-like receptors, and engagement of these receptors by the corresponding ligands activates ILC3s[59]. In addition, human ILC2s express the NCR NKp30 (ref. 60). Engagement of NKp30 by the ligand B7-H6 leads to the activation of skin-derived ILC2s. Interestingly, B7-H6 is expressed on keratinocytes, and its expression is upregulated in atopic dermatitis[60]. Therefore, although similar cytokines activate mouse ILCs and human ILCs, human ILCs might receive additional signals through Toll-like receptors or NCRs.

Given that mouse ILCs do not directly recognize pathogen-associated molecular patterns, the question remains of how ILCs sense tissue damage, infection and disruption of tissue homeostasis. Some molecules such as vitamins or metabolites are directly recognized by ILCs[61,62] (**Figs. 2** and **3**). Vitamin A includes fat-soluble compounds that are derived mainly from vegetables and are converted into functional ligands by oxidation. These include all-trans retinoic acid, which binds heterodimeric RAR or RXR receptors, and the 9-cis-retinoic acid isomer, which binds homodimeric RXR receptors in ILCs[63]. Through studies of genetic mouse models and various diets it has been demonstrated that constant sensing of vitamin A metabolites is crucial for the prenatal differentiation of LTi cells, the proper development of lymphoid organs and the generation of protective immune responses in these structures[61]. In adult mice, retinoic acid signals favor ILC3 responses and suppress ILC2 responses. Consequently, vitamin-A-deficient mice fail to control *Citrobacter rodentium* infection but mount a potent anti-helminth response[62].

Another metabolite-sensing nuclear receptor is Ahr ('aryl hydrocarbon receptor'), which is expressed by a variety of cells, including ILC3s[63]. Ligands for Ahr, such as the phytochemical indole-3-carbinol, are present in cruciferous vegetables such as cabbage and broccoli[63]. The population expansion of CCR6$^-$ ILC3s is strictly Ahr dependent, whereas CCR6$^+$ ILC3s develop in *Ahr*$^{-/-}$ mice but have functional defects such as reduced secretion of IL-22 (refs. 13,64). As a consequence, *Ahr*$^{-/-}$ mice lack cryptopatches and fail to control *C. rodentium* infection[64–66]. Although IL-22 is essential for the control of *C. rodentium* infection and ILC3s are the dominant source of IL-22 in the first week after infection, the importance of Ahr for ILC3-derived IL-22 versus T cell–derived IL-22 is incompletely understood[67]. Therefore, tools need to be developed that specifically target Ahr in all ILC3s but not in T cells, for delineation of the redundant functions of Ahr versus its non-redundant functions in these cells.

ILC2s also receive signals from the enteric nervous system. The neuropeptide VIP, which activates ILC2s, is secreted by enteric neurons, and its expression is regulated by circadian rhythm[68]. Therefore, ILC2 activation and eosinophil recruitment follow the circadian clock. In addition, ILC3s express molecules that might allow them to interact with the nervous system[33], which indicates that the biology of ILC–neuronal cell interactions might have a profound effect on tissue homeostasis.

In summary, when tissue homeostasis is disturbed, soluble mediators such as cytokines activate ILCs. Constant sensing of metabolites by ILCs provides essential survival or maturation signals. For better understanding of ILC biology, it will be crucial to know which metabolites and microbiota- or neuron-derived factors are detected by ILCs.

Effector molecules produced by ILCs

ILCs promote responses to various challenges by secreting soluble factors such as cytokines and other peptides. These include known effector cytokines, such as IFN-γ and TNF, for ILC1s[6]; IL-5, IL-9 and IL-13, for ILC2s[9,10,69]; and IL-17, IL-22, GM-CSF and IFN-γ, for ILC3s[20,46,70,71]. Although subsets of T cells, γδ T cells or NKT cells produce a similar array of cytokines, an important function for ILC-derived cytokines has been demonstrated in many disease systems, including but not limited to infection with *N. brasiliensis*[7,9,10], *C. rodentium*[19,72], rotaviruses[42,73] or *C. albicans*[17], atopic diseases, and colitis[20,34,47,74]. Together these findings suggest that there is a spatiotemporal 'division of labor', including ILCs that react quickly at mucosal tissues and T cells that are activated and proliferate in secondary lymphoid organs to combat infection at a later stage by similar mechanisms.

Beyond classic helper T cell cytokines, previously unknown ILC effector molecules have been described. For example, ILC2s secrete amphiregulin, which mediates tissue repair[11], and methionine-enkephalin[75], which induces beiging of adipocytes, while ILC3s secrete lymphotoxin-α (LTα), which stimulates the T cell–dependent production of immunoglobulin A[76,77].

Some effector molecules on ILCs require cell-to-cell contact. During development of lymphoid organs, engagement of the LTβ receptor (LTβR) on stromal or endothelial cells by membrane-bound LTαβ on CCR6$^+$ ILC3s (LTi cells) is essential for the formation of various lymphoid organs[78]. In adult mice, the interaction of LTαβ on CCR6$^+$ ILC3s with LTβR expressed on DCs in cryptopatches regulates an amplifying loop that results in the release of IL-23 by DCs and increased production of IL-22 by ILC3s during *C. rodentium* infection[76,79,80]. ILC3s also promote immunoglobulin production by B cells through diverse mechanisms that include engagement of LTβR on stromal cells or direct interaction of ILC3s and B cells through the ligand for the costimulatory receptor CD40, the Notch receptor ligand DLL1 and additional factors such as the B cell–activation factor BAFF[76,81,82].

In mesenteric lymph nodes, CCR6$^+$ ILC3s present to CD4$^+$ T cells peptides from commensal microflora loaded onto MHC class II. This interaction results in the deletion of potentially autoreactive T cells and the prevention of autoimmunity. This has therefore been called 'intestinal T cell selection'[30]. In contrast, stimulatory effects of MHC class II on ILC2s and ILC3s have also been described and are discussed further in the Perspective by Colonna and Bando in this Focus issue[32,83,84].

ILCs act mainly by secreting soluble mediators. Given that ILCs are tissue-resident cells, the anatomy of the tissue microenvironment might require rapidly diffusible effector molecules for signal transduction and amplification within the tissue. Effector mechanisms that require cell-to-cell contact seem to occur 'preferentially' in lymphoid structures such as cryptopatches or mesenteric lymph nodes.

Cross-regulation, inhibition and termination of ILC responses

The cross-regulatory and inhibitory pathways that control ILC responses are not well defined at present. Some studies have reported that inhibition by cytokines and competition for survival factors can limit ILC activation and thereby maintain mucosal homeostasis. For example, ILC2s are inhibited by type I and II interferons and IL-27, which are known to promote type 1 immunity. Consequently, administration of these cytokines reduces immunopathology in various models of airway inflammation induced by influenza A virus or an extract of the fungus *Alternaria alternata*[27,85,86].

Related to the findings discussed above, ILC2-promoting cytokines such as IL-25 and TSLP can suppress IL-22 secretion by ILC3s[87,88]. Commensal microflora induce the release of IL-25 from epithelial cells in the intestine that acts on CD11c$^+$ cells to limit ILC3-derived IL-22 secretion. Because IL-22 is critical for maintenance of the epithelial barrier, the administration of IL-25 increases the severity of dextran sulfate sodium (DSS)-induced colitis[87]. Moreover, mice with defects in non-canonical signaling via the transcription factor NF-κB due to specific deletion of the serine-threonine kinase IKKα in epithelial cells develop more-severe intestinal inflammation upon *C. rodentium* infection or DSS administration than that of mice with *loxP*-flanked alleles encoding IKKα. The immunopathology is diminished after IL-22 administration and is mechanistically explained by increased TSLP secretion by epithelial cells that dampens ILC3-derived IL-22 secretion[88].

Adaptive immune cells, in particular T_{reg} cells, control ILC responses via diverse mechanisms, one being competition for the uptake of local IL-2. Depletion of T_{reg} cells results in the proliferation of NK-cell-receptor-positive ILCs and ILC2s[30,89]. ILC2s are also regulated by mediators such as maresin or lipoxin A_4, which are secreted to resolve inflammation. In a model of allergic inflammation in the lung, maresin has been shown to induce the *de novo* generation of T_{reg} cells, which limits ILC2 responses and the severity of inflammation[90]. In addition, lipoxin A_4 dampens IL-13 production by binding the receptor ALX/FPR2 expressed by ILC2s[91]. Therefore, it appears that ILC responses are limited by cytokines that promote a different type of immune response, such as T_H1, T_H2 or T_H17, via competition with other lymphocytes for survival factors and via mediators that elicit the resolution of inflammation.

ILCs promote protective immunity to pathogens

It is well established that ILC2s mediate resistance to helminth infections. The observation that potent type 2 immune responses can be triggered in the absence of T cells was reported multiple times before the formal identification of ILC2s[92,93]. Most of the experiments demonstrating a non-redundant role for ILC2s in parasite infections were carried out with the nematode *N. brasiliensis*[7,9,10], but some reports have suggested that ILC2s might also contribute to the clearance of *Strongyloides venezuelensis* and *Trichuris muris*[94,95]. IL-25 and IL-33 are essential for ILC2 activation and worm expulsion in most circumstances[10,96]. IL-25 in the intestine is secreted by tuft cells and stimulates the release of IL-13, which induces tuft-cell hyperplasia in return[96–98]. Another important amplification mechanism in early ILC2 activation is the production of IL-9, which acts in an autocrine fashion on ILC2s[69]. When activated, ILC2s secrete IL-4, IL-5, IL-13 and amphiregulin to combat helminth infection. IL-5 is an important factor involved in eosinophil function, whereas amphiregulin mediates the repair of epithelial cells, although the mechanisms of action of amphiregulin in host defense are poorly defined. IL-13 induces

smooth-muscle contraction, mucus production by goblet cells, the recruitment of alternatively activated macrophages and the release of eotaxin, which together mediate worm expulsion[2,9,10,94].

ILC3s secrete IL-22, which mediates resistance to intestinal infection by acting directly on non-hematopoietic cells. IL-22 binds to the heterodimeric receptor IL-22Rα1–IL-10Rβ on epithelial cells. The binding of IL-22 triggers a signaling cascade that induces phosphorylation of the transcription factor STAT3, cell proliferation, secretion of antimicrobial peptides such as RegIIIβ, RegIIIγ, S108a and S109a, and the fucosylation of epithelial cells[40,70,99]. Fucosylation of the intestinal epithelium requires production of IL-22 and LTα by ILC3s, which protects against *Salmonella typhimurium* infection[77].

In addition, a synergistic requirement for IL-22 signaling on epithelial cells has been reported to support STAT1-mediated resistance to rotaviruses, which is mediated largely by IFN-λ. Rotaviral infection triggers the release of IL-1α from epithelial cells, which stimulates ILC3-derived IL-22 production. Consequently, *Il22*$^{-/-}$ mice are susceptible to rotaviral infection, whereas the administration of IL-22 leads to enhanced viral clearance[42,73].

Resistance to intestinal infection with *C. rodentium*, a model organism for attaching-and-effacing *Escherichia coli*, is strictly IL-22 dependent[40]. ILC3s are the predominant IL-22-producing population in the first week of *C. rodentium* infection[72,87]. The secretion of IL-23 by Notch2-dependent DCs or CX3CR1^{+} mononuclear phagocytes is required for proper ILC3 activation[41,100]. DCs are stimulated by engagement of LTβR by LTαβ on ILC3s localized in cryptopatches[79,80]. At later time points, T cell–derived IL-22 and B cells contribute substantially to the resistance to *C. rodentium*; therefore, whether ILC3-derived IL-22 is essential or could be compensated for by IL-22 from T_H17 cells has been debated[67,101]. It has been shown that *Il23a*$^{-/-}$ mice are more susceptible than *Il23a*$^{+/+}$ mice to infection with a high dose of *C. rodentium* but are not more susceptible to infection with a low dose of this pathogen[102]. Since IL-23 has been found to be essential for IL-22 production by ILC3s but not by T_H17 cells in this model, these data would indicate that ILC3s are important for resistance to a high dose of *C. rondentium* infection, but this does not rule out the possibility that ILC3s and T cells might have redundant functions. Experiments with *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mice (collectively called '*Rag*$^{-/-}$ mice' here), which lack T cells and B cells, have demonstrated an essential function for ILC3s in this infection model. In such analyses, *Rag*$^{-/-}$ mice are compared with *Rag*$^{-/-}$ *Il2rg*$^{-/-}$ mice, which lack all ILC subsets in addition to lacking T cells and B cells. Another approach often used is depletion of ILCs via injection of antibody to the alloantigen CD90 (Thy-1) (anti-CD90), which targets almost all ILCs. Collectively, the data have demonstrated that ILC3s are indispensable for resistance to *C. rodentium* on a lymphocyte-deficient background, because both *Rag*$^{-/-}$*Il2rg*$^{-/-}$ mice and *Rag*$^{-/-}$ mice that have undergone ILC depletion via anti-CD90 are more susceptible to *C. rodentium* infection than are *Rag*$^{-/-}$ mice[19,72]. *Rag*$^{-/-}$ mice together with ILC depletion via anti-CD90 are often used in ILC research to demonstrate essential functions of ILCs. Data obtained from such experiments might be criticized because T cells, which might compensate for ILC function, are absent in these mice. In addition, the localization and activation status of ILCs might be different in *Rag*$^{-/-}$ mice versus wild-type mice, and 30–50% of ILCs express RAG recombinase proteins. RAG proteins are known to regulate the cellular fitness of cNK cells, but their role in ILC2s and ILC3s is unknown[103]. In *Rag*$^{-/-}$*Il2rg*$^{-/-}$ mice, the lack of lymphoid organs might potentially be a confounding factor. Finally, the specificity of cell depletion via anti-CD90 has been criticized as well, because anti-CD90 might potentially elicit the depletion of other cells such as neurons.

To investigate ILC3 function in T cell–replete mice, two groups have generated more specific depletion systems. An *Nkp46*$^{Cre/+}$ system has been used to interfere with the development or function of NCR^{+} ILC3s[67,101]. The results have shown that NCR^{+} ILC3s are dispensable in the presence of T cells for the control of *C. rodentium* infection. This is in line with published data that have assigned an important function to CD4^{+} ILC3s on a *Rag*$^{-/-}$ background[72]. In one study, mice lacking NCR^{+} ILC3s were crossed to a background deficient in T cells (*Tcrb*$^{-/-}$*Tcrd*$^{-/-}$)[67]. On this background, mice deficient and sufficient in NCR^{+} ILC3s were equally susceptible to infection with *C. rodentium*. In contrast, another study of mice on a *Rag*$^{-/-}$ background genetically depleted of NCR^{+} ILC3s has reported that mice deficient in NCR^{+} ILC3s are more susceptible than *Rag*$^{-/-}$ mice to infection with *C. rodentium*[101]. These data might be explained by the different depletion strategies and the choice of a background deficient in T cells versus one deficient in T cells and B cells. Collectively, these data suggest a multilayered organization of innate and adaptive lymphocytes with complementary and redundant functions. A mouse model that ensures specific deletion of all ILC3s without targeting of T cells is required for full elucidation of the relative contributions of ILC3s and T cells.

The susceptibility of RORγ-deficient patients, who lack T_H17 cells and ILC3s, to *C. albicans* infection has attracted attention, but conclusions cannot be drawn about the contribution of ILC3s and T cells to anti-fungal immunity[104]. The role of ILC3s and T cells has been investigated in a mouse model of oropharyngeal infection with *C. albicans*. Similar to results obtained with human subjects, RORγt-deficient mice fail to control *C. albicans* infection. Interestingly, resistance to *C. albicans* infection is not T cell dependent, since *Rag*$^{-/-}$ mice control *C. albicans*. However, *Rag*$^{-/-}$ mice that have undergone depletion of ILCs and mice in which IL-17 or IL-23 has been neutralized are susceptible to infection, which suggests a pivotal role for CCR6^{+} ILC3s in this model[17]. Despite the clear data from this infection model, the question remains of how this infection model reflects human candidiasis. Results from the mouse model might be dependent on the infection route, and the importance of T cells might be underestimated because the infections are performed in naive mice[105].

ILCs are essential mediators of resistance to pathogens that use the mucosa to penetrate the host in mouse models, but little is known about human ILCs in host defense at barrier surfaces. It has been reported that the abundance of ILC2s is diminished in young children infected with *Schistosoma haematobium* and that anti-worm treatment restores ILC2 numbers[106]. However, a different study has found an increase in the number of c-Kit^{+} ILCs in filaria-infected patients[107]. Further research is needed for better delineation of the host-protective functions of human ILCs.

ILCs in allergy and autoimmunity

Chronic exposure to immune stimuli turns a tissue-protective response into immunopathology, although both are often mediated by similar effector mechanisms. Notably, genes linked to susceptibility to atopic disease, such as the genes encoding IL-33 and its receptor, as well as those encoding TSLP, IL-4, IL-5 and IL-13, are associated with ILC2 responses[108]. The association of detrimental ILC2 responses with atopic diseases such as asthma, atopic dermatitis and chronic rhinosinusitis is supported by data obtained from mouse models[34,47,109]. Protease allergens, including papain and house dust mite, are used to trigger non-infectious lung inflammation.

Remarkably, papain induces asthma-like symptoms in *Rag*$^{-/-}$ mice but not in *Rag*$^{-/-}$*Il2rg*$^{-/-}$ mice or *Rag*$^{-/-}$ mice that have undergone depletion of ILCs. In ILC-deficient mice reconstituted with ILC2s, challenge with allergen induces asthma-like symptoms[36,47]. Beyond IL-25 and IL-33, basophil-derived IL-4 stimulates ILC2s to promote inflammation in the lungs[36] and skin[110]. The ability of ILC2s to trigger airway hyper-reactivity is not only limited to non-infectious inflammation but is also reported after infection with influenza virus[111]. Skin lesions or nasal polyps of patients with atopic dermatitis or chronic rhinosinusitis, respectively, also show enrichment for activated ILC2s[57,112]. ILC2-dependent skin inflammation can also be induced in mice treated with the vitamin D analog calcipotriol, complexes of IL-2 and anti-IL-2 or overexpression of IL-33. ILC2 responses are elicited by TSLP or by IL-25 and IL-33 during skin inflammation[34,35,57,109]. Collectively, the data from genome-wide association studies of patients, coupled with functional studies of mouse model systems, suggest that ILC2s are involved in the pathogenesis of atopic diseases.

IL-23 is a potent activator of ILC3s, and this pathway is closely connected to inflammatory bowel disease[113–115]. Consistent with intestinal enrichment for ILC3s, the function of ILC3s has been established through the use of infection-induced or sterile inflammation models of the small or large bowel. Detrimental ILC3 responses have been reported in models of colitis induced by *Helicobacter hepaticus*, *Helicobacter typhlonius, S. typhimurium* or anti-CD40 and have been related to the activity of IL-17, GM-CSF and/or IFN-γ elicited by stimulation with IL-23 or IL-12 (refs. 13,20,67,74,116). In contrast, IL-22 protects mice from intestinal inflammation elicited by *C. rodentium* infection, DSS administration or the transfer of T cells[41,67,70,101]. A definitive role for ILCs in human inflammatory bowel disease has not been established, but several studies have reported altered number or function of ILCs in Crohn's disease. While greater IL-17 production by ILC3s from patients with Crohn's disease than by those from subjects without IBD has been reported[114], decreased numbers of ILC3s and accumulation of ILC1s have also been reported, some of which might be derived from ILC3s[21,117]. The expression of MHC class II on ILC3s was lower in a cohort of pediatric patients with Crohn's disease than in control subjects without inflammatory bowel disease. Interestingly, reduced expression of MHC class II is correlated with increased numbers of T_H17 cells, which would suggest that ILC3s might limit pathogenic T cells via MHC class II in Crohn's disease[30].

Despite the importance of ILC2s in many models of lung inflammation, airway hyper-reactivity induced by a high-fat diet is regulated by ILC3s. Studies of *Il17*$^{-/-}$ mice or *Rag*$^{-/-}$ mice depleted of ILCs by treatment with anti-CD90 have demonstrated that ILC3-derived IL-17 regulates airway hyper-reactivity stimulated by IL-1β dependent on the Nlrp3 inflammasome[43]. ILC3s might also be involved in the pathogenesis of skin inflammation, because skin lesions of patients with psoriasis vulgaris show enrichment for these cells. Data obtained with a mouse model of psoriasis induced by the imiquimod Aldara have confirmed that ILC3s contribute to the disease phenotype, but γδ T cells seem to be the main source of IL-22 in this model[118–120]. Given that the Aldara mouse model, like many models, recapitulates only certain aspects of the human disease, more detailed analysis of ILC3s in psoriasis is needed to establish a role for ILC3s. The importance of ILCs in chronic inflammation provides a potential basis for therapeutic intervention, as discussed in a related Commentary by Eberl and colleagues in this issue of *Nature Immunology*[121].

Organogenesis and maintenance of tissue integrity

The localization of ILC2s at epithelial surfaces and the fact that they react to alarmins released by damaged or dying epithelial cells suggests close interaction of these two cell types. Indeed, ILC2s express amphiregulin, which regulates cell proliferation or differentiation by binding to the epidermal-growth-factor receptor expressed by various cell types. Tissue repair of the airways after infection with influenza virus or repair of the intestinal epithelium after DSS-induced damage is mediated by amphiregulin secreted by IL-33-stimulated ILC2s[11,122]. ILC2s also promote wound repair in the skin through an as-yet-undefined mechanism[123]. Detrimental effects of ILC2-dependent tissue remodeling have been reported in the context of liver fibrosis after chemical injury, and IL-33-stimulated ILC2s secrete IL-13, which induces fibrosis mediated by liver stellate cells[124]; this indicates that in certain disease states, ILC2s might be a target for limiting excessive tissue-remodeling responses.

LTi cells, now identified as a constituent of the CCR6$^+$ ILC3 subset, were originally described in the 1990s as hematopoietic CD45$^+$CD3$^-$CD4$^+$ lymphocytes that are essential for lymphoid-tissue formation[16]. Mice lacking LTi cells due to deficiency in key transcription factors such as Id2 or RORγt do not develop lymph nodes, Peyer's patches or cryptopatches[15,125]. LTi cells are recruited into clusters as early as embryonic day 12.5, attracted by the chemokine CXCL13 from non-hematopoietic mesenchymal lymphoid organizer cells. When stimulated by the TNF-family cytokine TRANCE, IL-7 and retinoic acid, LTi cells express LTα1β2, which engages LTβR on mesenchymal cells. This interaction triggers release of the chemokines CXCL13, CCL19 and CCL21, which attract T cells and B cells, and increased expression of the adhesion molecules VCAM-1, ICAM-1 and MadCAM-1, which results in the formation of lymph nodes[78].

In adult mice, one striking difference between CCR6$^-$ ILC3s and CCR6$^+$ ILC3s is that the latter reside mainly in cryptopatches and are less proliferative[13,15]. CCR6$^+$ ILC3s are reported to be partially radio-resistant[126,127]. After damage to the epithelium elicited by irradiation, graft-versus-host disease or treatment with methotrexate, ILC3-derived IL-22 mediates the regeneration of epithelial cells in the intestine or thymus by directly acting on intestinal stem cells or thymic epithelial cells that express the IL-22 receptor subunit IL-22Rα1 (refs. 126–129). At steady state, the intestinal epithelium requires constant IL-22 signaling for the maintenance of barrier integrity and containment of commensal bacteria. IL-22 deficiency results in dissemination of the bacteria *Alcaligenes xylosoxidans*, which resides in lymphoid organs and triggers chronic inflammation[130]. Another study has reported that the pro-inflammatory mediator prostaglandin E_2 is essential for epithelial barrier integrity and prevention of systemic inflammation. Prostaglandin E_2 engages the receptor EP4 on ILC3s and triggers IL-22 production. This might have some implications for the treatment of human disease, because EP4 expression and prostaglandin E_2 synthesis are reduced in patients with systemic inflammation[131]. Of note, ILCs interact with non-immune cells to mediate tissue formation and remodeling. However, a more comprehensive analysis of ILC–parenchymal cell crosstalk will be crucial for better understanding of how ILCs differentially regulate inflammation versus repair in distinct tissues.

ILCs and cancer

Anti-cancer and cancer-promoting effects of ILC3s have been described, whereas a role for ILC2s in cancer biology has been suggested but not formally proven[132,133]. ILC1-like cells have been described in a mouse cancer model[134], as further discussed by Spits

and colleagues in this issue of *Nature Immunology*[1]. Through the use of subcutaneous injection of melanoma cells that express IL-12, it has been demonstrated that IL-12 secretion results in the rejection of melanoma. Experiments with several strains of knockout mice have suggested that ILC3s are required for tumor rejection. Strikingly, intra-tumoral injection of ILC3s inhibits tumor growth and accompanies changes in tumor microvasculature[135]. Interestingly, NCR$^+$ ILC3s are also found in human non-small-cell lung cancer, in which they are thought to promote the formation of protective tertiary lymphoid structures[136].

Despite the beneficial effects of IL-22 in many infection models, it has been reported that in certain circumstances, IL-22 promotes tumor growth, which would indicate that ILC3s might regulate tumor formation. In a model of colitis-associated cancer after DSS administration, it has been shown that IL-22 reduces epithelial damage and inflammation-associated cancer in the acute phase, whereas IL-22 has detrimental effects in the recovery phase. Bioactive IL-22 is tightly regulated by binding to its decoy receptor IL-22-binding protein, which has high expression at steady state or in the recovery phase but not when tissue is damaged, to allow the physiological function of IL-22. The role of IL-22 for tumor development has been further investigated with APC^{min} mice, which express a truncated form of the tumor suppressor APC and therefore develop colorectal cancer. Consequently, in the APC^{min} model, mice deficient in IL-22-binding protein develop more colon tumors, whereas mice deficient in IL-22 have fewer tumors[137]. Studies of a different model of colitis-associated cancer induced by infection with *H. hepaticus* and administration of the carcinogen azoxymethane to *Rag*$^{-/-}$ 129SvEv mice have reported that CCR6$^+$ ILC3s are the main source of IL-22 and that either depletion of ILCs or neutralization of IL-22 substantially reduces the tumor burden[138]. Beyond the bimodal role of IL-22 in the development and progression of cancer, the mechanisms by which ILCs interact with malignant cells remain poorly defined and require further analysis, especially in the context of human tumors.

ILCs and metabolic homeostasis

Low-grade chronic inflammation in white adipose tissue (WAT) induced by obesity increases metabolic risks. Type 2 responses promote energy expenditure, in part through the beiging of WAT. Such beiging is characterized by the appearance of beige adipocytes that can uncouple the electrochemical gradient in mitochondria from ATP synthesis by expression of the uncoupling protein UCP-1. Type 2 responses are associated with the recruitment of alternatively activated macrophages, which secrete epinephrine and catecholamines to regulate energy expenditure by adipocytes. IL-4 secretion by eosinophils and NKT cells, as well as IL-13, promotes the recruitment of alternatively activated macrophages. As eosinophils require IL-5 for proliferation, activation and migration to WAT, IL-5-secreting ILC2s seem to be important regulators of energy expenditure[139–141]. Interestingly, WAT shows enrichment for ILC2s, but ILC2s are decreased in obesity or in mice fed a high-fat diet. Gain- and loss-of-function experiments have confirmed the importance of IL-33- or IL-25-stimulated ILC2s in WAT homeostasis[75,142–144]. Two different mechanisms for this have been proposed. First, secretion of IL-5 and IL-13 by ILC2s results in the secretion of IL-4 by eosinophils. IL-4 directly controls the fate of PDGFRα$^+$ adipocyte precursors that express the IL-4 receptor and promotes the differentiation of beige adipocytes[144]. Second, the processing of proenkephalin A to methionine-enkephalin is dependent on the prohormone convertase PC1, which induces the upregulation of UCP-1 and beiging of WAT[75]. Whether ILCs other than ILC2s are involved in the regulation of metabolism is not clear at present. However, a role for IL-22 in metabolic homeostasis has been shown, which suggests a potential role for ILC3s in this process[145]. While a role for ILC2s in metabolic homeostasis in mice has been established, the mechanism by which ILC2s interact with adipocytes needs more definition. In addition, specific deletion of ILC2s would be needed to demonstrate a non-redundant function for these cells in metabolic homeostasis. Finally, the importance of ILC2s in human metabolic syndromes requires more investigation, although ILC2s are known to be present in the adipose tissue of humans and are decreased in abundance in obese people.

Summary and future perspectives

The identification of various ILC subsets and further insight into their tissue distribution have highlighted important functions for ILCs in triggering immunity, inflammation and tissue repair. ILC1s, ILC2s and ILC3s have multiple interactions with the microbiota, nutrients, metabolites, neurons and parenchymal cells. The molecular mechanisms underlying these interactions and their consequences for tissue homeostasis remain poorly defined. Future efforts to elucidate these processes will lead to a more integrated view of how ILCs regulate immunological and other physiological responses.

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The authors declare no competing financial interests.

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