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Origins and Evolution of Innate Lymphoid Cells: Wardens of Barrier Immunity

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Summary

The identification, in the late 2000's, of innate lymphoid cells (ILCs) as a new class of non-B, non-T lymphocytes has led to global efforts to understand their functions, plasticity, and evolutionary origins and to define their place within the leukocyte family. Although this work has uncovered striking similarities in the developmental cues, lineage-specific transcription factors and functional capacities of innate and adaptive lymphocytes, it has become clear that ILCs play a unique and defining role as stewards of barrier defence and that this sets them apart from their adaptive cousins. This review will explore how the dynamic environment of barrier surfaces has shaped ILC evolution and functionality. We highlight the critical importance of the microbiome and the unique role of ILCs as environmental sensors. We reflect on how these factors may have influenced the development of ILC2s and barrier immunity in the context of exposure to helminth parasites that have been driving forces of our evolution throughout human history. Finally, we argue that the plasticity of ILC function reflects their role as first-responders to environmental change.

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1. Introduction

The groundbreaking discovery of lymphoid and myeloid cells as distinct leukocyte subsets with complementary functions has formed the basis for introductory immunology lectures for first year undergraduate students for over a century. The pleasingly simple notion of myeloid cells with innate functions supported by lymphoid cells providing immune memory was complicated by the discovery first of Natural Killer (NK) cells, lymphoid cells capable of cytotoxic effector function without prior immunization (1), and later of lymphoid tissue inducer (LTi) cells that play key roles in the development of the architecture of the lymphatics (2). More recently, the discovery of three independent, but related, classes of non-B, non-T lymphocytes lacking antigen specificity and with innate functions (3) has led to a new appreciation for the wide-ranging roles played by the various lymphocyte subsets in diverse processes including defence against bacterial (4), viral (5) and parasitic infection (6), maintenance of barrier function (7), wound healing (8), repair (5), maintenance of metabolic homeostasis (9) and environmental sensing (7). The discovery of the innate lymphoid cell (ILC) 1, 2, and 3 subsets has led to questions about the relationship of each class to one another and to the other innate and adaptive lymphocytes and has sparked interest in the evolutionary factors shaping the development of the lymphoid lineage.

1.1. ILC subsets and functions

ILCs can be found in lymphoid and non-lymphoid tissue but are particularly abundant at mucosal and non-mucosal barriers (10, 11). From here they act as central coordinators for immune responses, integrating signals from the milieu, relayed via the epithelium, resident immune cells, the microbiome, pathogens or by sensing of the local availability of nutrients. ILC1, expressing the T-box transcription factor T-bet, produce IFN-γ and contribute to defence against bacterial and protozoan infection (12-14). ILC2, expressing GATA-binding protein 3 (GATA-3) (15) and RAR-related orphan receptor alpha (ROR-α) (16), produce the canonical type 2 cytokine IL-4, IL-5, IL-9 and IL-13 and are instrumental in responses to helminth infection (6, 17, 18), as well as coordinating tissue repair(5) and maintaining metabolic homeostasis (9). ILC3, expressing ROR-γt, produce IL-17A and IL-22(19, 20) and contribute to both antibacterial immunity (21) and tissue homeostasis (22).

This review aims to put ILCs in the context of the ever-changing environment of barrier surfaces where dietary signals, microbiome interactions and pathogen exposure shape immune responses. A major focus will be ILC2 cells and an exploration of how their anti-

helminthic functions overlap with their roles as environmental sensors, but where useful we will draw on studies of other ILC subsets.

1.2. ILC2 and helminth infection

ILC2s had initially been referred to as a non-B non-T-cell (NBNT) that relied upon IL-25 for survival and activation (23). Deletion of IL-25 or its receptor IL-25R (IL-17E-R) resulted in an absence of these cells and thus loss of protective responses to infection with *Nippostrongylus brasilensis.* Thereafter, a series of papers published in 2010 described IL-13-producing innate lymphoid cells in the mouse (6, 17, 18). The papers characterized the cells in different biological systems and the three identified populations were initially given different names, although all have since been grouped in the ILC2 family (3). ILC2 produce type 2 cytokines in response to local cues, including the alarmin cytokines IL-25 and IL-33 (6). The role of ILC2 in defence against helminth infection is multifaceted and mediated through production of type 2 cytokines and also by direct interactions with T cells. Via production of IL-5 and IL-13, ILC2 contribute to key anti-helminthic functions including eosinophilia (24) and goblet cell hyperplasia (6, 17). The parasite clearance defect in *Nippostrongylus brasiliensis*-infected mice lacking IL-25 and IL-33 receptors can be reversed by adoptive transfer of IL-13-producing ILC2 (6). Importantly, IL-25 administration alone does not induce worm expulsion in T- and B-cell deficient *rag2*$^{-/-}\gamma c^{-/-}$ mice in the absence of ILC2s (18), identifying ILC2 as the key IL-25-responsive cell in *N. brasiliensis* infection.

ILC2 play a further role in helminth infection via interaction with Th2 cells (25). MHCII-expressing ILC2s interact with antigen-specific T cells that in turn produce IL-2 to encourage ILC2 proliferation and type 2 cytokine production. Deletion of ILC2 MHCII attenuates the ability of ILC2 to stimulate *N. brasiliensis* expulsion, highlighting the importance of ILC2-T cell crosstalk.

1.3. ILCs at barrier surfaces

The relative enrichment of ILCs at barrier sites such as the lung and intestines reflects their key roles as modulators of the host-environment interface, as ‘first responders’ in the event of infection and as supporters of dendritic cells in the development of adaptive immune responses . The following sections will explore how ILC function at barrier surfaces may have evolved to serve these purposes.

1.4. ILCs and the microbiome

The last decade has seen an explosion in research into the role of the human microbiome in health and disease. The respiratory and intestinal tracts, and the skin are home to complex microbial communities and are also major sites of ILC activity, and it has become apparent that sensing changes in these microbial communities is a key function of ILCs – perhaps to prepare the host environment in advance of infection. Much of the attention is this area has been given to ILC3 and this work has led to some important emerging concepts. New evidence suggests that analogous processes may shape ILC2 responses at the interface with microbial populations.

The ILC3-derived cytokines IL-17A and IL-22 have potent tissue protective effects via coordination of defence against extracellular bacteria (26) and promotion of tissue repair (27). By contrast, excessive or uncontrolled production of these cytokines by ILC3 has been linked with inflammatory diseases of the bowel (19) and skin (28), and ILC3 GM-CSF production has been associated with acute intestinal inflammation (29). ILC3-derived IL-17A sustains inflammation in models of innate inflammatory bowel disease (19, 30) and IL-17A-producing ILC3s accumulate in the inflamed gut of Crohn's disease patients (19, 31). The regulation of ILC3 functionality at the barrier surface is therefore key to health.

The microbiome, and microbial products, play a major role in the regulation of intestinal IL-22 production (32) and, in turn, IL-22 supports intestinal barrier integrity, preventing dissemination of commensal flora (33). IL-22-deficient mice are highly susceptible to disease caused by the attaching and effacing bacterial pathogen *Citrobacter rodentium* and infection is associated with a loss of colonic epithelial cell integrity and results in systemic bacterial spread (33). Similarly, commensal *Alcaligenes* species residing in lymphoid tissues are restricted to this niche by LTi-derived IL-22, thus preventing systemic inflammation (32, 34). IL-22-producing T helper cells (Th22) play an important role in late-stage *C. rodentium* infection but it is not clear whether, as the predominant intestinal IL-22-producers, ILC3s are also involved. Indeed, recent findings suggest that, in the presence of T cells, IL-22-producing NKp46+ ILC3 are dispensable for control of *C. rodentium* infection but are required for protection of the cecum (22).

Further intimate links between microbiome and ILCs are highlighted by the finding that ILC3 expand and produce IL-22 in response to alterations in the microbiota following dextran sodium sulfate administration, and this response is concomitant with a return to normal barrier function (35). A recent study by Gury-Ben Ari and colleagues reveals the complexity of microbiome-induced regulation of ILC functions (36). RNA-seq and ChIP-seq analysis of

ILC subsets from Germ-free and antibiotic-treated mice revealed altered profiles for hundreds of transcripts and a markedly altered chromatin landscape relative to mice with undisturbed intestinal microbial communities. ILC1 and ILC2 were notably more affected by these microbial changes than ILC3 and adopted a transcriptional signature similar to that of ILC3. This work builds on the previous insights of Sawa *et al.* regarding ILC3 expansion following microbiome depletion (35), and suggests that a steady state microbiome may inhibit ILC3-associated transcriptional signatures in intestinal ILC populations. Upon microbiome depletion, release of this inhibition allows ILC3 to expand and maintain barrier function. The alterations in the chromatin landscape in antibiotic-treated mice described by Gury-Ben Ari *et al.* took place over just a few weeks, demonstrating that ILCs can read the state of microbial colonization and rapidly adjust their activity accordingly, via changes in the enhancer landscape and transcription-factor binding site accessibility. These epigenetic changes are in line with the emerging concept of learned immunity that is being recognized as critical in determining specificity in innate immune responses (37).

A groundbreaking recent paper has described how helminth infection can alter the composition of the intestinal microbiome (38). Mice deficient in the Crohn's disease susceptibility gene *Nod2* develop abnormalities in the small intestine that are driven by *Bacteroides vulgatus*, a constituent of the gut microbiota (39). Mice infected with the roundworm *Trichuris muris* are resistant to *Bacteroides* colonisation and accordingly do not develop intestinal abnormalities (38). *T. muris*-induced changes in intestinal microbiota are transitory and reverse in the weeks following clearance of infection (40). Crucially, administration of recombinant IL-13 or IL-4 to *Nod2*$^{-/-}$ mice reproduced the effect of helminth infection (38), demonstrating that colonisation resistance is the result of induction of type 2 immunity. The contribution of ILC2s to colonisation resistance has not been assessed, but as a major source of intestinal type 2 cytokine they have potential to play a key role. Indeed, the ability of IL-13 and IL-22 – key ILC2 and ILC3 cytokines respectively - to drive goblet cell hyperproliferation and mucus production during helminth infection (41, 42) is likely to influence bacterial colonisation of the intestine (43). Furthermore, Zaiss and colleagues have demonstrated that the ability of helminth infection to modulate allergic asthma is dependent upon changes in the intestinal microbiome and, particularly an increase in Clostridiales species that produce short chain fatty acids (44). Given the importance of fatty acid metabolism to ILC2 function (45) (discussed in section 1.6. below) this may point to an intriguing interplay between helminths, ILC2s and the microbiome, with ILC2 finely tuning their function to the linked changes in helminth infection status and composition of the microbiome.

ILC3 possess an analogous mechanism to sense and control the microbial environment via IL-22 production linked to lipid antigen engagement of surface-expressed CD1d (46). Antibody-mediated cross-linking of CD1d on ILC3 is sufficient to induce an IL-22 response, but engagement of CD1d also acts synergistically with IL-23 to further enhance IL-22 production. IL-22 plays a central role in epithelial barrier function and tissue repair and thus, ILC3 integration of signals obtained through CD1d, along with those received from cytokines in the local inflammatory milieu, may contribute to maintenance of homeostasis and to regulation of immune responses. Environmental sensing via recognition of microbial-derived lipids through CD1d likely contributes to maintenance of barrier function by ILC3. The influence of lipids and lipid metabolism on the function of both ILC2 (45) and ILC3 (46) suggests that environmental sensing is a core and conserved function of ILCs lineages.

It is clear, therefore, that a major role for ILCs in the intestine is the maintenance of barrier homeostasis. This is maintained both by microbe-mediated changes in ILC effector function and by ILC-driven changes in the composition of the microbiota. Maintaining the vast and complex microbial communities within the intestine requires a delicate balance between promotion and inhibition of immune responses directed against the microbiota. This is evidenced by the existence of functionally discrete ILC3 subsets, including those that present antigen and inhibit microbiota-directed T cell immunity (47) and those that promote anti-microbiota T cell responses via IL-22 production (48).

1.5. ILC2 and nutrient sensing

Just as ILC2 and ILC3 acquire signals from the microbiota that help shape their function, so ILC2 utilise nutritional sensing to adapt to changes at barrier surfaces. ILC2s are particularly adept at responding to dietary changes, tailoring immune responses accordingly. This nutritional sensing is intimately intertwined with the key role of ILC2 in defence against helminth infection.

Parasitic infections, particularly gastrointestinal nematodes, can have a significant impact on host nutrition and contribute to macro- and micronutrient deficiencies. Malnutrition is the predominant cause of immunosuppression worldwide (49), and is particularly common in areas where soil-transmitted helminth infection is endemic (50, 51). More than three quarters of the countries with moderate or severe vitamin A deficiency in children are also considered by the World Health Organisation to be at risk of soil-transmitted helminth infection and in need of periodic preventive chemotherapy (52); this represents some 250

million children. It is notable, therefore, that a recent cross-sectional study of pre-school and school-age children in urban slums in Nairobi, Kenya found significant associations between soil-transmitted helminth infection in pre-school children and both vitamin A and iron deficiencies (53). However, identifying a cause and effect relationship between infection and macro- and micronutrient deficiencies has not been straightforward. Interventional studies, including randomized-controlled trials utilizing vitamin A supplementation in combination with deworming, have failed to show significant benefits on serum retinol levels (54, 55). Meta-analyses have further exposed the complexity of the interaction between infection and malnutrition. One review encompassing experimental and observational studies of soil-transmitted helminths, schistosomiasis and a number of micronutrient measures identified no effect of antihelminthic treatment on vitamin A status but did describe a negative association between helminth infection and serum retinol (56).

Mechanistic understanding of the relationship between infection and nutrient deficiencies is also lacking, but it is likely that reduced appetite (57), impaired nutrient absorption following helminth-induced tissue damage (58) and increased nutrient loss all play a role (59). With regards to vitamin A deficiencies, helminths including *Ascaris lumbricoides* express retinol and retinoic acid-binding and degrading proteins that they utilize for their growth and development (60). Retinoic acid is highly enriched in the gastrointestinal tract and is key to a functioning adaptive immune system (61-63). T helper cell subsets are notably reduced in the gastrointestinal tract of vitamin A deficient mice (62, 64).

ILC play a key role in sensing such infection-induced changes in host nutritional status or metabolism and tailoring immune responses accordingly. Spencer and colleagues have described the relative enrichment of intestinal ILC2s in vitamin-A deficient mice and the corresponding reduction in ILC3 populations (7). ILC3 and ILC3-derived IL-22 and IL-17A were substantially reduced in vitamin A deficient mice, alongside increases in ILC2 and ILC2-derived IL-4, IL-5 and IL-13. This effect could be reproduced by inhibition of retinoic acid signaling in wild type animals and reversed by retinoic acid treatment of vitamin A deficient mice. These processes occurred independently of commensal bacterial flora, suggesting a different mechanism of environmental sensing to that utilized by ILC3 to respond to changes in intestinal flora.

Retinoic acid has a cell-intrinsic suppressive role on ILC2 maturation (7). Addition of retinoic acid impairs ILC2 development from ILC2 common progenitors in culture and inhibition of retinoic acid signaling increases ILC2 development and cytokine production. Common lymphoid progenitors transferred to ILC-deficient mice give rise to both ILC2 and ILC3 in the intestine but the balance can be switched towards dominant accumulation of ILC3 or ILC2 by

promotion or inhibition of retinoic acid signaling respectively. The ILC2-ILC3 balance is of functional significance for intestinal health, as the relative impairment of ILC3 in vitamin A-deficient mice or those treated with retinoic acid blockers was associated with increased susceptibility to infection with *Citrobacter rodentium*.

Although retinoic acid appears unable to convert mature ILC2s into ILC3s, it does encourage IL-22 production from ILC3 and inhibit ILC2 responses, in part by reducing IL-7Rα expression on ILC2s and their progenitors. Inhibition of retinoic acid reverses these effects and encourages increased IL-7 responsiveness in ILC2. Thus, in conditions of vitamin A scarcity, innate type 2 immunity is enhanced at the intestinal barrier. Heightened intestinal mucus production is evident in mice following vitamin A withdrawal (64) and vitamin A-deficient mice undergo goblet cell hyperplasia and increased RELM-β production, driven by ILC2-derived IL-13. Retinoic acid inhibition in mice infected with *Trichuris muris* did not compromise control of infection, as the defective T_H2 response was offset by increased IL-13-producing ILC2s (7).

Thus, vitamin A deficiency is associated both with resistance to nematode infection and increased susceptibility to bacterial infection. Spencer and colleagues argue that this switch in immune priorities may provide a means of maintaining barrier immunity in the face of dietary challenge. Vitamin A acts as a warning signal, allowing immunological adaptation to compensate for the impairment of adaptive immunity that is associated with retinoic acid scarcity. By boosting innate type 2 immunity, barrier defence is maintained via increased mucus production, tissue repair and promotion of immunomodulatory responses (65). We may speculate that ILC2-driven defenses against helminths evolved in tandem with human development in resource poor settings; where the problems of chronic malnutrition and endemic helminth infection overlap.

1.6. ILC2 and fatty acid metabolism

The Belkaid lab have followed up their findings with a description of the metabolic changes within ILC2 that underpin their functional enhancement in vitamin A deficiency. Building on previous work identifying the expression of genes associated with fatty acid metabolism as a key feature of ILC2s (66), Wilhelm *et al.* demonstrate that ILCs constitutively acquire fatty acids at barrier sites (45). This process is not essential for ILC maintenance, as blockade of fatty acid oxidation does not alter steady-state ILC numbers. However, blockade of fatty acid oxidation in the context of *T. muris* infection results in reduced ILC2 accumulation and

reduced infection-related increases in IL-5 and IL-13. A similar reliance on fatty acid oxidation for immune cell function during helminth expulsion has previously been demonstrated for alternatively activated macrophages (67, 68).

Mobilisation of fatty acids for use as an energy source is a physiological safety net in times of dietary restriction when glucose is scarce (69). It appears that ILCs, particularly ILC2, also show an increased reliance on fatty acid oxidation in periods of nutritional shortage (45). The increased ILC2 functionality in vitamin A deficient or retinoic acid inhibitor treated mice is mediated through increased fatty acid acquisition, and reduced retinoic acid signaling induces transcriptional changes in ILC2 that reflect an increased reliance on fatty acid oxidation. Intriguingly, blockade of fatty acid oxidation in vitamin A deficient mice reverses the usual enhancement of ILC2 IL-13 production afforded by reduced retinoic acid signaling but ILC2 IL-5 and IL-9 production is unaffected (45). This finding lends further credence to the idea that ILC2 have evolved to selectively maintain IL-13 levels during times of dietary stress due to its unique barrier-protective properties.

1.7. The role of brown adipose tissue in ILC2 function

Brown adipose tissue (BAT) utilises energy stores and is responsible for the physiological response to cold (70), driving the use of fat stores in times of poor resources. In 2011, it was shown that IL-4-producing eosinophils were present in the fat tissues of normal mice, and those fed high fat diets harbored lower numbers of these cells (71). Moreover, transgenic mice overexpressing IL-5 had higher number of eosinophils infiltrating into the adipose tissue and the percentage of eosinophils was found to negatively correlate with the total body weight. The presence of these cells was directly responsible for the generation of alternatively activated macrophages within BAT. Macrophage recruitment was not altered in the absence of eosinophils but their activation was changed in animals lacking IL-4/IL-13. An extension of this work demonstrated that the presence of ILC2 cells was necessary for the initial production of IL-5 required to promote eosinophils (24). Moreover, the presence and function of ILC2 cells was responsive to caloric input and hormonal cues, e.g. leptin. The ILC2 population within adipose tissue was found to be responsive to IL-33 injection and their effects on adipose tissue were recapitulated upon experimental infection with *N. brasiliensis*. These findings suggest that BAT could be promoted and sustained via helminth infection, offering a plausible, albeit untested, mechanism for the low rates of metabolic syndromes in countries with high rates of helminth infection. Evidence underlying this can be gleaned from two earlier studies by McDermott et al and Worthington et al (72, 73). Experimental infection

with *Trichinella spiralis* was shown to increase the levels of cholecystokinin - a regulatory peptide decreasing food intake - at the peak of intestinal inflammation. Neutralisation of CD4 T-cells resulted in food intake levels equivalent to pre-infection and a non-resolving parasite burden (72). Further work with *T. spiralis* has shown there to be a bi-phasic decrease in food intake coincident with the development of the adaptive immune response. Transfer of CD4 T-cells was sufficient to restore the food intake changes in T- and B-cell deficient *RAG*$^{-/-}$ infected mice. The second phase of hypophagia was dependent upon TNF signaling causing a decrease in leptin. Artificial maintenance of pre-infection leptin levels resulted in delayed worm expulsion (73). Collectively, these studies demonstrate the intimate link between the resolution of parasite infection and the pre-existence or generation of a nutrient poor intestinal environment. Key to this link is the ability of ILC2 to orchestrate anti-helminth immunity via tuning their responsiveness to their nutritional setting.

1.8. Tuft cells and ILC2

A further link between environmental sensing and ILC2 responses was recently put forward by three independent studies demonstrating the role of taste-chemosensory tuft cells (74) in driving the IL-25 dependent expansion of ILC2 cells in the intestine (75-77). von Moltke *et al* demonstrated that tuft cells in the intestinal epithelium constitutively express IL-25, thus sustaining ILC2s, and that *N. brasiliensis* infection drives tuft cell expansion and leads to a consequent rise in the number of ILC2s (77). Gerbe and colleagues further demonstrated that *pou2f3*$^{-/-}$ mice, lacking the Pou domain class 2 transcription factor 3, were deficient in tuft cells and thus lacked the IL-25 levels within the intestine required to maintain ILC2 numbers. However, rIL-25 was sufficient to restore this defect, leading to worm clearance (75). A positive feedback loop whereby tuft cell derived IL-25 drives ILC2s and ILC2 derived IL-13 drives tuft cell expansion ensures amplification of type 2 immunity during helminth infection (76). This response is reliant on signaling through the chemosensory receptor TRPM5 as *trpm5*$^{-/-}$ mice fail to expand tuft cells during *Heligmosomoides polygyrus* infection, do not increase tuft cell IL-25 production or ILC2 numbers and harbor higher parasite burdens than wild type controls (76). The question then arises; if tuft cells can sense invading helminths can they detect the metabolic or environmental cues that also give rise to ILC2? At present, it is not clear whether tuft cells sense the local microbial environment of merely integrate signals from the mucosal immune system.

1.9. ILCs and dendritic cells

In addition to providing a first line of defence against infection, innate immunity shapes the ensuing adaptive response via antigen presentation, costimulation and creation of an inflammatory milieu favouring differentiation of particular T helper cell subsets. The role of ILCs in directing adaptive immunity is best described in the case of allergic airway inflammation, where it has been demonstrated that ILC2s are critical for robust T helper (Th) 2 responses to the protease-allergen papain (78). Whilst IL-4 is dispensable for generation of papain-induced Th2 responses, ILC2-derived IL-13 is required to drive migration of lung dendritic cells to the draining lymph nodes where Th2 differentiation results. More recently, an analogous role for ILC2 in directing Th2 memory responses in the lung has been described, whereby ILC2-derived IL-13 was shown to stimulate dendritic cell production of the Th2-recruiting chemokine CCL17 (79). Upon rechallenge of papain-sensitised mice, Th2 cell recruitment to lung was significantly compromised both in ILC2-deficient mice and in those lacking expression of IL-13Rα1 expression on dendritic cells. The role of ILCs in coordination of adaptive immunity at the intestinal barrier is less well described, but it is notable that administration of IL-33 or IL-13 leads to an increase in intestinal $CCL17^{+}$ dendritic cells and that the IL-33 effect is abrogated in ILC2-deficient mice (79). Thus, the function of sentinel dendritic cells at barrier sites appears to be dependent upon ILC activity, providing a further example of the unique role played by ILCs in sensing environmental change and responding rapidly to shape local immune responses.

2. Conclusions and future directions

Collectively, the recent advances in our understanding of ILC2 biology at barrier surfaces paint a complex picture of integration of signals coming from pathogens, the microbiome, the epithelium and the local availability of nutrients (Figure 1). The evolution of our immune system has taken place in the context of repeated and sustained exposure to bacteria, viruses and parasites. Strategies have developed to distinguish pathogens from commensals and to limit tissue pathology during infection. In both cases, the barrier surfaces have been key battlegrounds, as they represent sites rich in commensal organisms and where tissue damage can quickly lead to systemic infection. Furthermore, the barrier sites of the skin, and gastrointestinal and respiratory tracts are major interfaces with the environment, and thus offer the opportunity for immune surveillance of a rapidly changing resource pool. Accordingly, immune cells such as ILCs that operate at barrier surfaces have evolved tools to adjust their function in response to fluctuations in their niche. ILC3 sense

changes in the composition of the microbiota that promote or inhibit their effector functions, while ILC2 are uniquely sensitive to changes in nutritional status. This ability may have evolved to enable compensatory immune function in conditions where competition between parasite and host leads to local nutritional deprivation. The capacity to boost ILC2 numbers and their activity in times of nutrient scarcity may provide three benefits, firstly as a compensatory mechanism for the impairment of adaptive immunity that occurs in low vitamin A settings (62), secondly to reinforce immunity against helminth parasites that compete with their host for nutrients, and finally to boost colonization resistance and prevent enteric bacterial infection. Entwined with this is the need to minimize the impact of damage caused by the migration of large metazoan parasites through host tissues. In this regard, it is notable that the boost to ILC2 responses in reduced vitamin A conditions is offset by a reduction in the numbers and effectiveness of ILC3s (7). As key producers of the inflammatory cytokines IL-17A and IL-22, their inhibition may reflect a prioritizing of tissue repair and wound healing responses. The promotion of these processes without excessive compromise of defence against infection requires intimate communication between the inflammatory and tolerogenic arms of the immune system (65). Indeed, the need to maintain a balance between immune defence and immune tolerance has been a driving force in the evolution of both helminths and their human hosts (80-83). As the field progresses, several key questions remain to be addressed. Mechanistically, do ILC2 integrate both helminth and nutritional cues simultaneously, and structurally are there any similarities in the molecules that act as these cues? A key translational question, requiring careful examination, is can these interactions be manipulated effectively to either boost resistance or enhance current chemotherapeutic efforts at parasite control? The intimate links between diet and immunity offer potential for affordable, non-invasive therapies designed to tune our immune systems to particular challenges in a variety of environments. This is an exciting emerging field of research, and consideration will need to be given to the role of ILC in any such intervention therapies.

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