Review

Interleukin-33 and the function of innate lymphoid cells

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Interleukin (IL)-33 is a member of the IL-1 cytokine family that has been shown to play an important role in the induction and effector phases of type 2 immune responses. Both innate and adaptive immunity are regulated by IL-33, and many studies have shown disease-associated functions for this cytokine. Recently, IL-33 has been implicated in the function of novel innate lymphocyte populations that regulate both protective responses in parasitic infections and allergic airway inflammation. Here, we discuss recent data highlighting the dual roles of IL-33 in protective and deleterious immune responses.

At the beginning

In 1998, an attempt to discover specific markers for T helper (Th)1 and Th2 cells using differential display polymerase chain reaction (PCR), showed that IL-18 receptor and ST2 were selectively expressed on Th1 and Th2 cells, respectively [1,2]. Both IL-18 receptor and ST2 are members of the IL-1 receptor family and are important for the function of Th1 and Th2 cells respectively [1–3]. ST2 is an orphan gene reported in 1989 in both mice (called ST2L) [4] and rats (called T1) [5]. Subsequently, in 2005, using a computer-data-based search for genes with homology to the IL-1 family, the ligand for ST2 was identified and named IL-33 [6], which is identical to NF-HEV, a nuclear factor highly expressed in high endothelial venules [7].

ST2 exists in several forms including transmembrane bound and soluble variants (sST2). Although binding of IL-33 to the transmembrane form of ST2 transmits signals leading to nuclear factor (NF)-κB activation [6], complex formation of IL-33 with sST2 leads to cytokine inactivation, suggesting a role for this variant form as a decoy receptor [8]. Furthermore, ST2L downregulates Toll-like receptor (TLR) signalling by competitively binding myeloid differentiation primary response gene 88 (MyD88), and hence performs an important regulatory role preventing over-exuberant inflammatory response during microbial infection [9,10].

Since its first description, important roles of IL-33 as both a traditional cytokine and a nuclear factor have emerged. The recent reports of novel innate cell populations that are potently activated by this cytokine have further expanded the field of IL-33. Here, we summarise the biology of IL-33 and review the recently discovered role of IL-33 in the induction and function of innate lymphoid cells (ILCs).

Cellular sources of IL-33

Il33 mRNA is expressed in many stromal cells of organs including the central nervous system, the lung and the gut [6]. The expression of IL-33 in different cell types has been confirmed in individual studies (Table 1). In resting cells, IL-33 is mainly found in the nucleus of the cell. IL-33 has been shown to be a chromatin-associated nuclear factor in chronically inflamed synovium and intestine [11]. Furthermore, a similarity between the chromatin-binding sequence of IL-33 and the Kaposi herpes virus latency-associated nuclear antigen (LANA) suggests that IL-33 interacts with chromatin at the H2A–H2B dimer on the surface of the nucleosome, affecting chromatin compaction [12]. Recently, nuclear IL-33 has been shown to bind NF-κB directly, sequestering it and reducing its ability to turn on gene transcription [13], suggesting that a principle role for intracellular IL-33 is to dampen gene activation.

IL-33 expression is increased in inflamed tissues in a variety of diseases and *in vitro* studies have shown that this phenomenon is linked to the induction of *Il33* mRNA expression in dendritic cells (DCs), macrophages and fibroblasts by TLR stimulation [14]. Recently, in an influenza model of airway inflammation, increased levels of IL-33 in the lung were secondary to an increase in alveolar macrophages expressing this cytokine in response to H3N1 virus [15].

Much controversy has surrounded the mechanism of IL-33 release from cells. It has been proposed that IL-33 may be released during cellular necrosis thereby acting as an 'alarmin' [16,17]. By contrast, during apoptotic cell death, IL-33 is thought to be rendered inactive by caspases [18]. In this regard and unlike IL-1 and IL-18, IL-33 is not activated by caspase 1 cleavage as initially proposed. In fact, IL-33 exists as a full-length, biologically active form that is inactivated by caspase cleavage [16,17]. Recently, IL-33 has been shown to traffic out of the nucleus though nuclear pores into membrane-bound cytoplasmic vesicles [19]. Moreover, fibroblasts under mechanical strain secrete IL-33 in the absence of necrosis, demonstrating that IL-33 could exit the cell in a similar manner to high-mobility group box 1 (HMGB1) protein [20] and in response to mechanical strain, similar to IL-1α [21].

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1471-4906/$ – see front matter http://dx.doi.org/10.1016/j.it.2012.04.005 Trends in Immunology, August 2012, Vol. 33, No. 8

Table 1. Tissue and cellular IL-33 expression.

Organ	Mouse	Human	Reference
Eye	RNA and protein	RNA and protein	[78,79]
Liver	RNA	ND	[14]
Colon	RNA and protein	RNA and protein	[63,80–82]
Small bowel	RNA and protein	ND	[64]
Stomach	RNA	Protein	[6,83]
Joints	RNA and protein	Protein	[40,84,85]
Lungs	RNA and protein	RNA and protein	[6,15,38,65,66,83]
Heart	RNA and protein	ND	[6,8,39]
Skin	RNA and protein	RNA and protein	[6,83,86,87]
Central nervous system	RNA and protein	RNA and protein	[6,88–90]
Spleen	RNA	ND	[6,14]
Kidney	RNA	Protein	[6,83]
Lymph node	RNA	RNA and protein	[6,7,83]
Cell type			
Macrophages	RNA and protein	RNA	[6,14,15,82,91]
Dendritic cells	RNA and protein	RNA	[6,14,91,92]
Mast cells	RNA and protein	ND	[91,93]
Epithelial cells	**RNA and protein**	RNA and protein	[6,29,64,83,94]
Smooth muscle cells	RNA	RNA and protein	[6,66,83]
Fibroblasts	RNA and protein	Protein	[8,14]
Myofibroblasts	ND	RNA and protein	[80,81]
Endothelial cells	RNA and protein	RNA and protein	[7,11,39,83]
Glial cells	RNA and protein	ND	[88]
Osteoblasts	RNA and protein	RNA	[95,96]
Adipocytes	ND	RNA	[97]

Abbreviation: ND, not determined.

IL-33 signalling and targets

IL-33 shares many key features with other IL-1 superfamily cytokines including a shared signalling pathway (Figure 1). To function as a classical cytokine, IL-33 requires the expression of both the ST2 receptor (also called IL-33 receptor) and the IL-1 receptor accessory protein (AcP) [22,23]. Similar to IL-1 and IL-18, recruitment of the toll/IL-1 receptor (TIR) adaptor myeloid differentiation primary response gene (MyD)88 to the membrane-associated receptor complex is essential for IL-33-induced cytokine production [6,24]. Recruitment of IL-1 receptor-associated kinase (IRAK) 1 and 4 ensues with downstream phosphorylation of several signalling molecules including extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and inhibitor of NFκB-α (IκB-α), which in turn, allows NF-κB activation [6] and results in target gene activation. Cell-type-specific variations in the signalling pathways of IL-33 exist, with signalling in basophils not requiring the phosphorylation of the tumor necrosis factor (TNF)-receptor-associated factor (TRAF)6 [24]; a step that has been shown to be key in both fibroblasts [25] and endothelial cells [26]. Similarly, the expression of the receptor tyrosine kinase, c-Kit is essential for optimal IL-33 signalling in mast cells [27].

ST2 is expressed in both innate and adaptive immune cells and is predominantly associated with type 2 immune responses (Table 2). Macrophages respond to IL-33 and short-term preincubation of macrophages with IL-33 enhances lipopolysaccharide (LPS)-induced cytokine production [28]. Importantly, IL-33 has been shown to enhance polarisation of macrophages to the alternatively activated phenotype and these cells have been shown to contribute to allergic airway inflammation [29]. In a similar manner, DCs are activated by IL-33, inducing the expression of co-stimulatory molecules including CD80 and OX40L and the production of proinflammatory cytokines [30]. Mast cells and basophils have both been shown to respond to IL-33 with increased production of proinflammatory cytokines and chemokines [31–33]. Interestingly, IL-33 alone is insufficient to induce mast cell degranulation but induces production of IL-6, TNFα and monocyte chemotactic protein (MCP)-1 [33] and synergises with IgE to induce increased cytokine production [34,35]. IL-33 induces profound eosinophilia *in vivo* [6] through stimulating high levels of IL-5 production by several cell types (see below). However, IL-33 also activates eosinophils directly inducing differentiation from bone marrow cells [36] and stimulating cytokine production [31,37].

IL-33 also regulates the adaptive immune system. In particular, IL-33 polarises Th cells to an atypical Th2 phenotype characterised by production of IL-5 and IL-13 but not IL-4 [38–40]. Similar to other IL-1 cytokines, IL-33 can induce cytokine production in effector Th2 cells independent of T cell receptor (TCR) triggering; a process enhanced by the presence of a signal transducer and activator of transcription (STAT) 5 activating cytokine [41]. More recently, IL-33 in combination with transforming growth factor (TGF)-β, has been shown to enhance IL-9 production from CD4 T cells [42,43]. IL-33 also regulates the activity of cytotoxic CD8 T cells. CD8 T cells upregulate ST2 expression in response to combined IL-12 and TCR stimulation *in vitro* [44], whereas in a lymphocytic choriomeningitis virus model, IL-33 released from non-haematopoietic cells resulted in elevated ST2 expression and enhanced the antiviral

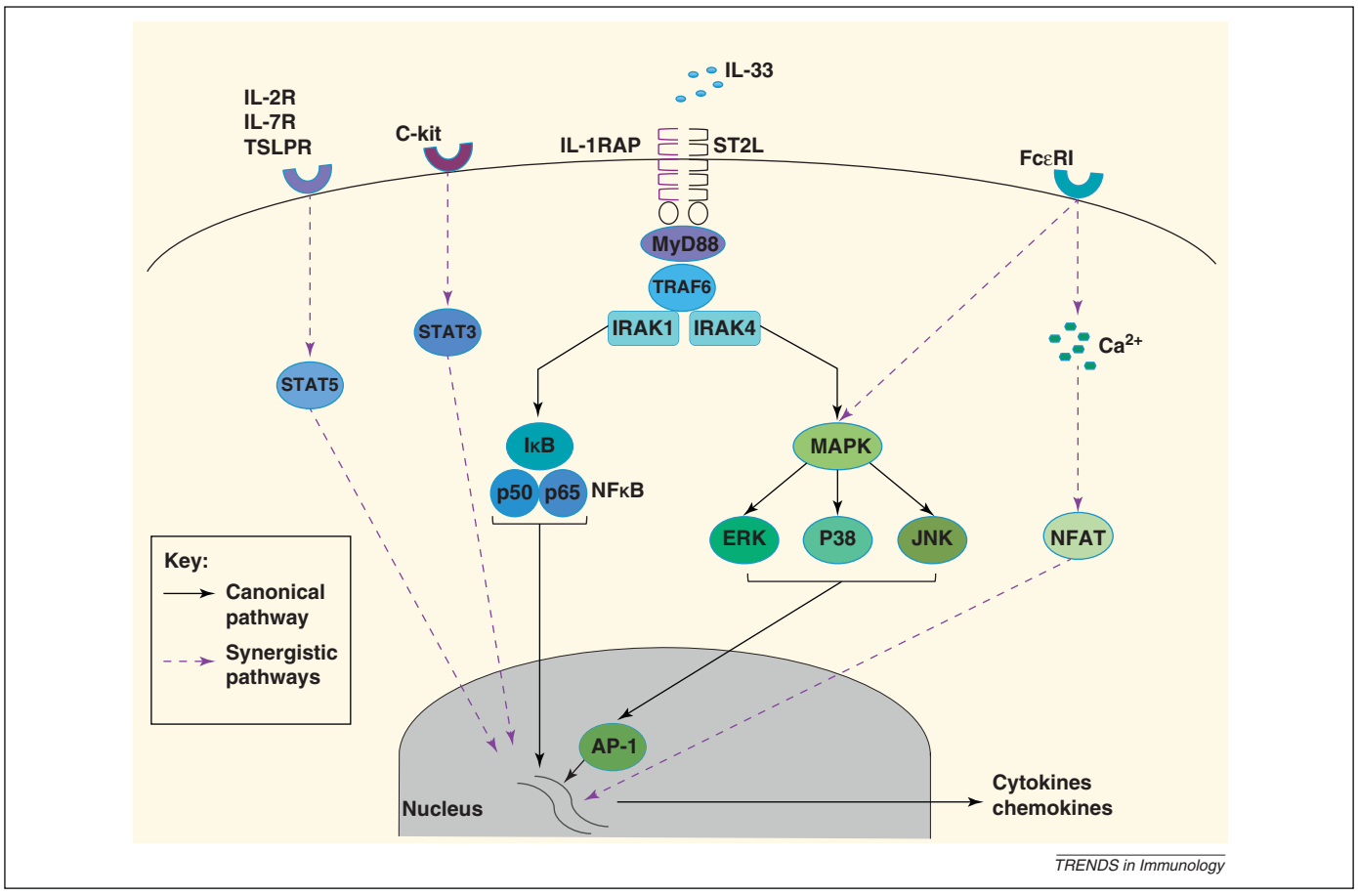

Figure 1. IL-33-induced signalling pathways. IL-33 binds a cell surface receptor composed of ST2 and IL-1 receptor AcP and induces the recruitment of MyD88 to the receptor complex. Receptor-associated MyD88 facilitates the activation of IRAK1 and IRAK4 by a process that, in some cell types, depends on TRAF6 recruitment. IRAKs induce downstream phosphorylation and degradation of IκB-a, resulting in NF-κB nuclear translocation, and the activation of ERK, C-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways that, in turn, induce activator protein (AP)-1 activation. NF-κB and AP-1 transcription factors induce gene expression in the nucleus, resulting in the biological responses of cells to IL-33. Heterologous receptor ligation can synergise with the canonical IL-33-dependent signalling pathway. Cytokines including IL-2, IL-7 and thymic stomal lymphopoeitin (TSLP) synergise with IL-33 to induce gene expression in target cells in a signal transducer and activator of transcription (STAT)5-dependent manner. Similarly, in mast cells, c-Kit signalling via STAT3 can synergise with the canonical IL-33 signalling pathway. Finally, FcεR1 and IL-33 receptors synergistically activate MAPK pathways, whereas FcεR1 additionally elevates responses to IL-33 via the mobilisation of intracellular Ca^{2+} and activation of nuclear factor of activated T cells (NFAT) transcription factors.

function of CD8 T cells [45]. B cells are also targets of IL-33. In particular, B1 cells have been shown to respond robustly to IL-33 treatment *in vivo* and *in vitro* with increased cytokine and IgM production [46].

Many nonimmune cells respond to IL-33, including endothelial cells that have been shown to produce NO in response to this cytokine in an Akt- and endothelial NO synthase (eNOS)-dependent manner [26]. Both epithelial cells endothelial cells produce IL-6 and IL-8 as well as MCP-1 in response to IL-33 *in vitro* [47] and murine fibroblasts respond in a similar manner to IL-33-producing chemotactic factors and proinflammatory cytokines [25].

Table 2. ST2-expressing cell types.

Cell type	Mouse	Human	Reference
Th2 cells	RNA and protein	RNA and protein	[1,3,98–100]
Cytotoxic T cells	RNA and protein	ND	[44,45]
B cells	RNA	RNA and protein	[87,98,101]
NK cells	Protein	RNA	[101,102]
Monocytes	RNA	Protein	[98,103]
Macrophages	RNA and protein	Protein	[9,29,87]
Mast cells	RNA and protein	Protein	[6,104]
Eosinophils	Protein	RNA and protein	[36,105,106]
Dendritic cells	RNA and protein	ND	[107]
Neutrophils	RNA and protein	ND	[108]
ILCs	Protein	RNA and protein	[49,55,61]
Fibroblasts	RNA and protein	ND	[4,25]
Epithelial cells	ND	RNA and protein	[47,64]
Osteoblasts	RNA	ND	[96]
Endothelial cells	RNA and protein	RNA and protein	[26,39,47]

Novel innate immune cells respond to IL-33

In the past 2 years, the discovery of novel subsets of innate immune cells, which respond to IL-33 and other innate cytokines, has greatly increased the interest in IL-33. Initially, a group of non-B non-T cells were found in fat-associated lymphoid clusters (FALCs) that expressed ST2 and produced IL-5 and IL-13 in response to IL-33 *in vitro* and *in vivo* [48]. These innate cells required IL-7 for their survival *in vivo* and were named natural helper cells (NHCs) in view of their ability to support B1 cell antibody production and self-renewal. Furthermore, using mice devoid of FALC ($gc^{-/-}$ $Rag1^{-/-}$ mice), a role for NHCs in helminth expulsion was elucidated.

Soon after, experiments in IL-13-reporter mice [49] allowed the identification of a population of IL-13-positive and lineage-negative cells that expanded in response to IL-33 or IL-25 treatment *in vivo*. The investigators named these cells 'nuocytes' in view of their IL-13 expression (Nu = 13th letter of the Greek alphabet) and demonstrated that, in *Nippostrongylus brasiliensis* infection, nuocytes were the main source of IL-13 [49]. Moreover, using $il\text{-}17R^{-/-}$ $Il\text{-}1r1^{-/-}$ mice, they showed that nuocytes were key to worm expulsion in their model and that this was IL-13-dependent. Similarly to NHCs, these cells proliferated in response to IL-7 *in vitro* and intracellular cytokine analysis of these cells demonstrated IL-5 and IL-13 production. These findings were in keeping with a similar innate immune cell population that was identified using IL-4 reporter mice. Similarly to nuocytes, these cells were key to worm expulsion, and transcriptome analysis of these cells clearly showed they were a novel cell type, distinct from basophils and Th2 cells. Termed innate type 2 helper (Ih2) cells, these cells were systemically dispersed and expanded in response to IL-33 *in vivo* but not in response to any γc-binding cytokines. The surface markers expressed by both nuocytes and Ih2 cells were very similar, suggesting that the populations were closely related. However, unlike nuocytes, Ih2 cells did not appear to express the stem-cell marker Sca-1.

In these initial studies, the role for the novel innate cells in the expulsion of parasites from the gut was explored, yet their role in other organs had not been investigated. Ih2 cells have been shown to be present in the lung, and subsequently the vital role of these cells in viral-induced airway hyperresponsiveness (AHR) has been demonstrated [15]. Asthma exacerbations are often triggered by viral infections and the mechanism for this has remained elusive. Using a model of influenza-driven airway inflammation, it has been demonstrated that AHR is induced by the H3N1 virus independent of adaptive immunity. Additionally, alveolar macrophage IL-33 levels are increased following viral infection, which in turn, enhances NHC expansion in an ST2-dependent manner. Using an adoptive transfer model, it has been shown that wild-type NHCs are able to reconstitute viral-induced AHR in recipient $il\text{-}13^{-/-}$ mice showing that NHC-dependent IL-13 production is sufficient for this process.

Further insight into the pathogenic roles of these novel innate cells has recently been provided by the demonstration of the expansion of these cells in ovalbumin- [50], glycolipid- [51] and fungus-induced [52] models of allergic inflammation, as well as in a model of parasite-driven eosinophilic pulmonary inflammation [53]. Interestingly, these innate cells have been demonstrated to be important sources of IL-9 during airway inflammation [49,54]. T cells have long been known to be able to produce IL-9 *in vitro* [42], however, the sources of IL-9 *in vivo* have remained elusive. It has been demonstrated using an IL-9-reporter mouse that ST2-positive lineage-negative innate cells were the main source of IL-9 in a papain-induced model of airway inflammation. IL-33 was shown to induce potently the accumulation of innate cells in the lung that subsequently produced IL-9 in response to IL-2-dependent signals [54]. The requirement for IL-2 in this model firmly linked the function of these innate cells to the adaptive immune system.

The importance of type 2 cytokines not only in parasite clearance and airway inflammation but also in tissue remodelling is well established. Consistent with this homeostatic role for type 2 immunity, IL-33-responsive innate cells have been shown to play an important role in tissue repair. In an influenza-virus-induced model of airway inflammation, IL-33-driven ILC-derived factors, including the epidermal-growth-factor-related cytokine amphiregulin, are essential to stimulate postviral epithelial repair [55]. Importantly, specific deletion of these cells during influenza infection results in loss of tissue repair and continued impairment of lung function. Taken together, these studies have demonstrated the functional relevance of the type 2 cytokines produced by these cells *in vivo*. ILCs have been shown to produce additional cytokines *in vitro* including IL-6 and IL-10 [49]. The roles of IL-6 and IL-10 production by ILCs *in vivo* have yet to be determined.

These data have shown that phenotypically similar innate cells that respond to IL-33 play a vital role in type 2 immunity. An important question remains as to whether these populations represent one single type of innate cell at different stage of development/differentiation or several distinct lineages. NHCs have been suggested to be of lymphocytic origin by virtue of the need for fms-related tyrosine kinase (Flt)3 expression for their development [56]. Flt3 is a cytokine receptor that is highly expressed in bone marrow lymphoid progenitors and also in rare myeloid progenitors with lymphoid potential [57]. Recent data have shown that nuocytes are derived from common lymphoid progenitors (CLPs) in the bone marrow [56,58]. Development of nuocytes from CLPs has been shown to require IL-7 and Notch signalling. Interestingly, nuocyte development requires cell-intrinsic expression of the transcription factor retinoic acid orphan receptor (ROR)-α [58], distinguishing nuocytes from IL-22-producing gut ILCs that require ROR-γ [59]. GATA binding transcription factor 3 (GATA3) is expressed in NHCs, nuocytes and Ih2 cells [48,49,60]. However, whether GATA3 is required for type 2 innate cell differentiation or function is unclear. Additionally, lung-derived ILCs are dependent on the transcription factor inhibitor of differentiation (Id)2 because their development is impaired in $Id2^{-/-}$ mice [55].

The presence in humans of cells that resemble these murine type 2 ILCs has also been reported. ILCs that respond to IL-33 and IL-25 have been demonstrated in

Table 3. Roles of IL-33 in disease.

Disease	Role of IL-33	Reference
Parasitic infection	• Enhanced parasite expulsion by regulation of Th2 gut responses • Upregulated in *Toxoplasma gondii* infected brain and important for the prevention of encephalitis in this model • Induction of ILC, production of IL-5 and IL-13 to allow parasite clearance	[48,49,63,90]
Bacterial infection	• Enhanced neutrophil chemotaxis via regulation of CXCR2 expression	[108]
Viral infection	• Increased expression of IL-33 in influenza lung infection • ILC induction leading to AHR and tissue repair post-infection	[15,55]
Fungal infection	• Increased IL-33 levels in the lungs of mice infected with *Aspergillus fumigatus* • Attenuated allergic response to *A. fumigatus* with ST2L-blocking antibody in combination with a TLR9 agonist • *Alternaria alternata* lung exposure induces ILC and airway inflammation	[52,109]
Asthma	• Increased production of IL-33 by alveolar macrophages in experimental pulmonary influenza • Induction of M2 macrophages in experimental asthma, disease exacerbation • Induction of ILCs and AHR in influenza infection • Induction of ILC-mediated tissue repair in the lung via amphiregulin • Increased levels in asthmatic lungs, correlating with disease severity • Induction of eosinophilia • IL-33 knockout mice have reduced airway inflammation in models of asthma	[6,15,29,55,65,66,69]
Cardiovascular	• Inhibits the formation of atherosclerotic plaques, protective type-2 immune responses • Improves outcomes in models of myocardial infarction • Protective effects in murine obesity-driven metabolic syndrome	[6,39,71–73]
Arthritis	• Exacerbates inflammation in murine arthritis models and is reduced by anti-TNF therapy • Elevated levels measured in sera and synovial fluid of rheumatoid arthritis patients • Elevated sera levels respond to anti-TNF treatment and correlate with disease activity	[40,76,84,85,110]
Central nervous system	• Increased expression in activated glial cells • Increased levels in experimental encephalitis • Gene polymorphisms associated with Alzheimer's disease	[88–90]

resting human lung tissue [55], as well as in foetal gut. Interestingly, elevated numbers of these ILCs have been shown to be present in nasal polyps [61], suggesting a role for these cells in allergic inflammation. Furthermore, a population of IL-13$^+$ cells was identified that were negative for T cell, mast cell and eosinophil markers and were only present in severe asthmatic lung biopsies but not in healthy controls, indicating the possibility of these cells being important mediators or markers of severe, rather than mild asthma [62].

IL-33 in disease

IL-33 is a potent activator of the immune system with roles in a variety of diseases (Table 3). Within the mucosal organs, IL-33 is important in the clearance of bacterial, fungal and parasitic infections [63], and IL-33 expression is increased in ulcerative colitis and Crohn's disease as well as in animal models of colitis [64]. As described above, IL-33 is implicated in the induction of airway inflammation in a variety of allergic models via the activation of eosinophils [36], macrophages [29], DCs [30] and Th2 cells [38]. Importantly, IL-33 levels have also been shown to correlate with asthma severity [65,66] and genetic variants of IL-33 have been implicated in susceptibility to hay fever [67] and the risk of asthma development [68]. The relevance of IL-33 in mucosal immune diseases has been confirmed by the use of IL-33 knockout mice in these models [69]. The upregulation of IL-33 during disease suggests it plays an active role in type 2 immune response induction. Although this is a protective mechanism in infections, it can be a deleterious process in other diseases such as asthma. In fact, as a potential alarmin, IL-33 fulfils its role as immune response trigger as well as key homeostatic factor, allowing tissue repair and damage containment, as demonstrated by its role in respiratory viral infections [15,55].

In the cardiovascular setting, IL-33 is protective in atherosclerosis by reducing the formation of atherosclerotic plaques [39] and reducing foam cell formation [70]. IL-33 also improves outcomes in a model of myocardial infarction [71], although there was no correlation with serum levels of IL-33 and post-infarction outcomes in a cohort of patients [72]. Additionally, a protective role of IL-33 in a model of obesity-driven metabolic disease has also been shown via the production of type 2 cytokines [73]. Recent research suggests a protective role for IL-33 in cardiovascular disease because it tilts the balance towards a type 2 immune response (reviewed in [74]).

IL-33 is also implicated in the homeostasis and pathogenesis of musculoskeletal disorders. IL-33 plays a protective role in bone metabolism, preventing bone resorption and affecting osteoclast differentiation [75]. However, IL-33 can also drive autoantibody-induced arthritis via mast cells [40] and levels of IL-33 are increased in patients with rheumatoid arthritis [76] and ankylosing spondylitis [77] but not psoriatic arthritis. As in other organs, IL-33 plays a dichotomous role in the musculoskeletal system, both as a homeostatic or a pathological factor, depending on the immune context.

Concluding remarks

Our understanding of IL-33-mediated responses has developed at a rapid rate since its discovery. Although its roles in type 2 immunity are well-documented, the recent discovery of novel innate immune cells that respond strongly to this cytokine have emphasised and helped clarify important roles for IL-33 in parasitic infection

and allergic inflammation. Furthermore, additional roles in less-expected areas including antiviral cytotoxic T cell responses are increasingly being described.

It is clear that IL-33 acts as a protective cytokine in certain infections and diseases and has deleterious effects in others. Therefore, the potential beneficial effects of manipulating IL-33-dependent processes in the clinic must be tempered by an appreciation of the possibility for detrimental outcomes. Nonetheless, judicious application or blocking of IL-33 may be of therapeutic value in specific diseases in the future.

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