Published in final edited form as:

Trends Immunol. 2018 February ; 39(2): 99–111. doi:10.1016/j.it.2017.10.004.

Sentinels of the type 2 immune response

Jakob von Moltke¹ and Marion Pepper¹

¹Department of Immunology, University of Washington School of Medicine, 750 Republican Street, Seattle, WA 98109, USA.

Abstract

Type 2 immune responses have evolved to sense and respond to large, non-replicating infections or non-microbial noxious compounds in the tissues. The development of these responses therefore depends upon highly coordinated, tightly-regulated tissue-residing cellular sensors and responders. Multiple exposures to Th2-inducing stimuli further enhances both the diversity and potency of the response. This review discusses advances in our understanding of the interacting cellular subsets that comprise both a primary and secondary type 2 response. Current knowledge regarding type 2 immune responses in the lung are initially presented and then contrasted with what is known about the small intestine. The studies described portray an immune response that depends upon well-organized tissue structures and suggest their modulation as a therapeutic strategy.

Specificities of type 2 responses

The requirements and objectives of type 1 and type 2 immunity differ greatly. While type 1 immune responses target small, invasive, and rapidly replicating microbial pathogens, type 2 immunity is triggered by macroscopic parasites and non-microbial noxious stimuli. Of note, while individual immune cells can target and degrade type 1 pathogens and infected cells, type 2 immune responses engage the entire tissue. For example, helminth expulsion requires a ‘weep and sweep’ response, that involves immune cells, mucus secretion by the epithelium, and increased contractility generated by the underlying smooth muscle. Type 2 cytokine secreting ILCs and Th2 cells in the tissues (described in this review) drive the recruitment and activation of numerous cell types including eosinophils, mast cells and basophils that secrete type 2 effector molecules. Accordingly, tissue-derived signals and tissue resident cells are of primary importance throughout type 2 immunity.

In this review we describe tissue sentinels of the type 2 immune response and consider the paradigms governing their activation. Using the lung as a model, we highlight the sentinel functions of non-hematopoietic cells (with a particular focus on epithelium, neurons and smooth muscle), tissue resident memory T cells, and B cells, and discuss how their relative contributions evolve with exposure. We begin with a discussion of group 2 innate lymphoid

*Correspondence: mpepper@uw.edu (M. Pepper).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

cells (ILC2s) as a hub for integration of tissue-derived innate type 2 signals. Next we discuss the multi-step nature of type 2 CD4 helper T cell (Th2) priming and activation, highlighting how Th2 cells adopt ILC2-like features as they differentiate and how tissue-derived signals are again critical for licensing the type 2 response. Once acute inflammation has resolved, tissue resident CD4 memory cells (Trm), B cells, and IgE-loaded mast cells become the dominant type 2 sentinels. In particular, we describe the recent identification of type 2 Trms and their dominant contribution to lung pathology on re-exposure. Long-term inflammation also drives emergence of tertiary lymphoid tissue that can support tissue resident adaptive cells and perhaps even lead to lymph node-independent T cell priming, thereby establishing a truly tissue-intrinsic type 2 immune response. Finally, we compare and contrast our relatively advanced understanding of type 2 responses in the lung with those in the small intestines during helminth infection or food allergy. Our hypotheses suggest that therapeutics for atopic disease such as asthma or food allergy must target the tissue resident cells and associated microenvironments that initiate and maintain the underlying immune response.

Innate type 2 immune sensing

Determining how the innate immune system senses and distinguishes exogenous (and some endogenous) stimuli is fundamental to our understanding of the immune response and accordingly to our therapeutic interventions. Sparked by the discovery of Toll-like receptors in the 1990s, our understanding of type 1 innate immune sensing of bacteria and viruses is now quite advanced. By contrast, our understanding of type 2 immune sensing remains significantly incomplete, perhaps in part because the lessons learned from type 1 sensing do not always translate to the type 2 context. Compared to bacterial and viral immune ligands, the set of stimuli that trigger a type 2 immune response is in fact even more diverse, ranging from macroscopic live worms to inert microscopic particles (e.g. chitin) to soluble enzymes (e.g. proteases). While there are some examples of classical pattern recognition receptors such as DC-SIGN or TLR4 contributing to type 2 immune responses [1,2], in general the type 1 paradigm of innate immune sensing has failed to explain the initiation of type 2 immunity. As an alternative, it has been widely proposed that type 2 innate immune sentinels respond to disruptions in tissue homeostasis that are common to the diverse set of type 2 stimuli.

ILC2s as a hub for type 2 initiation

The canonical cytokines that drive type 2 inflammation in the tissue are IL-5, IL-9, and IL-13. IL-4 is also a canonical type 2 cytokine and its expression in lymphoid tissue is absolutely critical for class switching to IgE, but its expression is minimal in peripheral tissues during early type 2 responses [3–8]. Th2 cells, eosinophils, mast cells, and basophils can all express type 2 cytokines to varying degrees, but are rare in the lungs of naïve mice [9–12]. Instead, tissue resident group 2 innate lymphoid cells (ILC2s) are the dominant early source of IL-5, IL-9, and IL-13 in the lung [13]. Furthermore, ILC2s are long-lived cells that are seeded to peripheral tissues during development, do not recirculate, and occupy niches in the tissue that poise them to coordinate a rapid response (e.g. near blood vessels and neurons) [14,15]. Understanding how ILC2s are activated is therefore central to uncovering the mechanisms of type 2 sensing.

While the transcriptional landscape of ILC2s is remarkably similar to effector Th2 cells, ILC2s lack a T cell receptor (TCR). There is also little evidence that ILC2s directly sense type 2 agonists via pattern recognition receptors. Instead, ILC2s are activated by numerous locally produced host signals that include cytokines (IL-25, thymic stromal lymphopoietin (TSLP), IL-33, TNF-like ligand 1A (TL1A)), lipids (leukotrienes, prostaglandins), and neuropeptides (vasoactive intestinal peptide, neuromedin U) [13,16–19]. As a general model, we and others propose that ILC2s serve as a sensing hub by integrating signals associated with disruptions in tissue homeostasis that are common to the broad set of type 2 agonists [16,20]. The release of IL-33 as an indication of tissue damage or stretching is currently the best example to support this model [21,22]. What kinds of tissue perturbations (e.g. hypoxia, acidification, metabolic stress, etc.) the other ILC2 activating signals might indicate remains poorly defined. One prediction of the model, however, is that tissue perturbation without an exogenous stimulus would be sufficient to induce type 2 responses, and this appears to be the case. For example, the first breaths taken by neonatal mice are believed to induce a transient IL-33 dependent activation of ILC2s [23], and sterile wounding is also sufficient to induce type 2 inflammation [24,25].

Another important aspect of the model for ILC2 activation is the integration of multiple signals, as was supported by our recent study demonstrating that optimal ILC2 activation in the helminth-infected lung requires both IL-33 and leukotrienes [10]. Integrating multiple signals provides an extra layer of regulatory control and can be explained at a transcriptional level. In adaptive T cells, signaling through the TCR induces the transcription factors NFAT, NFkB, and AP-1, which cooperatively drive cytokine transcription. In ILC2s, the combination of IL-33 (NFkB and AP-1) and leukotrienes (NFAT) seems to broadly replicate this TCR signal, and it is likely that other combinations of ILC2 activators function similarly. The source and regulation of ILC2 activators, however remains an area of active research.

Innate type 2 sentinels in the lung

Many of the canonical type 2 cells, including eosinophils, basophils, mast cells, and Th2s are either rare or completely absent in the naïve lung [9–12], leaving myeloid cells and non-hematopoietic cells as potential type 2 sentinels that activate ILC2s and initiate downstream type 2 immunity. IL-33 expression has been reported in alveolar macrophages [26], but their direct contribution to ILC2 activation remains undefined. Here we focus on the role of non-hematopoietic type 2 sentinels.

Epithelium and other non-hematopoietic cells—The epithelium is usually the site where exogenous agonists are first encountered and as such it is a critical type 2 sentinel. To date, most models and reviews of ILC2 activation depict upstream signals emerging non-specifically from the “epithelium”, but it is becoming clear that this represents an oversimplification. First, the epithelium consists of multiple distinct cellular lineages with varying contributions to the type 2 response. In the lung, for example, IL-33 and TSLP are predominantly expressed in type 2 pneumocytes [27,28], while IL-25 is restricted to tuft cells [29]. Second, the focus on epithelial cells excludes many other non-hematopoietic cells that might secrete ILC2 activators and thereby serve as type 2 sentinels. In particular, airway

smooth muscle and endothelial cells have been reported to express TSLP [30,31], endothelial cells are a predominant source of TL1A [32], and many fibroblasts are IL-33⁺ [33,34]. With so many potential sources for ILC2 activators, future studies will need to identify which are physiologically relevant *in vivo* during type 2 inflammation.

The mechanisms that link type 2 agonist detection in non-hematopoietic cells to induction and release of ILC2 activating signals are not yet fully characterized. TSLP regulation is mostly transcriptional and a broad range of type 2 agonists have been reported to induce TSLP through pattern recognition receptors, protease activated receptors, and others [35]. In general, these signals lead to the activation of NFkB and AP-1, both of which have binding sites in the TSLP locus [36]. Similar to TSLP, the cytokine TL1A is transcriptionally regulated and can be induced by signaling through Fc and pattern recognition receptors [32,37], but its regulation by type 2 agonists has not been well studied. IL-33 expression is generally constitutive, but the cytokine is sequestered in the nucleus of viable cells [22]. IL-33 signaling is therefore regulated predominantly by its release and extracellular proteolytic processing [38,39]. Currently, the only known mechanisms of IL-33 release are necrotic cell death or cell stretching [21,22]. Whether non-hematopoietic cells also synthesize leukotrienes or prostaglandins during type 2 initiation and how this might be regulated remains completely unknown.

Neurons—The interaction of the nervous and immune systems is generally an area of active research, and links to type 2 immunity were first suggested by studies that broadly disrupted sensory neuron function. These sensory neurons can be subdivided based on their expression of membrane channels, which also dictate their ligand specificities. Transient receptor potential channel subfamily A, member 1 (Trpa1), for example, is a promiscuous sensor that can be activated by a large number of noxious chemicals, including some, such as cigarette smoke, that are associated with allergic airway inflammation [40]. Of note, *Trpa1*^{−/−} mice have reduced airway infiltrate and type 2 cytokine production in an ovalbumin (OVA) model of asthma [41]. Similar results were seen when sensory neurons were more broadly ablated (through the targeting of neurons expressing the voltage-gated sodium channel Na_v1.8, associated with nociception, in Na_v1.8-Cre;DTA mice) or pharmacologically inhibited [42]. Notably, ILC2 frequency and IL-13 production were reduced at early timepoints following OVA challenge in Na_v1.8-Cre;DTA mice, suggesting a possible role for sensory neurons in type 2 initiation.

Attention has now turned to uncovering the mechanisms that link neuronal sensing to initiation of type 2 immune responses, with a focus on activation of ILC2s. One study found that ILC2s express VPAC2, a receptor for the neuropeptide VIP, and that stimulation with VIP can induce cytokine production [14]. Of note, at least some TRPA1 neurons express the neuropeptide VIP [42]. More recently, three papers reported the expression of NMUR1, the receptor for the neuropeptide NMU, on both lung and intestinal ILC2s. ILC2s were visualized in close association with NMU⁺ neurons and type 2 immune responses are attenuated in helminth-infected or house dust mite (HDM)-challenged *Nmu*^{−/−} or *Nmur1*^{−/−} mice [17–19]. How neurons sense type 2 stimuli remains to be determined. Intriguingly, activation of sensory neurons by the canonical type 2 cytokines TSLP, IL-4 and IL-33

underlies itch sensations in some models, suggesting a complex interaction of the nervous system with type 2 immunity [43–45].

Beyond the primary response

The innate sensing pathways described above predominate during initiation of primary type 2 immune responses in mice housed under specific pathogen free conditions, in which the immune system mimics that of a human neonate (Fig. 1) [46]. In most cases, including secondary infections and mice or humans that have been exposed to a wide range of exogenous stimuli, the immune context for type 2 responses is far more complex and the relative contributions of type 2 sentinels shift accordingly. In the following sections we focus on the type 2 sentinels that predominate once adaptive immunity has been established (Figure 1).

Adaptive type 2 sentinels in the lung: multi-step Th2 differentiation

The adaptive immune system depends upon tightly regulated cellular interactions that ideally generate tailored, functional responses to eradicate a pathogen without causing pathology. Induction of a primary CD4⁺ T cell response occurs when a small number of cells with the appropriate T cell receptors (TCR) bind peptide:MHC Class II complexes in the presence of co-stimulatory signals and cytokines. Paradigms based on Th1 biology would suggest that these activated CD4⁺ T cells proliferate and differentiate in the lymphoid organs, generating an expanded population of cytokine-producing effector cells that can subsequently migrate to sites of inflammation throughout the body [47–49]. Th2 differentiation follows different rules, perhaps due to the evolution of type 2 responses against predominantly tissue-residing (as opposed to blood-borne) pathogens or mucosal-sensed venoms or proteases.

In both murine models of worm infection and allergy, Th2 effector cell differentiation and cytokine production (IL-4, IL-5 and IL-13) is initiated in lymphoid organs (reviewed in [50]), but Th2 effector cell differentiation takes place after tissue entry [3–6]. The use of various Th2 cytokine reporters, largely generated by Locksley and colleagues, and models in which antigen-specific cells can be identified using TCR transgenic CD4⁺ T cells or tetramers have allowed the visualization of this multi-step potentiation. In these studies, lymphoid residing CXCR5⁺ and CXCR5⁻ T cells activated in a Th2-inducing environment can express high levels of IL-4 transcript (using the 4get reporter strain, that contains an IRES-GFP downstream of IL-4, and enables production of GFP if IL-4 is transcribed), yet only B cell-helping CXCR5⁺ T follicular helper (Tfh) CD4⁺ T cells express IL-4 protein (using the KN2 reporter, where human CD2 protein reports IL-4 translation) [3–8,51,52]. These findings raise interesting questions about studies describing IL-4 producing Th2 cells in the spleen and suggest a reanalysis of these models using more recently defined markers of Tfh cells including CXCR5 and BCL6. Further interrogation of the regulation of IL-4 expression by CXCR5⁺ Tfh cells in the lymphoid organs and CXCR5⁻ Th2 T effector cells in the lungs demonstrated that IL-4 expression by Tfh cells in the lymphoid organs requires the *IL4* enhancer HSV (hypersensitive site V), while IL-4 producing cells in the tissues were less dependent upon this region of the IL-4 locus suggesting different transcriptional regulation of IL-4 production in these two cell types [52]. IL-5 reporter mice have also been

used to show that IL-5 is expressed primarily by a subset of IL-4 transcript positive cells in the lung, again supporting the idea that IL-4 transcription is initiated in the lymphoid organs, but cytokine expression coincides with migration to the tissues and increased GATA3 expression [51]. Intravascular labeling techniques in a murine model of allergic airway inflammation have further defined the localization requirements for Th2 differentiation as only cells found in the lung parenchyma produced IL-4 and IL-13, demonstrating a requirement for tissue entry in this process [6].

Although the cues that drive the terminal differentiation of Th2 cells in the tissues are still being determined, several cytokines including IL-33, TSLP and IL-25 have been implicated in this process [50,51]. Specifically, IL-33 or a combination of IL-2 and IL-25 can induce TCR-independent epigenetic modifications of the IL-5 locus and its subsequent expression in *in vitro* activated Th2 cells in a murine model of airway inflammation. IL-33 can also augment IL-5 production in tissue-derived cells from nasal polyps, although remarkably this is not the case with CD4⁺ PBMCs from the same patients, again highlighting the heterogeneous states of Th2 differentiation throughout the body and the requirement of tissue residency [53]. As described above, one source of Th2 licensing cytokines appears to be the lung epithelial cells, which can produce IL-33 and TSLP in response to chitin or worm infection [27,28,51].

Whether and by what mechanisms ILC2s contribute to priming and/or licensing of Th2 cells remains controversial. Because ILC2s lack a unique marker and are transcriptionally so similar to Th2 cells, a genetic mouse model that selectively ablates ILC2s has been elusive. Several groups have now generated ILC2-deficient models using genetic and technical work-arounds [5,51,54,55], but the results are contradictory. In three studies ILC2 depletion lead to attenuated Th2 activation and recruitment [5,54,55], but another study found no defect in Th2 responses in the absence of ILC2s [51]. It remains unclear what might account for these differences but it is worth noting that one ILC2 deletion strategy relied on a cytokine CRE driver that may not be expressed in all cells in early immune responses [51], while other strategies relied on retinoic acid related-orphan receptor (*Rora*^{sg/sg}) mutants and CRE drivers that may have off-target effects [5,54,55].

How ILC2s might regulate Th2 biology also remains uncertain. Three studies suggest contact-dependent ILC2/Th2 interactions mediated by MHC-II or PD-L1 expression on ILC2s [55–57], but additional studies are needed to determine if and where such interactions occur *in vivo*. Of note, another study found no role for MHC-II expression on ILC2s in Th2 activation [51]. An indirect role for ILC2-derived IL-13 in promoting migration of dendritic cells to the lymph node for Th2 priming has also been reported [5]. Clearly, more work is needed to address the mechanisms that drive the transition from primary to secondary type 2 immune responses, especially once primed Th2 cells arrive in the tissue. Additionally, future studies will need to address if similar cues are required for the maintenance of Th2 memory cells in the tissues and the secondary reactivation of Th2 tissue resident memory cells. Nonetheless, a general theme of tissue-derived (e.g. cytokines) or tissue-localized (e.g. cell-cell interactions) signals required for licensing or enhancement of type 2 immune responses is emerging. As an additional example, two recent papers reported that sensing of defense collagens (e.g. surfactant protein A or complement component C1q) and apoptotic cells is

required in combination with IL-4 signaling to fully induce the tissue reparative properties of macrophages [58,59]. The licensing of type 2 responses by these tissue- or even microenvironment-restricted signals could provide an important layer of regulatory control.

Th2 effector cells become more like ILC2s

The potentiation of Th2 cell function not only occurs in the tissues, but also endows them with properties that are shared with their innate, tissue-residing counterparts. Differentiated Th2 cells and ILC2s share the expression of effector molecules (IL-5 and IL-13), surface molecules (IL-33R, TSLPR, etc...) and not surprisingly transcription factors (such as ROR α) with overlapping modules of genetic regulation [60]. Measures of genome-wide chromatin accessibility and regulatory elements in ILC2s and Th2s demonstrate that while ILC2s have largely accessible effector gene loci, Th2 cells progressively gain regulomes that overlap and converge with ILC2 regulomes [60]. Similar findings were reported in studies in which ATAC-seq was performed on either 4get+ Th2 cells from the lungs, lymphoid cells or ILC2s, which again demonstrated that lung 4get+ cells more closely resemble ILC2s than LN T cells [51]. Intriguingly, a recently identified pathological subset of human Th2 cells (Th2A) are highly mature effector cells that similarly upregulate receptors for IL-33 and IL-25 [61]. This sharing of regulatory circuitry between ILCs and differentiated Th2 cells in the tissues may be a common and effective tactic across all ILC and T helper subsets. Interestingly in studies comparing human ILC and Th subsets in the *lymphoid* organs, differences in ILC and Th regulomes were highlighted, perhaps again supporting the requirement of T cell *tissue* entry to gain this permissive state [62]. As the system gains potency due to both increased diversity of recruited cell subsets (ex. IL-5 recruited eosinophils) and gain of effector functions (both Th2 cells and IgE activated mast cells and basophils), this additional requirement of antigen specificity may be an important regulatory mechanism. Clearly, the antigen specificity of Th2 cells also remains important, and future studies must address the relative contributions of antigen and innate type 2 signals in the maintenance and activation of tissue Th2 cells.

Lung-residing Th2 memory cells

Resolution of infection results in a robust contraction of the effector lymphocyte pool. Although 90% of the effector cells die via apoptosis, a small population of memory cells persists that retains the characteristics of the effector cells that successfully controlled the infection. The development of lymphocyte memory involves a complex program of epigenetic modifications, transcription factor expression, metabolic conversion, and changes in localization. These changes allow a memory response to the same pathogen to be initiated more efficiently, with robust effector molecule expression occurring within hours instead of days [49]. At least three different types of memory T cell subsets have been described. Circulating memory subsets consist of the central and effector memory cells which transit from the blood and lymphatics to lymphoid organs or tissues, respectively, and were originally delineated based on expression of the CCR7 chemokine receptor [63]. A third non-circulating population of memory cells (Trm) has been the focus of intense research over the past decade. In both mice and humans, it has been shown that there are more memory cells residing in the tissues than in circulation [64,65]. Furthermore, both our work

and the work of others has demonstrated that Th2 Trm cells can act as pathological sentinels in response to allergen as described below.

Th2 Trm cells are pathological sentinels

Human lungs are constantly exposed to high levels of antigen from a diverse array of sources including allergens, respiratory pathogens, and non-microbial air pollutants. It is therefore not surprising that resident memory lymphocyte populations increase in number in the human lung with age, and presumably exposure [66]. Importantly, while many of these cells may be protective against respiratory pathogens, others are drivers of pathology such as allergy [67]. Some of the earliest evidence of pathological lung resident Th2 cells come from human studies in which it was observed that asthma can be transferred with lung transplant [68]. While it is difficult to track alterations in human lung resident T cell populations after antigen-exposure, the kinetics of lung resident T cell populations can be closely monitored in murine models of airway inflammation and infection. These studies have demonstrated that naïve mice housed in specific pathogen free conditions contain few Trms, yet both lung-resident CD8 and CD4 T can be found in abundance in murine lungs after infection or allergen exposure [69]. Importantly, these lung residing Trm cells are a source of rapid protection against respiratory pathogens or immunopathology to allergens [6,67,69–73]. Analysis of allergen-specific cells in mice housed in SPF-conditions also demonstrate that these Th2 Trm cells do not circulate and are retained for many months after exposure [6]. These cells express some markers in common with CD8 Trm cells (ex. CD69) yet the majority do not express another common marker of CD8 Trm, CD103 [6]. Th2 Trm cells can be rapidly reactivated to generate IL-5, IL-13, and IL-4 and can go from a quiescent memory cell to a responsive effector cell within the lung parenchyma, as previously shown with influenza infection [6,74]. Understanding where these cells reside and the signals that allow them to survive within the lung tissue is therefore critical to suppressing their associated pathology.

Type 2 microenvironments in the lung

Although there is still little known about Trm niches in the tissues, there is increasing evidence that CD4 Trm cells in the lungs reside in tertiary lymphoid structures referred to as inducible Bronchus-Associated Lymphoid Tissue or iBALT. iBALT formation in humans can be induced by infection, chronic inflammation (such as chronic obstructive pulmonary disease, COPD) or autoimmunity [75]. The presence of lymphoid clusters containing quiescent CD45RO⁺ T cells and CD20⁺ B cells have also been described in lungs from asthmatic patients [76]. iBALT can also form in murine models of airway inflammation and infection [6,67,77–82]. iBALT is characterized by the presence of loosely organized T cell and B cell areas (sometimes including germinal centers), a variety of dendritic cell subsets including follicular DCs, often high endothelial venules and even lymphatics [83]. These structures surround the blood vessels and airways and can persist for several months after an allergen or pathogen encounter. Recent evidence has demonstrated that these structures position highly functional memory T cells in close proximity to antigen-presenting cells (DCs and B cells), allowing rapid secondary memory cell activation and effector cell function after allergen-exposure in the tissues. Furthermore, much like a secondary lymphoid organ, stromal cells (and in some cases dendritic cells) within these structures

secrete chemokines (CXCL13, CCL19 and CCL21) and cytokines (IL-7, IL-33) that contribute to memory T cell entry, retention, and survival [78,84,85]. After infection the formation of iBALT also allows for the recruitment and priming of naïve T cells within the lung tissue, therefore propagating and perhaps diversifying the response [86,87]. The importance of these structures in the propagation of Th2 responses is currently under investigation, but recent studies in mice in which iBALT formation was prevented demonstrated that IL-5 expressing Th2 cells did not enter the lung or cause pathology [67].

Tertiary lymphoid organs in the lungs not only position CD4⁺ T cells in close proximity to the airways, but also in close proximity to B cells which can rapidly present antigen and behave as Th2-associated antibody-expressing effector cells. There is little known about the allergen-specific B cells that inhabit these sites within the lung, although localized IgE production in response to nasal allergen challenge has been described for many years (reviewed in [88]). Co-localization of antigen experienced CD4⁺ T cells and B cells may be a common strategy for rapid activation of both cellular and humoral immunity to control invading pathogens within mucosal sites. These localized antibody secreting B cells would also provide activating signals to mast cells and other high affinity IgE receptor expressing cells that inhabit the site. The presence of these sites therefore creates an additional layer of surveillance within the tissues that can provide rapid protection or pathology, depending upon the antigenic insult. Understanding how these structures form and are maintained, and the survival cues that they provide for pathogenic Th2 and B cells will be important for the development of novel therapeutics.

Type 2 immune sentinels in the small intestine

As highlighted above, the type 2 immune response induced in lung tissue by worm infection or during allergic inflammation has been studied quite extensively. By contrast, less is known about the mechanisms that regulate type 2 inflammation in the small intestine. Mouse genetics have revealed key pathways that regulate the intestinal type 2 response, but technical challenges have limited the ability to do careful mechanistic studies. Tissue viability is greatly reduced in type 2 inflamed small intestine, making it difficult or even impossible to isolate viable cells for flow cytometry or other *in vitro* assays. Technical advances will be needed to address these limitations.

On a broad level, the principles that govern type 2 inflammation in the small intestine are analogous to those in the lung. Here too, ILC2s monitor signals derived from their surrounding tissue and contribute significantly to early responses. With time and exposure, adaptive cells predominate and account for both anamnestic responses to pathogens and the intestinal pathology associated with food allergies. Nonetheless, in many respects the lung and intestine are more different than similar (Table 1).

One of the most notable distinctions between the lung and small intestine is their environmental exposure. The lung must contend with inhaled particulates and allergens, but microbial colonization is modest [89]. By contrast, the small intestine is constantly exposed to high concentrations of dietary constituents and microbial commensals, which must be mostly ignored or even actively tolerated while still maintaining responsiveness to

pathogens. In support of and in part due to this constitutive immune activity, the small intestine is also the site of abundant tertiary lymphoid structures such as Peyer's patches, isolated lymphoid follicles, and cryptopatches. Importantly, these lymphoid structures provide the opportunity for local priming, licensing, and/or reactivation of adaptive immune responses, all of which may integrate particularly effectively with the largely mucosal nature of type 2 agonists.

Another consequence of constitutive immune responses in the small intestine is that the set of type 2 immune sentinels differs from the lung. Along with a predominant role for tuft cells in the intestine (discussed below), the basal frequency of mast cells is also much higher in the intestinal lamina propria than anywhere in the lung [12]. As a result, mast cells can contribute to innate type 2 sensing even before B cells provide them with IgE. Mast cells both secrete and respond to IL-33, with activation of NFAT leading to upregulation of IL-33 independently of IgE [90,91]. In one model of intestinal worm infection, mast cell-derived IL-33 contributes to early ILC2 activation [92].

Indeed, the mechanisms of ILC2 activation differ significantly in the lung and small intestine. In the naïve lung, ILC2s uniformly express the IL-33 receptor, but only weakly express the IL-25 receptor [93]. Accordingly, type 2 inflammation is significantly attenuated in the lung when IL-33 signaling is ablated [10,94–97]. By contrast, in the small intestine ILC2s highly express the IL-25 receptor and IL-33 receptor expression is relatively low [98]. Accordingly, while epithelial cells are an abundant source of IL-33 in the lung, skin and numerous other tissues, both reporter mice and validated antibodies have failed to detect IL-33 in the small intestinal epithelium of naïve mice [29,34,99]. Instead, IL-33 is abundant in pericryptal fibroblasts of the small intestine [34] and worm clearance is regulated predominantly by IL-25 [100–102]. A greater role has been reported for IL-33 in models of food allergy, but it is not clear where and when it is required in this context [103–105].

Consistent with the high expression of IL-25 receptor on intestinal ILC2s, worm clearance is delayed by several days in the absence of IL-25 signaling [100]. Recently, three papers identified epithelial tuft cells as the predominant source of intestinal IL-25 [29,106,107]. Tuft cells are normally very rare, representing <1% of the intestinal epithelium, but during type 2 inflammation their frequency increases >10-fold. This tuft cell hyperplasia is driven by a signaling circuit in which tuft cell-derived IL-25 induces IL-13 production in lamina propria ILC2s and Th2 cells. IL-13 in turn signals in undifferentiated epithelial progenitors, biasing their lineage commitment towards tuft and goblet cell fates. Since the intestinal epithelium is completely replaced in ~5 days, induction of IL-13 rapidly leads to profound epithelial remodeling from an enterocyte dominated absorptive state to a tuft and goblet cell enriched phenotype that contributes to worm clearance. Although there are IL-25⁺ tuft cells in the trachea and nasal passages [29], hyperplasia has not been reported and it remains unclear if the signaling circuit described in the intestine is also functional in the airways. Notably, the half-life of tracheal epithelium is ~6 months [108], so the kind of remodeling observed in the small intestine would take many months.

Lastly, many aspects of intestinal type 2 immunity remain completely unexplored, such as (1) the role of tuft cells and ILC2s in adaptive immunity; (2) activation and licensing of Th2

effector cells in the tissue; and (3) the differentiation, maintenance, and activation of resident memory Th2 cells. Much also remains to be learned about how ILC2s are first seeded to tissue and the cell-cell interactions that keep them in their niche. Interestingly, unlike T-cells and other ILCs, intestinal homing is imprinted in a subset of ILC2s during development and this process is retinoic acid independent [109]. How ILC2s are directed to the lung and other peripheral tissues remains unknown.

In general, the ability to translate paradigms between different tissue types will depend both upon the specific cell types residing within a given tissue, as well as the extent of exposure of a given tissue to the surrounding environment. For example, the differences we highlight between the lung and the small intestine are largely derived from studies of mice that are housed under specific pathogen free conditions and may not exist in “wild” mice constantly exposed to respiratory pathogens and allergens [46].

Concluding Remarks

The findings presented in this review highlight recent advances that unravel the complexity of the type 2 immune response and emphasize the important contributions of tissue-resident cells. While immune cells and cytokines remain the key effectors of type 2 inflammation, the critical contributions of non-immune cells, especially as sentinels, are becoming clearer. The recognition that numerous and varied cell types are involved in both primary and secondary sensing during type 2 responses presents the field with many challenges, but also many novel targets for therapeutic intervention.

References

1. Gringhuis SI, et al. Fucose-specific DC-SIGN signalling directs T helper cell type-2 responses via IKK ϵ - and CYLD-dependent Bcl3 activation. *Nat. Commun.* 2014; 5:ncomms4898.
2. Hammad H, et al. House dust mite allergen induces asthma via TLR4 triggering of airway structural cells. *Nat. Med.* 2009; 15:410–416. [PubMed: 19330007]
3. Liang H-E, et al. Divergent expression patterns of IL-4 and IL-13 define unique functions in allergic immunity. *Nat. Immunol.* 2012; 13:58–66.
4. Reinhardt RL, et al. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* 2009; 10:385–393. [PubMed: 19252490]
5. Halim TYF, et al. Group 2 innate lymphoid cells are critical for the initiation of adaptive T helper 2 cell-mediated allergic lung inflammation. *Immunity.* 2014; 40:425–435. [PubMed: 24613091]
6. Hondowicz BD, et al. Interleukin-2-Dependent Allergen-Specific Tissue-Resident Memory Cells Drive Asthma. *Immunity.* 2016; 44:155–166. [PubMed: 26750312]
7. Glatman Zaretsky A, et al. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. *J. Exp. Med.* 2009; 206:991–999. [PubMed: 19380637]
8. King IL, Mohrs M. IL-4-producing CD4⁺ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. *J. Exp. Med.* 2009; 206:1001–1007. [PubMed: 19380638]
9. Anderson KG, et al. Intravascular staining for discrimination of vascular and tissue leukocytes. *Nat. Protoc.* 2014; 9:209–222. [PubMed: 24385150]
10. Von Moltke J, et al. Leukotrienes provide an NFAT-dependent signal that synergizes with IL-33 to activate ILC2s. *J. Exp. Med.* 2017; 214:27–37. [PubMed: 28011865]
11. Wakahara K, et al. Basophils are recruited to inflamed lungs and exacerbate memory Th2 responses in mice and humans. *Allergy.* 2013; 68:180–189. [PubMed: 23205591]
12. Gurish MF, et al. Intestinal mast cell progenitors require CD49 β 7 (alpha4beta7 integrin) for tissue-specific homing. *J. Exp. Med.* 2001; 194:1243–1252. [PubMed: 11696590]

13. Halim TYF. Group 2 innate lymphoid cells in disease. *Int. Immunol.* 2016; 28:13–22. [PubMed: 26306498]
14. Nussbaum JC, et al. Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature.* 2013; 502:245–248. [PubMed: 24037376]
15. Gasteiger G, et al. Tissue residency of innate lymphoid cells in lymphoid and nonlymphoid organs. *Science.* 2015; 350:981–985. [PubMed: 26472762]
16. Von Moltke J, Locksley RM. I-L-C-2 it: type 2 immunity and group 2 innate lymphoid cells in homeostasis. *Curr. Opin. Immunol.* 2014; 31:58–65. [PubMed: 25458996]
17. Cardoso V, et al. Neuronal regulation of type 2 innate lymphoid cells via neuromedin U. *Nature.* 2017; doi: 10.1038/nature23469
18. Klose CSN, et al. The neuropeptide neuromedin U stimulates innate lymphoid cells and type 2 inflammation. *Nature.* 2017; doi: 10.1038/nature23676
19. Wallrapp A, et al. The neuropeptide NMU amplifies ILC2-driven allergic lung inflammation. *Nature.* 2017; doi: 10.1038/nature24029
20. Palm NW, et al. Allergic host defences. *Nature.* 2012; 484:465–472. [PubMed: 22538607]
21. Kakkar R, et al. Interleukin 33 as a mechanically responsive cytokine secreted by living cells. *J. Biol. Chem.* 2012; 287:6941–6948. [PubMed: 22215666]
22. Moussion C, et al. The IL-1-Like Cytokine IL-33 Is Constitutively Expressed in the Nucleus of Endothelial Cells and Epithelial Cells In Vivo: A Novel “Alarmin”? *PLoS ONE.* 2008; 3
23. Saluzzo S, et al. First-Breath-Induced Type 2 Pathways Shape the Lung Immune Environment. *Cell Rep.* 2017; 18:1893–1905. [PubMed: 28228256]
24. Loke P, et al. Alternative activation is an innate response to injury that requires CD4+ T cells to be sustained during chronic infection. *J. Immunol. Baltim. Md 1950.* 2007; 179:3926–3936.
25. Xu J, et al. IL33-mediated ILC2 activation and neutrophil IL5 production in the lung response after severe trauma: A reverse translation study from a human cohort to a mouse trauma model. *PLOS Med.* 2017; 14:e1002365. [PubMed: 28742815]
26. Chang Y-J, et al. Innate lymphoid cells mediate influenza-induced airway hyperreactivity independently of adaptive immunity. *Nat. Immunol.* 2011; 12:631–638. [PubMed: 21623379]
27. Mohapatra A, et al. Group 2 innate lymphoid cells utilize the IRF4-IL-9 module to coordinate epithelial cell maintenance of lung homeostasis. *Mucosal Immunol.* 2016; 9:275–286. [PubMed: 26129648]
28. Hardman CS, et al. IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation. *Eur. J. Immunol.* 2013; 43:488–498. [PubMed: 23169007]
29. Von Moltke J, et al. Tuft-cell-derived IL-25 regulates an intestinal ILC2-epithelial response circuit. *Nature.* 2016; 529:221–225. [PubMed: 26675736]
30. Zhang K, et al. Constitutive and inducible thymic stromal lymphopoietin expression in human airway smooth muscle cells: role in chronic obstructive pulmonary disease. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2007; 293:L375–382. [PubMed: 17513456]
31. Kimura S, et al. Increased Expression and Role of Thymic Stromal Lymphopoietin in Nasal Polyposis. *Allergy Asthma Immunol. Res.* 2011; 3:186–193. [PubMed: 21738884]
32. Migone T-S, et al. TL1A Is a TNF-like Ligand for DR3 and TR6/DcR3 and Functions as a T Cell Costimulator. *Immunity.* 2002; 16:479–492. [PubMed: 11911831]
33. Schmitz J, et al. IL-33, an Interleukin-1-like Cytokine that Signals via the IL-1 Receptor-Related Protein ST2 and Induces T Helper Type 2-Associated Cytokines. *Immunity.* 2005; 23:479–490. [PubMed: 16286016]
34. Mahapatro M, et al. Programming of Intestinal Epithelial Differentiation by IL-33 Derived from Pericryptal Fibroblasts in Response to Systemic Infection. *Cell Rep.* 2016; 15:1743–1756. [PubMed: 27184849]
35. Takai T. TSLP expression: cellular sources, triggers, and regulatory mechanisms. *Allergol. Int. Off. J. Jpn. Soc. Allergol.* 2012; 61:3–17.
36. Redhu NS, et al. Essential role of NF- κ B and AP-1 transcription factors in TNF- α -induced TSLP expression in human airway smooth muscle cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2011; 300:L479–485. [PubMed: 21148792]

37. Prehn JL, et al. The T cell costimulator TL1A is induced by FcγR signaling in human monocytes and dendritic cells. *J. Immunol. Baltim. Md 1950.* 2007; 178:4033–4038.
38. Lefrançois E, et al. Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells. *Proc. Natl. Acad. Sci. U. S. A.* 2014; 111:15502–15507. [PubMed: 25313073]
39. Lefrançois E, et al. IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G. *Proc. Natl. Acad. Sci. U. S. A.* 2012; 109:1673–1678. [PubMed: 22307629]
40. Kichko TI, et al. Cigarette smoke has sensory effects through nicotinic and TRPA1 but not TRPV1 receptors on the isolated mouse trachea and larynx. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 2015; 309:L812–820. [PubMed: 26472811]
41. Caceres AI, et al. A sensory neuronal ion channel essential for airway inflammation and hyperreactivity in asthma. *Proc. Natl. Acad. Sci. U. S. A.* 2009; 106:9099–9104. [PubMed: 19458046]
42. Talbot S, et al. Silencing Nociceptor Neurons Reduces Allergic Airway Inflammation. *Neuron.* 2015; 87:341–354. [PubMed: 26119026]
43. Liu B, et al. IL-33/ST2 signaling excites sensory neurons and mediates itch response in a mouse model of poison ivy contact allergy. *Proc. Natl. Acad. Sci. U. S. A.* 2016; 113:E7572–E7579. [PubMed: 27821781]
44. Wilson SR, et al. The epithelial cell-derived atopic dermatitis cytokine TSLP activates neurons to induce itch. *Cell.* 2013; 155:285–295. [PubMed: 24094650]
45. Oetjen LK, et al. Sensory Neurons Co-opt Classical Immune Signaling Pathways to Mediate Chronic Itch. *Cell.* 2017; 0
46. Beura LK, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature.* 2016; 532:512–516. [PubMed: 27096360]
47. Reinhardt RL, et al. Visualizing the generation of memory CD4 T cells in the whole body. *Nature.* 2001; 410:101–105. [PubMed: 11242050]
48. McLachlan JB, et al. Dendritic cell antigen presentation drives simultaneous cytokine production by effector and regulatory T cells in inflamed skin. *Immunity.* 2009; 30:277–288. [PubMed: 19200757]
49. Pepper M, et al. Different routes of bacterial infection induce long-lived TH1 memory cells and short-lived TH17 cells. *Nat. Immunol.* 2010; 11:83–89. [PubMed: 19935657]
50. Nakayama T, et al. Th2 Cells in Health and Disease. *Annu. Rev. Immunol.* 2017; 35:53–84. [PubMed: 27912316]
51. Van Dyken SJ, et al. A tissue checkpoint regulates type 2 immunity. *Nat. Immunol.* 2016; doi: 10.1038/ni.3582
52. Vijayanand P, et al. Interleukin-4 production by follicular helper T cells requires the conserved IL4 enhancer hypersensitivity site V. *Immunity.* 2012; 36:175–187. [PubMed: 22326582]
53. Endo Y, Nakayama T. PATHOGENIC MEMORY Th2 CELLS AND ALLERGIC DISEASES. *Arerugi Allergy.* 2016; 65:1182–1186. [PubMed: 27885201]
54. Gold MJ, et al. Group 2 innate lymphoid cells facilitate sensitization to local, but not systemic, TH2-inducing allergen exposures. *J. Allergy Clin. Immunol.* 2014; 133:1142–1148. [PubMed: 24679471]
55. Oliphant CJ, et al. MHCII-mediated dialog between group 2 innate lymphoid cells and CD4(+) T cells potentiates type 2 immunity and promotes parasitic helminth expulsion. *Immunity.* 2014; 41:283–295. [PubMed: 25088770]
56. Mirchandani AS, et al. Type 2 innate lymphoid cells drive CD4+ Th2 cell responses. *J. Immunol. Baltim. Md 1950.* 2014; 192:2442–2448.
57. Schwartz C, et al. ILC2s regulate adaptive Th2 cell functions via PD-L1 checkpoint control. *J. Exp. Med.* 2017; doi: 10.1084/jem.20170051
58. Minutti CM, et al. Local amplifiers of IL-4Rα-mediated macrophage activation promote repair in lung and liver. *Science.* 2017; 356:1076–1080. [PubMed: 28495878]
59. Bosurgi L, et al. Macrophage function in tissue repair and remodeling requires IL-4 or IL-13 with apoptotic cells. *Science.* 2017; 356:1072–1076. [PubMed: 28495875]

60. Shih H-Y, et al. Developmental Acquisition of Regulomes Underlies Innate Lymphoid Cell Functionality. *Cell*. 2016; 165:1120–1133. [PubMed: 27156451]
61. Wambre E, et al. A phenotypically and functionally distinct human TH2 cell subpopulation is associated with allergic disorders. *Sci. Transl. Med.* 2017; 9
62. Koues OI, et al. Distinct Gene Regulatory Pathways for Human Innate versus Adaptive Lymphoid Cells. *Cell*. 2016; 165:1134–1146. [PubMed: 27156452]
63. Sallusto F, et al. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999; 401:708–712. [PubMed: 10537110]
64. Steinert EM, et al. Quantifying Memory CD8 T Cells Reveals Regionalization of Immunosurveillance. *Cell*. 2015; 161:737–749. [PubMed: 25957682]
65. Thome JJC, et al. Spatial map of human T cell compartmentalization and maintenance over decades of life. *Cell*. 2014; 159:814–828. [PubMed: 25417158]
66. Thome JJC, et al. Early-life compartmentalization of human T cell differentiation and regulatory function in mucosal and lymphoid tissues. *Nat. Med.* 2016; 22:72–77. [PubMed: 26657141]
67. Shinoda K, et al. Maintenance of pathogenic Th2 cells in allergic disorders. *Allergol. Int. Off. J. Jpn. Soc. Allergol.* 2017; 66:369–376.
68. Corris PA, Dark JH. Aetiology of asthma: lessons from lung transplantation. *Lancet Lond. Engl.* 1993; 341:1369–1371.
69. Zens KD, et al. Vaccine-generated lung tissue-resident memory T cells provide heterosubtypic protection to influenza infection. *JCI Insight*. 2016; 1
70. Teijaro JR, et al. Cutting edge: Tissue-retentive lung memory CD4 T cells mediate optimal protection to respiratory virus infection. *J. Immunol. Baltim. Md 1950.* 2011; 187:5510–5514.
71. Sakai S, et al. Cutting edge: control of *Mycobacterium tuberculosis* infection by a subset of lung parenchyma-homing CD4 T cells. *J. Immunol. Baltim. Md 1950.* 2014; 192:2965–2969.
72. Kinnear E, et al. Airway T cells protect against RSV infection in the absence of antibody. *Mucosal Immunol.* 2017; doi: 10.1038/mi.2017.46
73. Eliasson DG, et al. M2e-tetramer-specific memory CD4 T cells are broadly protective against influenza infection. *Mucosal Immunol.* 2017; doi: 10.1038/mi.2017.14
74. Turner DL, et al. Lung niches for the generation and maintenance of tissue-resident memory T cells. *Mucosal Immunol.* 2014; 7:501–510. [PubMed: 24064670]
75. Hwang JY, et al. Inducible Bronchus-Associated Lymphoid Tissue: Taming Inflammation in the Lung. *Front. Immunol.* 2016; 7:258. [PubMed: 27446088]
76. Elliot JG, et al. Aggregations of lymphoid cells in the airways of nonsmokers, smokers, and subjects with asthma. *Am. J. Respir. Crit. Care Med.* 2004; 169:712–718. [PubMed: 14711796]
77. Guest IC, Sell S. Bronchial lesions of mouse model of asthma are preceded by immune complex vasculitis and induced bronchial associated lymphoid tissue (iBALT). *Lab. Investig. J. Tech. Methods Pathol.* 2015; 95:886–902.
78. Shinoda K, et al. Thy1+IL-7+ lymphatic endothelial cells in iBALT provide a survival niche for memory T-helper cells in allergic airway inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 2016; 113:E2842–2851. [PubMed: 27140620]
79. Moyron-Quiroz JE, et al. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. *Nat. Med.* 2004; 10:927–934. [PubMed: 15311275]
80. GeurtsvanKessel CH, et al. Dendritic cells are crucial for maintenance of tertiary lymphoid structures in the lung of influenza virus-infected mice. *J. Exp. Med.* 2009; 206:2339–2349. [PubMed: 19808255]
81. Fleige H, et al. IL-17-induced CXCL12 recruits B cells and induces follicle formation in BALT in the absence of differentiated FDCs. *J. Exp. Med.* 2014; 211:643–651. [PubMed: 24663215]
82. Eddens T, et al. Pneumocystis-Driven Inducible Bronchus-Associated Lymphoid Tissue Formation Requires Th2 and Th17 Immunity. *Cell Rep.* 2017; 18:3078–3090. [PubMed: 28355561]
83. Randall TD. Bronchus-associated lymphoid tissue (BALT) structure and function. *Adv. Immunol.* 2010; 107:187–241. [PubMed: 21034975]
84. Cyster JG. Lymphoid organ development and cell migration. *Immunol. Rev.* 2003; 195:5–14. [PubMed: 12969306]

85. Rangel-Moreno J, et al. Pulmonary expression of CXC chemokine ligand 13, CC chemokine ligand 19, and CC chemokine ligand 21 is essential for local immunity to influenza. *Proc. Natl. Acad. Sci. U. S. A.* 2007; 104:10577–10582. [PubMed: 17563386]
86. Halle S, et al. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. *J. Exp. Med.* 2009; 206:2593–2601. [PubMed: 19917776]
87. Day TA, et al. Secondary lymphoid organs are dispensable for the development of T-cell-mediated immunity during tuberculosis. *Eur. J. Immunol.* 2010; 40:1663–1673. [PubMed: 20222088]
88. Campo P, et al. Local IgE in non-allergic rhinitis. *Clin. Exp. Allergy.* 2015; 45:872–881. [PubMed: 25495772]
89. Dickson RP, Huffnagle GB. The Lung Microbiome: New Principles for Respiratory Bacteriology in Health and Disease. *PLOS Pathog.* 2015; 11:e1004923. [PubMed: 26158874]
90. Hsu C-L, Bryce PJ. Inducible IL-33 expression by mast cells is regulated by a calcium-dependent pathway. *J. Immunol. Baltim. Md 1950.* 2012; 189:3421–3429.
91. Saluja R, et al. The role of IL-33 and mast cells in allergy and inflammation. *Clin. Transl. Allergy.* 2015; 5:33. [PubMed: 26425339]
92. Shimokawa C, et al. Mast Cells Are Crucial for Induction of Group 2 Innate Lymphoid Cells and Clearance of Helminth Infections. *Immunity.* 2017; 46:863–874.e4. [PubMed: 28514691]
93. Huang Y, et al. IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential “inflammatory” type 2 innate lymphoid cells. *Nat. Immunol.* 2015; 16:161–169. [PubMed: 25531830]
94. Oboki K, et al. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc. Natl. Acad. Sci.* 2010; 107:18581–18586. [PubMed: 20937871]
95. Bartemes KR, et al. IL-33-responsive lineage- CD25+ CD44(hi) lymphoid cells mediate innate type 2 immunity and allergic inflammation in the lungs. *J. Immunol. Baltim. Md 1950.* 2012; 188:1503–1513.
96. Barlow JL, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J. Allergy Clin. Immunol.* 2013; 132:933–941. [PubMed: 23810766]
97. Kamijo S, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. *J. Immunol. Baltim. Md 1950.* 2013; 190:4489–4499.
98. Hoyler T, et al. The transcription factor GATA3 controls cell fate and maintenance of type 2 innate lymphoid cells. *Immunity.* 2012; 37:634–648. [PubMed: 23063333]
99. Pichery M, et al. Endogenous IL-33 Is Highly Expressed in Mouse Epithelial Barrier Tissues, Lymphoid Organs, Brain, Embryos, and Inflamed Tissues: In Situ Analysis Using a Novel IL-33–LacZ Gene Trap Reporter Strain. *J. Immunol.* 2012; 188:3488–3495. [PubMed: 22371395]
100. Fallon PG, et al. Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J. Exp. Med.* 2006; 203:1105–1116. [PubMed: 16606668]
101. Neill DR, et al. Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature.* 2010; 464:1367–1370. [PubMed: 20200518]
102. Hung L-Y, et al. IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proc. Natl. Acad. Sci. U. S. A.* 2013; 110:282–287. [PubMed: 23248269]
103. Han H, et al. IL-33 promotes gastrointestinal allergy in a TSLP-independent manner. *Mucosal Immunol.* 2017; doi: 10.1038/mi.2017.61
104. Chu DK, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J. Allergy Clin. Immunol.* 2013; 131:187–200. e1–8. [PubMed: 23006545]
105. Galand C, et al. IL-33 promotes food anaphylaxis in epicutaneously sensitized mice by targeting mast cells. *J. Allergy Clin. Immunol.* 2016; 138:1356–1366. [PubMed: 27372570]
106. Gerbe F, et al. Intestinal epithelial tuft cells initiate type 2 mucosal immunity to helminth parasites. *Nature.* 2016; 529:226–230. [PubMed: 26762460]

107. Howitt MR, et al. Tuft cells, taste-chemosensory cells, orchestrate parasite type 2 immunity in the gut. *Science*. 2016; 351:1329–1333. [PubMed: 26847546]
108. Kauffman SL. Cell proliferation in the mammalian lung. *Int. Rev. Exp. Pathol.* 1980; 22:131–191. [PubMed: 7005143]
109. Kim MH, et al. Retinoic Acid Differentially Regulates the Migration of Innate Lymphoid Cell Subsets to the Gut. *Immunity*. 2015; 43:107–119. [PubMed: 26141583]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Trends Box

- Non-hematopoietic cells of the lung epithelium, nervous system, and stroma are the primary sentinels of type 2 inducers such as allergens and helminths.
- Group 2 innate lymphoid cells (ILC2s) integrate signals from non-hematopoietic cells and release cytokines to drive the downstream type 2 inflammatory response and perhaps adaptive type 2 immunity.
- Once antigen-specific responses are established, tissue-resident CD4 memory T-cells (Trm) and B-cells together with IgE-loaded mast cells become the dominant type 2 sentinels in the lung.
- Tertiary lymphoid structures develop in the lung with exposure and provide an important site for the maintenance and perhaps even priming of adaptive immune cells.

Outstanding Questions Box

- What are the physiologically relevant cellular sources of ILC2-activating signals? Are different cells important for different type 2 inducers?
- How is the production and release of ILC2-activating signals regulated by type 2 inducers?
- How are adaptive type 2 responses initiated? What is the role of ILC2s in this process?
- How are Trm maintained in the tissue? How are they reactivated? What are the relative contributions of innate signals vs. antigen?
- What are the similarities and differences between type 2 immune responses in the lung and intestine?
- Do neurons, tuft cells, and/or Trm contribute to food allergy?

Figure 1. Type 2 sentinels of the primary and secondary immune response

The primary sentinels of type 2 stimuli in the lungs of SPF mice and humans include a diverse array of non-hematopoietic cells including specialized cells found within the airway epithelial layer, smooth muscle cells and neurons. Upon tissue perturbation these sensors release effector cytokines (IL-25, thymic stromal lymphopoietin (TSLP), IL-33, TNF-like ligand 1A (TL1A)), lipids (leukotrienes, prostaglandins), and neuropeptides (vasoactive intestinal peptide, neuromedin U) that converge upon the ILC2s which act as an innate sensing hub. Activated ILC2s are early producers of the canonical type 2 cytokines (IL-5, IL-13 and IL9) and may contribute to the adaptive immune response through either contact dependent or independent mechanisms. Inflammation leads to the development of tertiary lymphoid structures within the lung called inducible bronchus associated lymphoid tissue (iBALT) that support the entry and maintenance of activated and differentiated Th2 cells and B cells that further propagate the response (right panel). These adaptive immune cells can be

maintained for long periods of time in the tissues where they lie in close proximity to each other and sites of antigen entry, therefore becoming major sentinels of a secondary type 2 stimulus that can rapidly clear a pathogen or cause pathology.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 1

	LUNG	SMALL INTESTINE
Environmental Exposure	Inhaled particulates; minimal microbial exposure, especially in distal lung	Dietary constituents; microbial commensals
Antigenic Tolerance	Minimal	Vital
Tertiary Lymphoid Structures	None at rest; iBALT during inflammation	Peyer's patches established during development; isolated lymphoid follicles expand during inflammation
Mast Cells	Naïve tissue: rare After exposure: present	Naïve tissue: present After exposure: present
Epithelial IL-33 expression	Naïve tissue: Yes After exposure: Yes	Naïve tissue: No After exposure: Maybe
Tuft Cells	Role in type 2 inflammation unknown	Undergo hyperplasia during worm infection; required for worm clearance
Epithelial turnover	Months	Days