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REVIEW

The Evolutionary Role of the IL-33/ST2 System in Host Immune Defence

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Abstract Interleukin (IL)-33 is a recently identified pleiotropic cytokine, which can orchestrate complex innate and adaptive immune responses in immunity and disease. It has been characterized as a cytokine of the IL-1 family and affects a wide range of immune cells by signalling through its receptor ST2L. Accumulating evidence suggests a crucial role of IL-33/ST2 in inducing and modifying host immune responses against a variety of pathogens including parasites, bacteria, viruses and fungi as well as sterile insults of both endogenous and exogenous source. In this review, we endeavour to give a comprehensive overview of the current knowledge about the role of IL-33 and its receptor ST2 in host defence against infections.

Keywords IL-33 · ST2 · Infection · Infectious disease · Host defence · Immune response

Introduction

Since its identification as an interleukin (IL)-1 family cytokine in 2005 IL-33 has attracted great attention due to its importance in the induction and modulation of immune responses. IL-33 has been implicated in a wide range of human inflammatory diseases, including allergic, cardiovascular and autoimmune conditions, where depending on the disease setting, it may elicit beneficial or detrimental effects.

The gene encoding IL-33 was originally identified during a screen for differentially expressed genes in vasospastic cerebral arteries after subarachnoid haemorrhage in dogs. Characterization of the encoded protein showed intracellular localization and changes of expression levels in response to inflammatory stimuli, which lead to the conclusion that the unknown gene termed DVS 27 would encode a nuclear protein that could be involved in inflammatory events (Onda et al. 1999). Subsequently, IL-33 was found to be expressed in the nuclei of a variety of cell types associated with heterochromatin via an evolutionarily conserved homeodomain-like helix–turn–helix motif and serve as a transcriptional repressor (Baekkevold et al. 2003; Carriere et al. 2007; Moussion et al. 2008). Intracellular IL-33 can further bind to the nuclear factor (NF)-κB subunit p65 which prevents expression of NF-κB target genes such as tumour necrosis factor (TNF)-α (Ali et al. 2011). Thus, intracellular IL-33 may have the ability to dampen pro-inflammatory signalling.

When searching for additional proteins containing the β-trefoil structure present in IL-1 and fibroblast growth factor-like proteins, IL-33 was identified as a member of the IL-1 family (Schmitz et al. 2005). In line with a cytokine function, IL-33 can be released into the extracellular space, but the mechanisms of IL-33 release stayed enigmatic for some time. At first it was postulated that IL-33 was processed similar to other IL-1 family members by caspase-1 and released as a mature protein (Schmitz et al. 2005). However, it soon became clear that the IL-33 protein only contains caspase-3 and caspase-7 but not caspase-1

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cleavage sites and that caspase-dependent processing may degrade rather than activate the protein when cells undergo active apoptosis (Cayrol and Girard 2009; Luthi et al. 2009). It has been suggested that IL-33 may be released in an unprocessed full-length form and may serve as an alarmin similar to high-mobility group protein B (HMGB)-1 and IL-1α (reviewed in Haraldsen et al. 2009). Comparable to HMGB-1 and IL-1α, IL-33 does not contain a classical leader sequence for secretion and is currently considered to be mainly released passively when cells undergo necrotic cell death (Smith 2010). A recent report indicates that the released full-length IL-33 may then be processed by extracellular neutrophil elastase and cathepsin G to generate smaller proteins with an even tenfold higher activity than the full-length form (Lefrancais et al. 2012). Released IL-33 binds to its receptor which consists of a heterodimer between ST2L and IL-1 receptor associated protein (IL-1RAcP). The ST2 gene was identified over 20 years ago and its protein product was found to be highly similar to the extracellular portion of IL-1 receptors type 1 and type 2 (Tominaga et al. 1991; Yanagisawa et al. 1993). Soon after, ST2 was shown to be expressed on type 2 but not on type 1 helper T cells and thus was suggested as a stable marker to distinguish between these two T cell lineages (Xu et al. 1998). Schmitz et al. (2005) also identified IL-33 as the ligand for the ST2L/IL-1RAcP heterodimer and showed that IL-33 binding to ST2 triggers the recruitment of adapter molecule MyD88 to ST2L which activates downstream NF-κB and MAP kinase pathways. This induces the expression of various target genes leading to the release of pro-inflammatory cytokines and activation of the immune system. In addition, ST2 has also been suggested to mediate effects independent of IL-33 (Gillibert-Duplantier et al. 2012; Nagata et al. 2012; Takezako et al. 2006).

Therefore, IL-33 leads a dual life, as its intra and extracellular form clearly have two opposing functions. Further, IL-33 and ST2 might not be an exclusive receptor–ligand pair which adds another dimension of complexity. All this needs to be taken into account when interpreting IL-33-related experimental data. In support of this, ST2 and IL-33 knockout mice show only some overlapping phenotypes and differ substantially in others, e.g. in their responses to experimental induction of autoimmune conditions (Oboki et al. 2010). Therefore, depending on the research field, experimental settings and the read-out aimed for, the choice between ST2 and IL-33-deficient animals needs careful consideration. ST2-deficient animals might, however, still be the more suitable choice to investigate the extracellular cytokine function of IL-33. Knocking out the IL-33 gene itself affects both intracellular suppressive as well as extracellular stimulatory role, which might cause substantial difficulties when trying to assign certain experimental outcomes to one or the other function.

Evolutionary Development of the IL-33/ST2 Axes

Mostly due to ongoing host–pathogen co-evolution and a corresponding high positive selection pressure, immune system genes usually evolve faster than the genomic average (Schlenke and Begun 2003; Stein et al. 2007). Nevertheless, some highly conserved immune system genes appeared early during evolution and represent an ancient mechanism of protection against infection or endogenous danger by sensing pathogen or danger-associated molecular patterns (PAMPs or DAMPs) (Hansen et al. 2011). The prototypical DAMP, the alarmin HMGB-1 (Klune et al. 2008), has a striking 99 % protein homology between human and mouse and orthologous genes can still be found in *Caenorhabditis elegans* (45 % homology; homologies available from: NCBI HomoloGene at http://www.ncbi.nlm.nih.gov/sites/entrez?db=homologene). Due to its pro-inflammatory properties when released upon necrosis, IL-33 has been compared to HMGB-1 and classified as alarmin (Moussion et al. 2008). However, although IL-33 still shows a 54 % protein sequence identity between human and mouse, conservation is comparably low considering examples such as HMGB-1. Further, IL-33 sequences have not been reported in any species older than rodents. BLAST searches (available at: http://blast.ncbi.nlm.nih.gov/Blast.cgi) using the human IL-33 mRNA sequence against sequences available from evolutionary old mammalian phyla such as monotremes (taxid:9255) and marsupials (taxid:9263) do not reveal sequences with significant homology to IL-33. This indicates that IL-33 is an evolutionary young protein that might have arisen recently after the divergence of placental from non-placental mammals. In line with this, it has been suggested previously, that not all mammalian IL-1 family genes have orthologs in fish, suggesting a recent evolutionary origin of these genes (Huising et al. 2004).

Sequences homologous to human ST2 exist in mouse (protein homology 67 %) and have also been identified in birds (chicken, *Gallus gallus*) and fish (zebra fish, *Danio rerio*) with a protein identity of 43 and 34 %, respectively. Besides a higher conservation which suggests an important function, this indicates that ST2 is the evolutionary older protein. It might have fulfilled an IL-33-independent function before appearance of IL-33, and evolved to recognize extracellular IL-33 as an alarmin in order to be able to sense necrotic cell death in surrounding cells and initiate an appropriate immune response. In line with this, a cleaved and soluble form of ST2 (sST2), has been reported to bind to monocytes and dendritic cells (DC), where it inhibits lipopolysaccharide (LPS) signalling and subsequent effector functions (Nagata et al. 2012; Takezako et al. 2006). Further, sST2 has also been shown to be a possible mediator of tumour metastasis (Gillibert-Duplantier

et al. 2012). These functions seem to be independent of the availability of extracellular IL-33 as ligand, supporting the notion that ST2 might have been present and functional before the appearance of IL-33.

The Effect of IL-33 on Immune Cell Functions

Accumulating evidence suggests that the IL-33 receptor ST2L might be expressed on all innate immune cells, but only on selective populations of adaptive immune cells (Table 1). Accordingly, IL-33 directly or indirectly affects a broad range of immune cells. IL-33 treatment of naive mice induces splenomegaly, eosinophilia, increased serum IgE, IL-5 and IL-13 production and histopathological changes in lungs and gastrointestinal tract (Schmitz et al. 2005).

The Effect of IL-33 on Innate Immune Cells

Among the innate cells responding to IL-33 are mast cells (Allakhverdi et al. 2007), basophils (Suzukawa et al. 2008), eosinophils (Pecaric-Petkovic et al. 2009), macrophages (Kurowska-Stolarska et al. 2009), neutrophils (Alves-Filho et al. 2010), DC (Besnard et al. 2011) and innate lymphoid cells (iLC) (Chang et al. 2011). Neutrophils show enhanced recruitment to inflammatory sites and increased phagocytosis and killing activity in response to IL-33 (Alves-Filho et al. 2010; Le et al. 2012). Basophils and eosinophils respond to IL-33 (synergistically with IL-3 and/or FcεRI-activation) with secretion of IL-4, IL-13 and IL-8 and enhanced FcεRI-induced mediator release. All these responses are mediated by NF-κB activation (Pecaric-Petkovic et al. 2009). Local tissue mast cells are potent mediators of acute inflammation. They recognize IL-33 released from necrotic cells and thereby play a key role in responding to tissue and cell injury. Upon binding of IL-33 to their surface ST2L receptors, mast cells secrete pro-inflammatory leukotrienes and cytokines and initiate protective immune responses as well as crucial wound healing processes (Allakhverdi et al. 2007; Enoksson et al. 2011). Innate lymphoid cells, a group of recently described non-T, non-B iLC populations, increase their production of IL-13 upon IL-33 stimulation (Chang et al. 2011). Similar to mast cells, iLC have also been shown to function not only in first-line innate immunity and inflammation but also in tissue remodelling, repair and homeostasis (Spits and Cupedo 2012). Thus, in addition to immune defence mechanisms, IL-33 seems to induce cell types which are relevant during tissue repair processes, such as iLC and mast cells, and might therefore also play a role in preventing tissue damage and immunopathology after infectious or sterile insults.

Effector molecules secreted by innate immune cells strongly shape the type of the subsequently induced adaptive immune response. The majority of cytokines

Table 1 ST2 expressing immune cell types and their responses to IL-33

Cell type	IL-33 effect	References
Innate		
Mast cells	Release of leukotrienes and cytokines for immune responses and wound healing	Allakhverdi et al. (2007)
Basophils	IL-4, IL-13 and IL-8 production	Suzukawa et al. (2008)
Eosinophils	Enhanced FcεRI-induced mediator release	Pecaric-Petkovic et al. (2009)
Neutrophils	Enhanced neutrophil recruitment	Alves-Filho et al. (2010)
	Enhanced phagocytosis and killing activity	Le et al. (2012)
Dendritic cells	Induction of Th2 T cell-inducing phenotype	Besnard et al. (2011)
Macrophages	Induction of alternatively activated M2 phenotype	Hazlett et al. (2010), Kurowska-Stolarska et al. (2009), Nelson et al. (2011)
	Enhanced LPS-induced cytokines (e.g. TNF-α, IL-1β)	Espinassous et al. (2009)
NK/NKT cells	Increased TCR-triggered IFN-γ production	Bourgeois et al. (2009)
iLC	Increased production of IL-13	Chang et al. (2011), Neill et al. (2010), Price et al. (2010), Yasuda et al. (2012)
Adaptive		
Th2 cells	Increased release of Th2 cytokines	Lohning et al. (1998), Schmitz et al. (2005)
	Induction of chemotaxis	Xu et al. (1998)
	TCR-independent IL-13 production	Guo et al. (2009)
Tc1 cells	Increased TCR-triggered IFN-γ production and Tc1 effector functions in synergy with IL-12	Yang et al. (2011)
B1 cells	Proliferation IgM, IL-5 and IL-13 production	Komai-Koma et al. (2011)

secreted in response to IL-33 (e.g. IL-4, IL-13, IL-8) are known to induce Th2-polarization in adaptive immune cell types. IL-33 further changes the phenotype of alveolar macrophages towards an alternatively activated macrophage (AAM) phenotype (Kurowska-Stolarska et al. 2009) and induces DC, which can prime naive lymphocytes to produce type 2 cytokines (Besnard et al. 2011). However, IL-33 has also been demonstrated to activate innate immune cells to induce type 1 responses. The innate lymphocytic cell types, natural killer (NK) and NKT cells, respond to IL-33 with increased interferon (IFN)-γ release upon T cell receptor (TCR) engagement leading to a pro-Th1 effect (Bourgeois et al. 2009). This suggests that depending on the respective setting, IL-33-stimulated innate cells may have the capacity to initiate and amplify both Th1- and Th2-oriented immune responses (Smithgall et al. 2008).

The Effect of IL-33 on Adaptive Immune Cells

Besides affecting the adaptive immune system indirectly via the activation of innate immune cells, IL-33 can also directly drive adaptive responses. Th2 cells were the first adaptive immune cell type identified to express ST2L (Xu et al. 1998). IL-33 was then shown to increase Th2-associated effector functions (Lohning et al. 1998; Schmitz et al. 2005) and act as selective Th2 chemoattractant (Komai-Koma et al. 2007). Interestingly, signalling through ST2L is crucial for Th2 function but not for Th2 differentiation (Kropf et al. 2003) supporting the importance of IL-33-induced innate cell types in inducing naive T cells to differentiate into the Th2 lineage. Recently, $CD8^+$ type 1 cytotoxic T cells (Tc1) have been demonstrated to express ST2L and respond to IL-33. In these cells, IL-33 enhances TCR-triggered IFN-γ release and synergizes with IL-12 to promote $CD8^+$ T cell cytokine production and cytotoxic function (Yang et al. 2011). In contrast, both Th1 and Th17 cells do not seem to respond to IL-33 directly (Xu et al. 1998, 2008), suggesting selective effects of IL-33 on different T cell subsets (Guo et al. 2009). Among the B cell population, B1 cells have been shown to express ST2L and IL-33 activates B1 cell proliferation and enhances their IgM, IL-5, and IL-13 production in vitro and in vivo in an ST2L-dependent manner (Komai-Koma et al. 2011).

The Role of IL-33 in Host Immune Defence

Due to its potent effects on a wide range of immune cell types, a significant impact of IL-33 on various inflammatory conditions is not surprising. A vast amount of research has been performed to define the role of IL-33/ST2 in human inflammatory diseases in order to find possible therapeutic applications. This has been extensively reviewed before (Liew 2012; Liew et al. 2010). As shown in Table 2, IL-33 and its receptor ST2 have also been demonstrated to play significant roles in a variety of infectious settings, most prominently in Th2-polarized immune responses which seems evident when considering general IL-33 effects. IL-33 together with IL-3 acts on human basophils to induce IL-4 production (Pecaric-Petkovic et al. 2009; Suzukawa et al. 2008) and IL-33 also activates mast cells (Allakhverdi et al. 2007). Stimulation of Th2 cells with IL-33 together with thymic stromal lymphopoietin or other STAT5 activators results in TCR-independent IL-13 production (Guo et al. 2009). IL-13 induces ST2L expression on macrophages, rendering them responsive to IL-33, which in turn promotes differentiation into AAMs (Kurowska-Stolarska et al. 2009). All these are aspects of prototypic type 2 responses where Th2 cells are activated to produce cytokines including IL-4, IL-5, IL-9, IL-13 and IL-25. IL-4 regulates B cell class switching to IgE, which forms immune complexes and activates innate immune cells including basophils and mast cells by cross-linking high-affinity Fc receptors for IgE (FcεRI) on their surfaces. Activated basophils and mast cells secrete cytokines, chemokines, histamine, heparin, serotonin and proteases, which among others induces further inflammatory cell recruitment (reviewed in Paul and Zhu 2010). Thus, IL-33 seems the perfect candidate to initiate and amplify type 2 responses. In addition, however, a few reports indicate that IL-33 can also be involved in inducing type 1 and even regulatory responses.

As depicted in Fig. 1, different aspects of the IL-33/ST2 axes have been implicated in different steps during the induction of an immune response. (1) The expression of intracellular IL-33 is upregulated in response to a variety of exogenous or endogenous noxious stimuli. (2) The same agents can cause necrotic cell death and release of IL-33 into the extracellular space. (3) There, IL-33 functions as an alarmin by directly and indirectly activating a broad range of immune cells. (4) IL-33 binding to its receptor ST2L can be blocked by the soluble form of ST2, adding another possibility for regulation.

Regulation of Intracellular IL-33 by Inflammatory Agents

A potential role of intracellular IL-33 during infections has been suggested by several reports showing upregulation of IL-33 mRNA and/or protein levels after stimulation of various types of cells with PAMPs, such as bacterial LPS, CpG oligonucleotides and viral DNA mimics (Hudson et al. 2008; Nile et al. 2010; Polumuri et al. 2012; Shimosato et al. 2010; Zhang et al. 2011). Hudson et al.

Table 2 Implication of IL-33 and its receptor ST2L in host immune defence

Organism/experimental setting	Evidence for IL-33 involvement	References
Parasites		
Leishmania major	ST2L expressing CD4 T cells localize at site of infection	Kropf et al. (2002)
	ST2L signalling regulates excessive type 1 responses	Kropf et al. (2003)
Toxoplasma gondii	Infection upregulates ST2 mRNA	Jones et al. (2010)
	$ST2^{-/-}$ mice are more susceptible to infection	
Trichuris muris	Infection upregulates IL-33 expression	Humphreys et al. (2008)
	IL-33 induces parasite expulsion and secretion of TSL, IL-4, IL-9, and IL-13	
Nippostrongylus brasiliensis	iLC expand in response to IL-33 and are sufficient for worm clearance	Neill et al. (2010), Price et al. (2010)
Strongyloides venezuelensis	Infection induces pulmonary accumulation of iLC which proliferate and produce IL-5 and IL-13 in response to IL-33	Yasuda et al. (2012)
Bacteria		
Bacterial TLR agonists and other bacterial PAMP mimics	Upregulation of IL-33 mRNA	Hudson et al. (2008), Nile et al. (2010), Polumuri et al. (2012), Shimosato et al. (2010), Zhang et al. (2011)
Lipopolysaccharides	IL-33 enhances LPS-induced inflammatory cytokine production by macrophages	Espinassous et al. (2009)
Pseudomonas aeruginosa	IL-33 dampens inflammation and tissue damage due to M2 macrophage polarization resistance against keratitis	Hazlett et al. (2010)
Experimental sepsis	Increased neutrophil recruitment and bacterial clearance	Alves-Filho et al. (2010)
	Enhanced phagocytosis and killing activity	Le et al. (2012)
Leptospirosis	Increased levels of sST2 are associated with bleeding and mortality in leptospirosis	Wagenaar et al. (2009)
Viral TLR agonists and other viral PAMP mimics	Upregulation of IL-33 mRNA	Hudson et al. (2008), Polumuri et al. (2012)
Influenza virus	Upregulation of IL-33 mRNA correlates with increase in pro-inflammatory cytokines	Le Goffic et al. (2011)
Dengue virus	sST2 levels are associated with disease severity	Houghton-Trivino et al. (2010)
	Negative correlation between sST2 serum levels and platelet/white blood cell count	Becerra et al. (2008)
LCMV	IL-33 mediates protective antiviral $CD8^{+}$ T cell responses	Bonilla et al. (2012)
Influenza virus	Increased IL-33/ST2 expression levels	Le Goffic et al. (2011)
	$ST2^{-/-}$ infected mice have decreased lung function, loss of airway epithelial integrity and impaired respiratory tissue remodelling	Monticelli et al. (2011)
	Infection induces IL-33 production by alveolar macrophages	Chang et al. (2011)
Pneumocystis murina	IL-33 induced M2 macrophages cause enhanced fungal clearance	Nelson et al. (2011)
Candida albicans	IL-33 enhanced neutrophil recruitment and neutrophil effector functions	Le et al. (2012)
Alternaria alternata	Infection-induced ATP release induces IL-33 secretion	Chaturvedi et al. (2006)

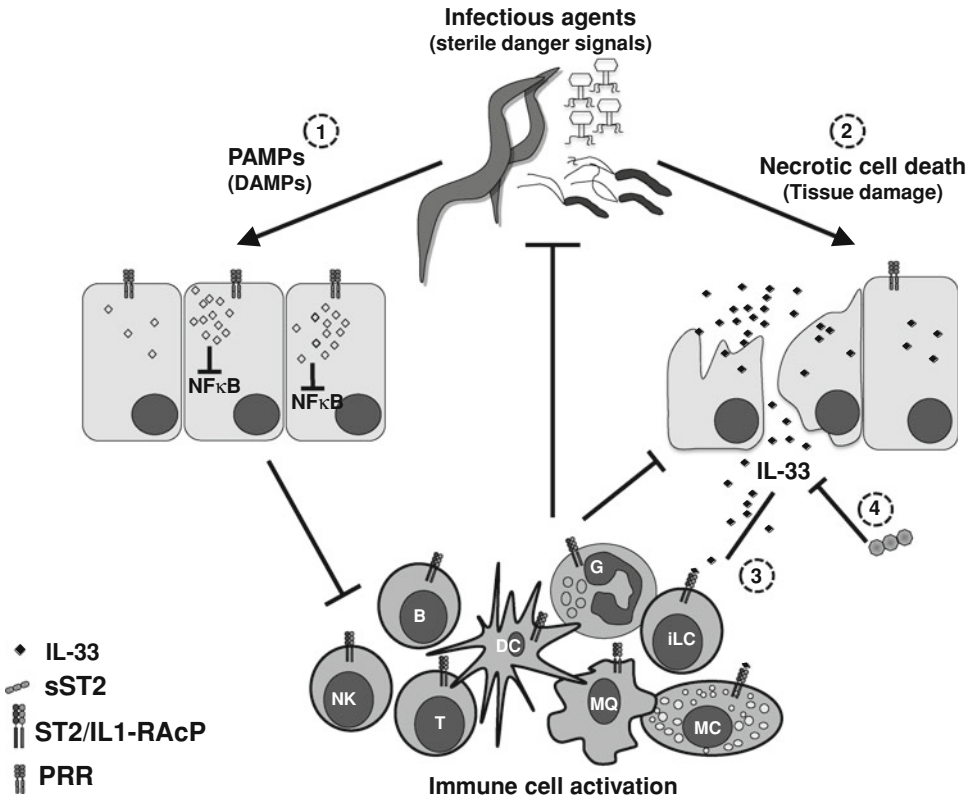

Fig. 1 IL-33 and its dual role during infections. *1* Cells upregulate intracellular IL-33 in response to molecules containing pathogen associated molecular patterns (PAMPs) and other pro-inflammatory stimuli. Intracellular IL-33 inhibits NF-κB and thereby prevents expression and release of pro-inflammatory cytokines as well as subsequent activation of an immune response. *2* At the same time, infectious (and other noxious) agents can cause cell and tissue damage leading to the release of intracellular IL-33 from the cells. *3* Extracellular IL-33 can bind to a variety of target cells expressing the IL-33 receptor (ST2L in a heterodimer with IL1-RAcP). In response to extracellular IL-33, these immune cells are activated, proliferate and exert their diverse effector functions in order to eliminate the initial infection and restore immune and tissue homeostasis. *4* Extracellular IL-33 can be sequestered by its decoy receptor sST2, adding a further regulatory mechanism. *DC* dendritic cells, *MQ* macrophages, *G* granulocytes, *B* B cells, *T* T cells, *NK* natural killer cells, *iLC* innate lymphoid cells, *MC* mast cells, *PRR* pattern recognition receptors

show that agonists of Toll-like receptor (TLR) ligands such LPS (TLR4), Pam3Cys (TLR2) and dsRNA (TLR3) as well as the pro-inflammatory cytokine IL-1β significantly increase IL-33 mRNA and protein expression in central nervous system glia (Hudson et al. 2008). Stimulation of splenocytes and peritoneal macrophages with CpG oligonucleotides (TLR9) also induced IL-33 mRNA expression (Shimosato et al. 2010) and human monocytes upregulate IL-33 mRNA and protein levels upon LPS stimulation (Nile et al. 2010). The TLR5 ligand flagellin induced IL-33 mRNA and protein upregulation in human corneal epithelium and cultured primary human corneal epithelial cells (Zhang et al. 2011). Finally, Polumuri et al. (2012) performed a comprehensive analysis of IL-33 transcriptional regulation by treating primary murine macrophages with a panel of TLR (TLR2, TLR3, TLR4 and TLR9) and non-TLR (MDA5, RIG-I) agonists. The corresponding results suggest a role for the transcription factor interferon regulatory factor-3 (IRF-3) and cAMP response element-binding protein but not protein kinase C or tyrosine kinases in the regulation of IL-33 expression. Although the various distinct stimuli used in this study activate entirely unrelated receptors in the cell membrane, endosome or cytosol, all these pathways lead to the activation of TANK-binding kinase 1 (TBK-1). TBK-1 activates transcription factor IRF-3, a key transcriptional activator in the innate immune system which is central to the transcriptional regulation of IFN-β and other pro-inflammatory cytokines (Polumuri et al. 2012).

Importantly, in addition to being upregulated in transcriptional studies in vitro using PAMP mimics, IL-33/ST2 expression levels have been demonstrated to be affected in vivo by infection with influenza virus (Le Goffic et al. 2011). Influenza A is a single-stranded RNA virus that can cause respiratory illness and severe lung tissue damage. Influenza activates infected cells to produce excessive and potentially lethal amounts of pro-inflammatory cytokines and chemokines, called the "cytokine storm" (Peiris et al.

2009). Infection with influenza A virus upregulates IL-33 mRNA in murine lungs, which positively correlates with a significant increase in the cytokines TNF-α, IFN-γ, IL-1β and IL-6. IL-33 protein is also upregulated in bronchoalveolar lavages as well as in alveolar epithelial and endothelial cells. Similarly, human epithelial cells upregulated IL-33 transcript levels after in vitro infection with different strains of influenza A virus (Le Goffic et al. 2011). Further, IL-33 mRNA and protein levels were increased in the brains of mice infected with the neurotropic virus Theiler's murine encephalomyelitis virus (Hudson et al. 2008) as well as in the gut during infection with the parasitic nematode *Trichuris muris* (Humphreys et al. 2008).

Care needs to be taken when expression is determined by mRNA levels only, as due to possible post-transcriptional processing and regulation these might not directly reflect protein levels. In general, however, it should be considered that changing expression levels of intracellular IL-33 protein primarily affect its role as transcriptional repressor. Intracellular upregulation of IL-33 in response to a broad range of inflammatory stimuli might be a negative feedback mechanism in order to suppress excessive inflammation. Only when upregulated or constitutively expressed IL-33 is released/secreted into the extracellular space, IL-33 can act as IL-1 family cytokine.

Release of IL-33 into the Extracellular Space

Data showing a direct effect of infection on the release of IL-33 from the infected cells is not available yet. However, infections cause tissue damage and IL-33 has been shown directly to be released upon cell necrosis and endothelial cell disruption mimicking tissue damage in experimental settings (Cayrol and Girard 2009; Luthi et al. 2009). Thus, it is feasible to assume a direct effect of infection-related tissue damage on IL-33 release. This makes IL-33 a prototypic alarmin; a protein with a potentially unrelated intracellular function, which the immune system evolved to recognize when released from cells in order to sense and appropriately react to surrounding danger. IL-33 is constitutively expressed in a variety of structural cell types (Moussion et al. 2008; Pichery et al. 2012) and upregulated in response to stimulation/infection (Polumuri et al. 2012). It is still a matter of controversy, whether IL-33 can also be actively secreted in addition to the major pathway of passive release. A few reports indicate that there might be ways of IL-33 release, which are independent of tissue damage and necrotic cell death. Kakkar et al. (2012) show that mechanical strain induced IL-33 secretion from murine fibroblasts in vitro and in vivo in the absence of cellular necrosis. Further, stimulation with adenosine triphosphate (ATP) might be involved in active IL-33 release. The environmental allergen, fungus *Alternaria alternata*, induces rapid release of ATP from airway epithelial cells, which in turn induces IL-33 release without evidence of cell death. Blocking purinergic receptors abrogates IL-33 release (Kouzaki et al. 2011). Further, brief ATP stimulation after PAMP treatment might induce active IL-33 secretion from central nervous system glia cells (Hudson et al. 2008). Although Hudson et al. (2008) did not confirm the absence of necrotic cell death, they provide evidence for active secretion by demonstrating that nuclear expression of IL-33 in living cells was decreased after ATP treatment. A similar stimulation has been shown previously to result in cell-lysis-independent secretion of IL-1α and might therefore also induce secretion of other IL-1 family cytokines without a caspase-1 cleavage site (Brough et al. 2002; Keller et al. 2008). ATP is considered an endogenous danger signal that can cause sterile inflammation in the absence of an infectious insult. Regulation of IL-33 release by ATP indicates that its role in host defence might not be limited to immune responses against infectious pathogens.

In summary, different passive as well as active mechanisms might be involved in the release of IL-33. If a system to detect extracellular IL-33 as alarmin is already in place, a way for active release of IL-33 in order to alarm surrounding cells without or before necrotic cell death has obvious evolutionary advantage.

The Role of Extracellular IL-33 and Signalling via ST2L

Once released into the extracellular space, IL-33 can act as alarmin and signal via its receptor ST2L. A considerable body of evidence suggests an important role of extracellular IL-33 and ST2L signalling in the immune defence against all kinds of pathogens including parasites, bacteria, viruses and fungi.

Parasitic Infection

Among the first reports demonstrating IL-33/ST2 involvement in host defence where reports of Th2 immune responses against parasites, including *Leishmania major* (Kropf et al. 2003), *T. muris* (Humphreys et al. 2008) and *Toxoplasma gondii* (Jones et al. 2010). *T. gondii* is a protozoan parasite which is ingested and then distributed throughout the body via the bloodstream. It can cause toxoplasmic encephalitis in immunocompromised patients, although acute infections of healthy individuals are mostly unsymptomatic (Robert-Gangneux and Darde 2012). Toxoplasma infection upregulates ST2L mRNA transcripts in the brain and ST2-deficient mice are significantly more susceptible to infection with *T. gondii*. Higher susceptibility correlates with increased pathology, greater parasite burden and increased levels of inducible nitric oxygen

species, IFN-γ and TNF-α, indicating that IL-33 signalling via ST2L plays a role in balancing Th1/Th2 responses for an effective control of parasite number and immunopathology (Jones et al. 2010). *Nippostrongylus brasiliensis* and *Strongyloides venezuelensis* are helminthic gastrointestinal parasitic worms in rodents, representative for human hookworms, which can cause protein and iron deficiency anaemia (Albonico and Savioli 1997). Innate lymphoid cells which have been demonstrated to expand in response to IL-33, represent the predominant early source of IL-13 during infection with *N. brasiliensis* and are sufficient for worm clearance (Neill et al. 2010; Price et al. 2010). Yasuda et al. (2012) recently confirmed a role for IL-33 in the activation of iLC during infection with the helminthic parasite *S. venezuelensis*. *S. venezuelensis* induces pulmonary accumulation of iLC, which proliferate and produce IL-5 and IL-13 in response to exogenous IL-33. Thereby, iLC in turn induce lung eosinophilic inflammation and help to expel worms. Endogenous IL-33 might be released from alveolar epithelial type II cells (ATII) which proliferate and upregulate IL-33 expression upon infection with *S. venezuelensis* (Yasuda et al. 2012). The parasitic nematode *T. muris* is a well-established model for the human gastrointestinal parasite *T. trichuria* (whipworm) and infects the large intestine where it embeds itself into the intestinal walls and causes considerable morbidity due to diarrhoea, iron deficiency anaemia and vitamin deficiencies (Antignano et al. 2011). During infection with *T. muris*, IL-33 mRNA expression has been shown to be upregulated in the gut and mice can be induced to expel the parasite by administration of exogenous IL-33. The immunological mechanism underlying this clinical outcome seems to be the IL-33-induced production of IL-4, IL-9 and IL-13 as well as epithelial cell-derived thymic stromal lymphopoietin. A thereby induced Th2 switch prevents an inappropriate parasite-specific Th1-polarized response and is essential for expulsion of the parasites (Humphreys et al. 2008).

Bacterial Infection

Besides being protective during worm infections, IL-33 involvement has also been suggested in bacterial infections and type 1 immune responses. Treatment of macrophages with extracellular IL-33 enhances LPS-induced inflammatory cytokine production (TNF-α, IL-6 and IL-1β) by increasing the expression of both LPS receptor components (myeloid differentiation protein 2 and TLR4), the soluble form of CD14 and the MyD88 adaptor molecule. In addition, IL-33 treatment also enhances the cytokine response to TLR2 (Espinassous et al. 2009). Interestingly, IL-33-dependent host protection during certain type 1 immune responses might be due to a rather regulatory effect of IL-33, as it has been suggested to be involved in dampening excessive type 1 pro-inflammatory responses and tissue damage. During infection with *Pseudomonas aeruginosa*, a Gram-negative bacterium and opportunistic human pathogen, IL-33 shifts macrophage polarization towards an M2 phenotype and thereby promotes resistance against keratitis, a major pathological side effect of an overactive Th1 immune response in *P. aeruginosa* infection (Hazlett et al. 2010). Treatment with IL-33 has also been shown to reduce mortality in mice with experimental sepsis, as IL-33-treated mice developed increased neutrophil influx into the peritoneal cavity and were therefore, more efficient in bacterial clearance. IL-33 induces increased neutrophil recruitment by preventing the TLR-induced downregulation of the neutrophil chemokine receptor CXCR2, which is crucial for recruitment of neutrophils from the circulation to the site of infection. Thereby, IL-33 caused an increased local but reduced systemic pro-inflammatory response (Alves-Filho et al. 2010). In contrast to that, ST2L signalling does not seem to be essential in pulmonary infection with *Mycobacterium tuberculosis*, as ST2-deficient mice display a normal host defence against this pathogen (Wieland et al. 2009). This indicates that the relevance of IL-33/ST2 signalling varies between different types of infections, possibly due to the different levels of IL-33 expressed within the infected cell type and the amount of cell death and tissue damage induced by the infection.

Viral Infection

IL-33 and ST2 also play significant roles during viral infections. As described above, during helminth infection ST2L expressing iLC have been shown to expand in response to IL-33 and produce IL-13 (Neill et al. 2010). iLC are also induced in murine lungs after infection with influenza A virus. The virus activates the NLRP3 inflammasome, which increases production of IL-33 by alveolar macrophages and in turn activates iLC to produce substantial amounts of IL-13 (Chang et al. 2011). Interestingly, IL-33-dependent induction of iLC has also been implicated in re-establishing tissue homeostasis in infected lungs. Blockage of ST2L signalling results in severely decreased lung function, loss of airway epithelial integrity and impaired respiratory tissue remodelling indicating a potential role of IL-33 in restoring tissue homeostasis (Monticelli et al. 2011). Thus, IL-33-induced iLC seem to be important not only in the initiation and effector phase of an immune response, but also in the subsequent resolution of inflammation and tissue repair. Therefore, it has been suggested that innate immune responses by iLC in general are a first line of defence coupled with regenerative potential, whereas exacerbation and disbalance causing

immunopathology seems to occur after involvement of adaptive T cells (Wilhelm and Stockinger 2011). Further, a direct protective effect of IL-33 during viral infections has been demonstrated, as IL-33 directly drives protective antiviral $CD8^+$ T cell (CTL) responses against lymphocytic choriomeningitis virus (LCMV) infection in mice. IL-33 signalling through ST2L on activated $CD8^+$ T cells enhanced clonal CTL expansion and was crucial for virus control. Efficient CTL responses after infection were dependent on the release of IL-33 from a radio-resistant and thus a non-hematopoietic cell type of the splenic T cell zone, likely fibroblastic reticular cells which are known targets of LCMV infection (Bonilla et al. 2012).

Fungal Infection

Finally, IL-33 has also been shown to have beneficial effects during host defence against fungal infections. Administration of exogenous IL-33 to mice infected with *Pneumocystis murina* resulted in enhanced fungal clearance from the lungs. This was due to enhanced anti-fungal activity of alveolar macrophages which in response to IL-33 switched to a M2 phenotype, as indicated by their expression of the M2 marker RELM-α and production of the chemokine CCL17 (Nelson et al. 2011). Further, during acute peritoneal infection with *Candida albicans*, IL-33 treatment induced rapid fungal clearance and reduced mortality. In this case, IL-33 enhanced phagocytosis and killing activity in neutrophils by enhancing recruitment, reversing TLR-induced CXCR2 downregulation and activating TLR and Dectin-1 signalling pathways (Le et al. 2012).

Taken together, extracellular IL-33 has a broad spectrum of functions and the final outcome of IL-33/ST2 signalling might depend on the initial type of response and the surrounding cytokine milieu of the respective inflammatory setting. By activating many different types of immune cells, including iLC, IL-33 seems to have a role in both immune resistance against the invading pathogen as well as in inducing host tolerance towards the infection in order to prevent detrimental immunopathology. Therefore, the IL-33/ST2 axes might be involved in maintaining an appropriate balance between efficient pathogen clearance and the level of resulting immunopathology which has been suggested to determine the optimal outcome of an immune response (Medzhitov et al. 2012).

Fine-Tuning of IL-33/ST2 Signalling by Soluble ST2

Extracellular IL-33 seems to have beneficial effects during the resistance against infections. However, any pro-inflammatory response needs to be tightly regulated in order to re-establish homeostasis after the infection has been cleared. Soluble ST2 might be part of this regulatory arm in the IL-33/ST2 system, as it blocks extracellular IL-33 and prevents further ST2L-mediated immune cell activation (Hayakawa et al. 2007). As so often the case, evidence for this arises from situations where the initially protective system seems to backfire and happens to be of disadvantage. Serum levels of sST2 have been shown to be significantly higher in mice that did not recover from experimental sepsis compared to those that did (Alves-Filho et al. 2010). In human patients with leptospirosis, which is caused by the spirochete bacteria *Leptospira* spp. inducing a biphasic disease starting with flu-like symptoms followed by meningitis, liver damage and renal failure, levels of sST2 are also associated with increased mortality (Wagenaar et al. 2009). A similar situation is found in dengue-infected patients. Dengue virus is a mosquito-borne single-stranded RNA virus that induces a human disease with a wide spectrum of clinical manifestations ranging from an acute self-limiting febrile illness (dengue fever) to severe disease (dengue hemorrhagic fever, DHF), which can result in a life-threatening syndrome (dengue shock syndrome) (Chaturvedi et al. 2006). In dengue patients with DHF sST2 levels are associated with disease severity as significantly higher levels of serum sST2 are observed in DHF patients compared to mild dengue fever patients and normal healthy control individuals (Houghton-Trivino et al. 2010). Further, a significant negative correlation was established between sST2 serum levels with platelet and white blood cell counts (Becerra et al. 2008). Assuming that sST2 mediates its effects via IL-33 under these inflammatory conditions, this suggests that IL-33 itself could have beneficial effects on disease development, but fails to do so as it is neutralized by increased levels of its decoy receptor sST2. However, by investigating sST2 levels only, it cannot be excluded that IL-33-independent mechanisms play a role as well.

Concluding Remarks

Interpretation of IL-33-related experimental data needs to integrate several layers of complex interactions. IL-33 in addition to its extracellular immune stimulatory capacity has an intracellular immune regulatory function. Its receptor ST2 exists in both a membrane-bound signalling form expressed on many immune cells and a soluble form which antagonises IL-33 function. Therefore, although IL-33 is generally considered a type 2 cytokine, the outcome of IL-33 signalling depends largely on the cytokine milieu of the respective disease condition or experimental setting. As depicted in Fig. 1, intracellular IL-33 is upregulated in response to PAMPs and DAMPs and inhibits NF-κB and,

therefore, NF-κB-dependent expression of pro-inflammatory cytokines (1). Simultaneously, tissue damage and necrotic cell death lead to the release of IL-33 into the extracellular space (2). Released IL-33 acts as alarmin and activates ST2L expressing innate and adaptive immune cells to exert their diverse effector functions in order to clear the initial insult, repair damaged tissues and re-establish homeostasis (3).

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