

REVIEW

IL-33: a tissue derived cytokine pathway involved in allergic inflammation and asthma

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Summary

Besides classic T cell-derived T-helper type 2 (Th2) cytokines such as IL-4, IL-5 and IL-13, tissue-produced cytokines such as thymic stromal lymphopoietin, IL-25 and IL-33 are now recognized as important contributors to allergic inflammation. IL-33 is produced by various tissue dwelling cells and broadly enhances allergic inflammation through its effects on hematopoietic cell types. The environmental or endogenous triggers that provoke IL-33 cellular release may be associated with infection, inflammation or tissue damage. This review summarizes the wide range of documented IL-33 activities on human cellular mediators of inflammation as well as genetic evidence that IL-33 contributes to disease. Finally, there will be a discussion of still unanswered questions regarding the mechanisms by which cytokine activity is generated and IL-33's relationship with other Th2-associated cytokines.

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Interleukin-33 background and expression pattern

IL-33 was originally identified as a protein expressed in lymph node-associated endothelial cells [1]. The cytokine properties of the molecule were not apparent at the time; however, other salient features were recognized: namely its propensity to localize to the nucleus of resting cells and to bind to chromatin. Both these activities are due to protein domains in its amino terminus, as shown in Fig. 1. The function of IL-33 in the cell nucleus is unclear; although recent studies indicate that it binds to the dimer formed by histones H2A and H2B at the surface of the nucleosomes and may exhibit transcriptional repressor activity [2, 3]. Expression of IL-33 in endothelial cells is dependent on high cell density and rapidly turned off in newly proliferating cells [4]. IL-33 intracellular function in these cells may thus be associated with the maintenance or support of mature blood vessels.

IL-33 is now known to be expressed more broadly and to have attributes consistent with an IL-1-like cytokine. The molecule was recognized in 2005 as the ligand for an orphan receptor, ST2 (or now sometimes referred to as IL-33R α), discovered 12 years prior [5, 6]. Cytokine activity resides in the carboxyl terminus of IL-33 (Fig. 1), which is predicted to fold as a β -trefoil structure, similar to IL-1 and IL-18. This conserved feature, as well as a conserved

organization of intron–exon junctions, likely reflects a common evolutionary ancestry (for a general review of IL-1, IL-18 and IL-33, see [7]). Relatively abundant IL-33 mRNA expression is found in multiple tissue-related cell types such as endothelial cells, smooth muscle, fibroblasts, cardiomyocytes, keratinocytes, adipocytes and mucosal epithelial cells [5, 8, 9]. Skin, gut and lung appear to be prominent IL-33-expressing organs and expression is also enriched in the brain and spinal cord [5, 9, 10]. IL-33 expression is not usually found in hematopoietic cells, though it does occur under certain circumstances [11, 12]. All immunohistochemical surveys of IL-33 protein expression in tissues have revealed predominant localization to the nucleus [1, 2, 4, 9]. This suggests that nuclear localization is likely a fundamental property of the molecule and not cell specific. Cytokine activity is presumably coupled to a process by which IL-33 is released from cells. It has thus been proposed that IL-33 represents a dual-function alarmin-like molecule whose release signals tissue damage to local immune cells, similar to other molecules such as IL-1 α and HMGB1 that possess both nuclear and extracellular activity [9, 13, 14].

Expression of cytokine activity is usually tightly regulated at both the mRNA and protein level. The regulation of IL-33 mRNA expression and its relationship to cytokine activity is likely to be complicated if the timing and

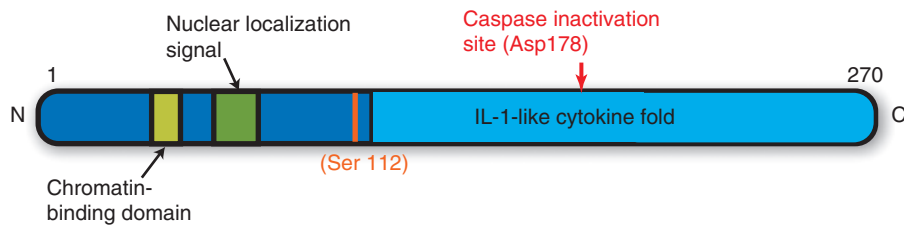


Fig. 1. Representation of IL-33 protein. IL-33 is a 270 amino acid protein that contains an IL-1-like cytokine fold in its carboxyl terminus. Most studies performed with recombinant IL-33 have utilized a molecule corresponding to amino acids 112–270 [5], however, caspase does not cleave at this site, nor is it known to be a naturally occurring amino terminus. Apoptosis-associated caspase activity does cleave and inactivate the protein at Asp178 within the cytokine domain [14]. Within the amino terminus of the full-length molecule are nuclear localization and chromatin-binding motifs, as described in the text. The protein does not contain a classical secretory leader sequence.

purpose for its expression varies. For example, in contrast to endothelial cells, expression of IL-33 mRNA in epithelial cells is positively regulated by inflammatory stimuli such as TNF or LPS [5]. In these cells, *de novo* expression of IL-33 may be meant for cytokine activity. Indeed, epithelial cells are positioned for contact with the environment and can be provoked to activate cytokine expression upon infection or exposure to pathogen-derived products, irritants or allergic substances [15, 16]. These types of stimuli have been linked to IL-33 induction. For example, pulmonary challenge with LPS-containing house dust mite led to epithelial-dependent release of IL-33 protein in the lungs of mice [17]. Likewise, IL-33 mRNA was increased in the colons of mice infected with the nematode parasite *Trichuris* [18]. Finally, in response to airway challenge with allergen, IL-33 protein concentrations were increased in the lung, however, the cellular origins were not identified [19]. Further studies are needed to more-completely understand the types of endogenous or environmental situations that lead to release of IL-33 cytokine.

ST2: properties of the interleukin-33 receptor

IL-33 exerts its cytokine activity through a signalling receptor known as ST2. The active receptor complex is comprised of IL-33-bound ST2 and a ubiquitously expressed, shared co-receptor termed IL-1R accessory protein (AcP) [20–24]. ST2 and AcP recruit the adaptor molecules MyD88 and IRAK to induce activation of the transcription factor NF- κ B as well as MAP kinases, such as p38 and JNK [5, 25–27]. This signalling mechanism distinguishes IL-33 from classical T-helper type 2 (Th2) cytokines, which signal through JAK–STAT pathways.

ST2 is expressed predominantly, but not exclusively, on immune cells associated with allergic inflammation. ST2 mRNA is expressed constitutively by mature mast cells and mast cell precursors under the control of Th2-associated GATA elements [28–30]. Th2- but not Th1-polarized T cells also express ST2 [31–33]. Studies with mouse cells indicate that expression of ST2 on Th2 cells is subsequent to IL-4 expression [34–36] and ST2 expression

on CD4⁺ T cells *in vivo* localizes with a Th2-dominated immune response at mucosal sites [37, 38]. The activities of IL-33 on these cells as well as additional ST2-expressing human immune cells are summarized in the following sections and in Fig. 2.

T-helper type 2 T cells

Consistent with their selective expression of ST2, Th2 T cell function is enhanced by IL-33. Human Th2-polarized cells from allergic donors respond to IL-33 in the presence of T cell receptor (TCR)-dependent antigen stimulation by producing elevated amounts of IL-5 and IL-13 [39]. IL-33 also induces Th2 cytokines in the absence of TCR stimulation [39], suggesting it can act as an endogenous activation signal for Th2 cells. This finding may be relevant in the context of allergen-independent inflammation, for example in non-atopic asthmatics. Moreover, administration of IL-33 *in vivo* has been shown to promote production of IgE, even in the absence of infection or allergens, and this may be due to its ability to directly enhance Th2 cytokine production [5]. However, IL-33 is not a pure Th2-promoting cytokine. For example, IL-33 also leads to the production of ng/mL amounts of IFN- γ from polarized human Th2 cells [21, 39]. This observation differs from the pure Th2 paradigm encountered with typical *in vitro* mouse cell polarization studies [5], however, it is consistent with other evidence for T cell plasticity *in vitro* and *in vivo* [40, 41]. The induction of both IL-13 and IFN- γ also mirrors the mixed Th1/Th2 cytokine profile found in some chronic human asthmatics [42, 43]. Finally, both human and mouse Th2 cells are selectively attracted to IL-33 through an unclear mechanism [44], indicating IL-33 can both recruit and activate Th2 cells.

The ability of IL-33 to activate naïve T cells and its capacity to regulate T cell development *in vivo* is not well understood. TCR activation of freshly isolated human CRTH2-positive T cells – likely entailing a memory Th2 population – rendered them responsive to activation by IL-33 [45]. This is consistent with ST2 expression being coincident with a committed Th2 phenotype. However, in some situations T cell activation may lead to transient ST2

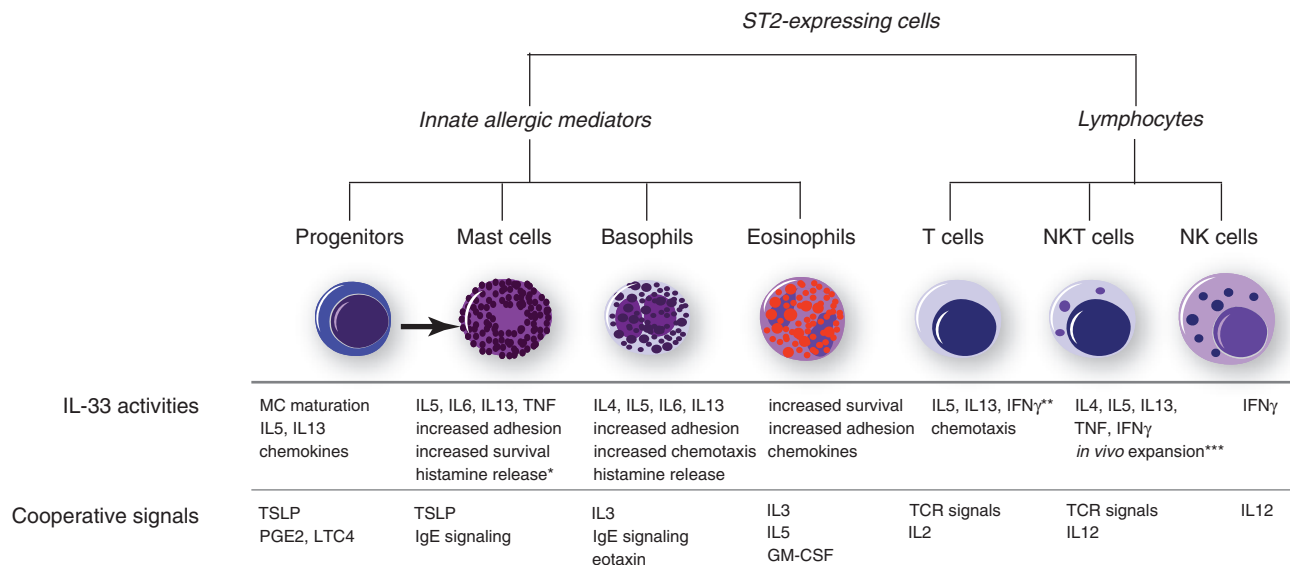


Fig. 2. Cellular activities of IL-33. Human lymphocytes and myeloid-derived (innate allergic) mediators for which biological responses to IL-33 have been described. Cytokines produced in response to IL-33 or other biological activities augmented by IL-33 are summarized, but not meant to be exhaustive. Cooperative signals indicate activating stimuli that have been shown to cooperate or synergize with IL-33. *Augmented histamine release from mast cells is variable among primary mast cell cultures. **IFN- γ production from T cells observed from primary human T cells only. ****In vivo* expansion of NKT cells observed in mice.

expression and IL-33-responsiveness by naïve T cells. For example, possibly in a DC-dependent mechanism, IL-33 induces IL-5 and IL-13 production from naïve mouse T cells [46]. Moreover, IL-33 promotes the development of an IL-5- and IL-13-expressing mouse T cell population *in vitro* and *in vivo* and, in contrast to classical Th2 responses, independent of IL-4 [26]. These attributes could in part explain why it is difficult to interpret results from Th2-driven lung inflammation models in which the T cell dependent effects of ST2 and IL-33 have been conflicting [26, 47].

Mast cells, basophils and myeloid progenitor cells

Among the innate allergic mediators, mast cells are central players in the elicitation of IgE-driven responses and contribute to the pathological features of asthma [48, 49]. Primary human mast cells stably express ST2 and respond directly to stimulation with IL-33 by producing several inflammatory cytokines and chemokines [27, 30]. These effects are enhanced when IL-33 is combined with a second stimulus, including either thymic stromal lymphopoietin (TSLP) [30], or IgE cross-linking [27]. Mouse mast cells are also selectively responsive to direct activation by IL-33 [50] and in one study, among over 40 cytokines tested, only IL-3, SCF and IL-33 were capable of directly inducing mast cell IL-6 and IL-13 production [51]. IL-33 by itself does not induce release of lipid mediators, including PGD₂ and LTC₄, or release of histamine via degranulation [27, 30], suggesting it cannot completely substitute for an IgE-delivered signal. However, IL-33 will directly activate degranulation of mast cells that have

been sensitized with free IgE [52]. This activity, along with its ability to promote IgE production, endows IL-33 with the capacity to mediate IgE-associated anaphylactic shock.

ST2 is expressed at several stages during development of the mast cell lineage, including on the earliest detectable committed precursors in the mouse [29]. Human CD34⁺ mast cell progenitors also express ST2 and addition of IL-33 markedly accelerated their maturation into tryptase-containing mast cells when cultured for 6–8 weeks [30]. This effect was already detectable after 1 week of culture and was further enhanced by TSLP. Moreover, freshly isolated circulating CD34⁺ progenitor cells respond to IL-33 by rapidly releasing very high levels of inflammatory Th2-like cytokines and chemokines [53]. These include IL-5, IL-13, granulocyte macrophage-colony forming factor (GM-CSF) and IL-6 as well as CXCL8, CCL1 and CCL17. In addition to differentiating into effector cells at sites of allergic inflammation, CD34⁺ progenitors may thus themselves exert a potent inflammatory activity, which is enhanced by IL-33 in synergy with other locally produced cytokines such as TSLP. Indeed, CD34⁺ progenitor cells producing IL-5 and IL-13 are detected in the sputum of asthmatic patients and their numbers increase following allergen challenge [53]. Interestingly, IL-33 augments human mast cell survival and adhesion to fibronectin [27], therefore it may provide a signal to not only mature and activate, but also maintain localization of tissue mast cells.

Basophils are related to mast cells and are also targets of IL-33 activity. Both cells share a common granulocytic

lineage and expression of the high-affinity FcεRI IgE receptor. Although extremely rare in circulation (<1% of blood leucocytes), basophils are recognized as important contributors to some Th2 immune responses [54]. This is attributable in part to the fact that they are early providers of IL-4 and they also produce other Th2-promoting cytokines such as IL-25 [55] and TSLP [56]. Freshly isolated human basophils express high amounts of ST2 RNA and respond to IL-33 activation by producing a number of cytokines, including IL-5, IL-6, IL-13 and IL-4 [39]. IL-33 activity on basophils is potently synergistic when combined with IL-3 [39, 57]. IL-33 was shown to enhance histamine, IL-4 and IL-13 release in response to IgE cross-linking [45, 58]. IL-33 also increases expression of CD11b to enhance the adhesive properties of basophils and dose-dependently augments migration towards eotaxin [58]. IL-33 can therefore contribute to basophil recruitment, adhesion and effector function, especially when coupled with other activating signals.

What are the implications for stable ST2 expression on these innate allergic mediators and the stimulatory effects of IL-33? In human asthma, mast cells secrete inflammatory cytokines and are a pivotal source of bronchial-constricting factors such as leukotrienes and PGD₂. Their documented localization in asthmatic bronchial smooth muscle links them to provoked and chronic airway constriction [59, 60]. Basophils have also been found in the airways of asthmatics [61] and high numbers are associated with fatal asthma [62, 63]. Released IL-33 from smooth muscle or other tissue cells could provide a local signal to sustain the accumulation, maturation and effector function of innate allergic mediators and their progenitors under pathological conditions. IL-33 could also promote mast cell- and basophil-driven inflammation and anaphylaxis, due to its ability to activate IgE-dependent and -independent effector responses.

Eosinophils

Pulmonary eosinophilia is a common form of allergic-associated inflammation and published data suggest eosinophils may sustain ongoing inflammation in the airways [64]. Although IL-5 is an important promoter of eosinophil function, these cells may be supported locally – for example in the lung – by factors besides IL-5 [65]. Several lines of early evidence indicated a link between IL-33 and eosinophil biology. For example, parasite-infected ST2-deficient mice had reduced numbers of lung infiltrating eosinophils [66, 67] and pulmonary challenge of naïve animals with IL-33 led to infiltration of large numbers of eosinophils [5, 57]. Finally, the induction of ST2 mRNA in *Aspergillus* allergen-challenged lungs was shown to be eosinophil dependent [68], suggesting an interplay between eosinophils and the IL-33 axis.

IL-33 has subsequently been shown to augment a variety of eosinophil functions. For example, it enhances eosinophil survival [69, 70] and like with basophils, IL-33 increases CD11b expression and eosinophil adhesion to extracellular matrix proteins [70]. IL-33 also activates eosinophil superoxide production more quickly and slightly more potently than IL-5 [69]. Although IL-33 does not act directly on neutrophils [69], it does promote eosinophil production of the neutrophil chemokine IL-8, particularly when combined with IL-3, IL-5 or GM-CSF [45, 69]. Whether IL-33 can induce eosinophil degranulation is unclear and may be donor-dependent, as one group found that it could [69] and another found that it could not [70]. IL-33 is not a direct chemoattractant for eosinophils [70], however, we have found that, unlike IL-25 or TSLP, IL-33 induces a shape change response in human eosinophils and dose-dependently enhances chemokinetic activity, similar to IL-5 (unpublished observations). Locally derived IL-33 could thus provide a potent signal to help mobilize, maintain and enhance the function of eosinophils within inflamed mucosal sites and perhaps systemically.

Natural killer T cells and natural killer cells

Thus far, the IL-33 activities discussed in this review are linked to classic cellular mediators of allergic-like inflammation. However, IL-33 acts directly on lymphocytes besides Th2 cells, including NKT cells and NK cells. NKT cells have emerged as a potentially important lymphocyte population in the context of asthma and airway responsiveness [71]. They are present in asthmatic lungs and able to rapidly produce large amounts of effector cytokines. Human NKT cells express ST2 RNA and in the presence of TCR stimulation, produce increased amounts of several Th1 and Th2 cytokines in response to IL-33 [39]. In the absence of TCR stimulation, IL-33, in cooperation with IL-12, directly induces NKT cells to make high amounts of IFN-γ [39]. This synergistic activity is also observed with purified human NK cells, in part due to the ability of IL-12 to increase ST2 expression [39]. The same effects were also demonstrated with mouse NKT and NK cells and, moreover, IL-33 expanded the number of these cells *in vivo* [72]. The IL-12-dependent production of IFN-γ is a prototypical Th1-like response and, on the one hand, this is difficult to reconcile with the Th2-like nature of allergic inflammation as well as the majority of IL-33's documented activities. On the other hand, it suggests that the outcome of IL-33 activity is context dependent and likely modulated by the local physiologic conditions. One of the more common inducers of exacerbations in asthma is viral infection, which typically induces a Th1-like cellular immune response [73, 74]. Interestingly, NKT cells have been implicated in viral-induced lung inflammation [75] and infection with respiratory syncytial virus has been

shown to provoke ST2-dependent pulmonary inflammation in mice [76]. The ability of IL-33 to promote both Th2 and Th1-associated responses thus may be particularly relevant in the context of infection.

Regulation of interleukin-33 activity

Because of its ability to drive potentially harmful tissue inflammation in many different ways, mechanisms have evolved to regulate the cytokine activity of IL-33. The most well-known mechanism involves the sequestration and neutralization of IL-33 by a soluble form of ST2. Soluble ST2 (sST2) is expressed by fibroblasts and other cells and arises via an alternatively spliced mRNA [77–79], which is induced by serum and other activating stimuli [79, 80] as well as anti-inflammatory signals such as vitamin D [81]. sST2 acts as a decoy receptor to bind and effectively inhibit IL-33 activity [82] and its potency in doing so is increased in the presence of the soluble co-receptor AcP [21]. An additional form of regulation was described involving the IL-1 receptor family member single Ig IL-1 receptor-related (SIGIRR) [83], a molecule previously implicated as a negative regulator of IL-1 and TLR signalling [84, 85]. SIGIRR associated with ST2 in cells and IL-33-driven Th2 responses were augmented both *in vitro* and *in vivo* in experiments with SIGIRR-deficient mice. IL-33 activity was therefore hypothesized to contribute to the mucosal inflammation exhibited by SIGIRR-deficient mice.

Links to human disease

There is accumulating evidence that the IL-33 pathway is associated with human disease. For example, the concentration of sST2 is significantly increased in asthmatics [86, 87] and idiopathic pulmonary fibrosis patients [88]. The elevation of soluble receptor in pathological situations provides indirect evidence for disease-associated pathway

activation. Additionally, two groups found that sST2 levels increase acutely, and in one study proportionally to the severity of, either allergen-induced attack or an asthmatic episode [87, 89], indicating that the IL-33 axis may be activated during short-term exacerbations. sST2 concentrations are also increased in other disease settings including arthritis [90–92], consistent with other evidence this pathway is not restricted to Th2- or pulmonary-associated inflammation [93, 94]. Less is known about circulating IL-33, however, a Japanese study reported that the average amount of IL-33 in healthy control sera is approximately 360 pg/mL, as compared with approximately 550 pg/mL allergic pollinosis sufferers [95] and IL-33 is elevated in atopic patients suffering anaphylaxis [52].

Genetic links have been made between asthma or allergy-related traits and polymorphisms in the genes encoding IL-33 and ST2 (gene name *IL1RL1*) (Table 1). Polymorphisms in the *IL33* gene (chromosome 9q) are associated with Japanese cedar pollinosis, the most common form of allergic rhinitis in Japan [95] and asthma-associated *IL33* single nucleotide polymorphisms (SNPs) were identified in a study of approximately 2500 individuals [96]. A SNP in the distal promoter of the *IL1RL1* gene (chromosome 2q) was associated with atopic dermatitis (AD) in a small population study and an even stronger association was found specifically in the AD patients with high IgE levels [97]. The same polymorphism was significantly associated with asthma in a population of Australian children presenting with acute symptoms [89]. Moreover, children homozygous for this allele had higher disease severity scores than heterozygotes or non-carriers. A functional correlate to the polymorphism was identified by demonstrating that the AD-associated ST2 promoter variant mediated double the transcriptional activity in human mast cells compared with the non-AD-associated allele [97]. This may result in carriers of this SNP having higher amounts of ST2 expression on their effector cells;

Table 1 Polymorphisms in ST2 (*IL1RL1*) and *IL33* associated with allergic disease and asthma

Locus	SNP	Association	Location	Effect	Allele frequency (pop)	References
<i>ST2 (IL1RL1)</i>	rs6543116[A]	Atopic dermatitis	Promoter	Increased transcription	0.41 (Japanese)	[97]
<i>ST2 (IL1RL1)</i>	rs6543116[A]	Childhood asthma	Promoter	Not tested	0.44 (Australia)	[89]
<i>ST2 (IL1RL1)</i>	rs1420101[A]	BHR	Intron	Unknown	0.38 (Dutch asthma)	[98]
<i>ST2 (IL1RL1)</i>	rs1921622[A]	BHR and asthma	Intron	Unknown	0.64 (Dutch asthma)	[98]
<i>ST2 (IL1RL1)</i>	rs1861246[C]	BHR and asthma	Intron	Unknown	0.78 (Dutch asthma)	[98]
<i>ST2 (IL1RL1)</i>	rs1420101[A]	Eosinophils and asthma	Intron	Unknown	0.34–0.40 (several)	[100]
<i>ST2 (IL1RL1)</i>	several	Chronic rhinosinusitis	Various	Unknown	0.17–0.40 (Quebec)	[99]
<i>IL33</i>	rs3939286[A]	Eosinophils and asthma	5' of ATG	Unknown	0.04–0.29 (several)	[100]
<i>IL33</i>	rs1929992[T]	Allergic rhinitis	Intron	Unknown	0.48 (Japanese)	[95]

Genetic polymorphisms identified in the *ST2 (IL1RL1)* and *IL33* genes and their association with asthma and allergy-related traits. SNP refers to the single nucleotide polymorphism identified in the study and indicated is the associated allele (A, G, C or T refers to the DNA sequence at the variable position). Allele frequency refers to the frequency of the trait- or disease-associated allele in the normal population studied, unless otherwise indicated.

however, this has not been tested. Disease-associated *IL1RL1* SNPs were also identified in a study of Dutch asthma families [98]. In this case, the polymorphisms were associated with asthma as well as IgE concentrations and bronchial hyperresponsiveness. Additional *IL1RL1* SNPs were identified in a different study that associated, in a protective manner, with chronic rhinosinusitis [99]. Finally, in a large-scale study designed to identify sequence variants contributing to elevated eosinophil numbers, *IL5* and *IL1RL1* were two of only five genes with SNPs that reached genome-wide significance (and an *IL33* SNP nearly reached genome-wide significance) [100]. Interestingly, both the *IL1RL1* and *IL33* variants were also significantly associated with asthma in a follow-on assessment with nearly 8000 asthmatics and 45 000 controls. The genetic link to eosinophil numbers and asthma is relevant considering both the biological connection between eosinophils and asthma as well as the ability of IL-33 to directly promote eosinophil function and IL-5 production from many cells (Fig. 2). Further study is required to determine how broadly these individual disease-associated polymorphisms replicate and whether functional correlates can be identified. However, genetic contributions to either disease susceptibility or symptoms from both the *IL1RL1* and *IL33* loci in multiple studies establishes a connection with the pathway itself and hence implies this cytokine axis is involved in human disease.

Remaining questions

The cellular activities summarized in this review indicate that IL-33 is capable of promoting a constellation of allergy-associated responses. In order to better understand how this pathway is integrated into protective immunity and disease processes, several key questions need to be addressed. For instance, in the context of allergic inflammation, what is the relationship between IL-33 and the classic Th2 cytokines such as IL-4, IL-5 and IL-13? IL-33 is distinguished by its unique tissue expression pattern (not expressed by T cells) and its employment of non-JAK-STAT signalling. However, as discussed earlier, it is unclear to what extent IL-33 is involved in the generation of Th2 T cell responses, a role usually ascribed to IL-4. It is also unclear if IL-33 activity is more important early in a Th2 response or more important later; however, most evidence suggests IL-33 acts to promote and amplify effector responses from already established Th2 T cells, likely in the affected tissue. IL-33 may thus represent a later-acting signal that increases production of, for example, IL-5 and IL-13 in local tissues. A better understanding of how or if Th2 cytokines themselves regulate IL-33 expression will further define the hierarchy of these cytokines. A related question concerns the relationship between IL-33 and other epithelial-derived cytokines, including TSLP and IL-25. Although this trio shares

similar localization and an overlapping range of target cells (see [101] for a recent review), it's unlikely they have identical functions in the context of inflammation or tissue homeostasis. They can, however, cooperate in their collective actions on immune cells [30, 55], suggesting there are instances when they operate in concert. Further study is needed to sort these questions out.

Finally, there is the question of how, when and where IL-33 cytokine activity is generated. In most cells, IL-33 is found in the nucleus, presumably as a full-length molecule. The nuclear function of IL-33 and its relationship to cytokine function is unknown. Nor is it clear if all IL-33-expressing cells can be induced to generate extracellular cytokine and what the important physiologic triggers are. Release of active IL-33 is postulated to be coupled with proteolytic processing and IL-33 was shown capable of acting as a caspase-1 substrate [5]. The caspase-1-mediated processing of IL-1 and IL-18 usually occurs in the context of the NLRP3 inflammasome complex [102] and there is some evidence for NLRP3-dependent IL-33 release from a monocytic cell line [12]. However, most of the released IL-33 was unprocessed and there was no evidence that cleavage created active cytokine. Moreover, as pointed out by others [2], the reported N-terminus of mature IL-33 does not match a typical caspase-1 cleavage site, nor is it conserved in all species. Subsequently, it has been shown that caspase-1 cleavage of IL-33 is not required for IL-33 activity [14, 103, 104] and that cleavage by apoptosis-associated caspases actually occurs within the cytokine domain (Fig. 1), leading to IL-33 inactivation [14, 103]. This finding is consistent with the fact that apoptotic cells typically do not induce an inflammatory response. It was also reported that cell necrosis or endothelial cell disruption – a surrogate for tissue damage – leads to the release of unprocessed, but apparently active IL-33 [14, 103]. In the latter study, although the authors suggested full-length IL-33 possessed comparable biological activity, the potency was markedly lower than a truncated form of IL-33 containing only the cytokine-like domain [103]. More recently it was shown that release of IL-33 from human epithelial cells was coincident with processing by the calcium-dependent protease calpain; however, the effect on biological activity was undetermined [105]. These findings raise questions of whether protease-processing is sometimes required for IL-33 release or perhaps for the extracellular generation of highly active cytokine. The answers to these questions will be important to more completely understand the physiological context and nature in which this pathway is activated.

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