Interleukin-33, friend and foe in type-2 immune responses

Clare Hardman^a and Graham Ogg^{a*}

^aMRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, NIHR Biomedical Research Centre, Radcliffe Department of Medicine, University of Oxford, Oxford, OX3 9DS, UK

*Corresponding author

Graham Ogg

MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Oxford, OX3 9DS, UK

Tel 0044 1865 222334

Fax 0044 1865 222502

Email address: graham.ogg@ndm.ox.ac.uk

Abstract

IL-33 is the most recent addition to the IL-1 cytokine family, identified in 2005 as the ligand of T1/ST2 and inducer of type-2 immune responses. IL-33 has been implicated in a wide range of disease settings, in anti-inflammatory responses and homeostasis, and thus signalling must be strictly regulated. Altered gene expression, post-translational modification, decoy receptor, and receptor signalling are all modulatory mechanisms used to control the IL-33 pathway. Understanding both the genetic and post-translational factors influencing IL-33 activity will be critical for provision of safe effective treatment of type-2 disorders.

Introduction

Discovered in 2005, as the ligand for TLR/IL1R super family receptor ST2, interleukin-33 (IL-33) is the most recent addition to the IL-1 cytokine family[1]. IL-33 is structurally similar to other IL-1 family members, with the published crystal structure showing the characteristic β -trefoil barrel at the C terminus, which is critical for classical cytokine receptor binding and signalling via MAP kinase or NF- κ B [2]. Through heterotrimeric binding to T1/ST2 and IL-1RAcP, IL-33 exerts influence over innate and adaptive type-2 immune responses.

IL-33 is described as a dual-function protein, because in addition to the C-terminal cytokine domain, IL-33 contains a nuclear localisation sequence and chromatin-binding domain at the N-terminus, allowing IL-33 to bind heterochromatin in the nucleus[3]. Based on the molecular mimicry observed between IL-33 and a Kaposi sarcoma herpes virus motif, IL-33 was thought to act as a transcriptional repressor, although specific gene targets were elusive[4]. However, emerging evidence suggests that IL-33 may act to repress transcription of the soluble IL-33 decoy receptor, sST2, and IL-6[5], and binds to the regulatory domains of the *IL13* gene, inducing expression[6].

IL-33 lacks a secretory signal peptide, an IL-1 family trait, which would mark the protein for release by the classical endoplasmic reticulum and Golgi pathway[1]. IL-33 is found preformed in the nucleus under steady state conditions, and as extracellular binding to ST2 is required for signalling, IL-33 is believed to act as an alarmin, signalling danger to the immune system. Indeed IL-33 is rapidly, passively released from damaged cells in response to stress conditions such as infection, injury and inflammation[7], detected in the asthmatic lung only during active disease[8] and following Influenza infection[9]. However, recent evidence suggests that IL-33 could be released without associated cellular necrosis. Mechanical stress induces release of biologically active IL-33 from fibroblasts[10]; and fungal aeroallergen challenge induces release of ATP from airway epithelial cells, which in an autocrine manner stimulates IL-33 secretion through triggering of P2 purinergic receptors[11].

Genome-wide association studies (GWAS) and murine models have implicated IL-33 signalling in numerous wide-ranging diseases including allergy[12], inflammatory bowel disease (IBD)[13], cardiac disease[14], rheumatological disease[15] and type-2 diabetes[16]. Candidate gene studies and GWAS showed that the *IL33* and ST2 (encoded by *IL1RL1*) loci, but not genes encoding associated adaptor or signalling molecules, are important for allergic asthma development. All eight identified asthma-associated SNPs are situated 5' of the gene, on chromosome 9, or within the first intron[17]. The *IL1RL1* gene was first associated with allergy when the SNP haplotype rs11685480, rs6543115, and rs6543116, which results in higher ST2 expression, was linked to atopic dermatitis[18]. Since then genetic studies have correlated 15 different SNPs in the *IL1RL1* gene with asthma[19]. It is likely that these studies may prove important in future approaches to predict treatment responses to IL-33 pathway modulation.

Expression of IL-33 and ST2

IL-33 was first identified in high endothelial venules, giving rise to the original nomenclature NF-HEV [nuclear factor (NF) in high endothelial venules (HEV)], where it was found to act as a transcriptional repressor[3]. Since then, high expression of IL-33 has been observed in the lung, GI tract, skin and brain[1], where a range of non-hematopoietic cells express the cytokine. IL-33 has been reported in epithelial cells of mucosal sites[20], fibroblasts[21], keratinocytes[22], SMC[23] and endothelial cells[24], thus bolstering the emerging view of such structural cells as an active part of the immune system, and not simply as a passive structural barrier. More recently cells of haematopoietic origin

have been observed to express IL-33, including mast cells[25], DCs[26], macrophages[27] and monocytes[28].

The action of IL-33 in inducing type-2 immune responses is coordinated through binding to T1/ST2, a complex of IL-1RL1/ST2 and IL-1RAcP accessory protein[29]. Many immune cells are responsive to IL-33 and carry cell surface T1/ST2. The main effects of IL-33 are in inducing type-2 immunity, including activation and accumulation of ILC2[30], Th2 cells and M2 polarised macrophages[31]. ST2 expression has also been documented on mast cells, eosinophils, basophils and iNKT cells. Recently an exciting role for IL-33 signalling has been suggested in activating ST2⁺ Tregs[32] and regulating Th1 and Th17 cells[33].

T1/ST2 is composed of an extracellular region of three Ig-like domains, a transmembrane region, and an intracellular Toll-like/IL-1 receptor domain. Upon ligand binding, MyD88 and subsequently IRAK1 and IRAK4 are recruited to the membrane signalling complex, whereupon phosphorylation of downstream molecules NF- κ B or MAPK pathways are activated[3], resulting in gene expression of targets, including type-2 cytokine genes. Through alternative splicing, three possible ST2 transcripts are expressed, the long isoform encoding the full cell-surface receptor ST2L and a short isoform encoding the soluble protein sST2, which acts as a decoy receptor by sequestering IL-33. Indeed treatment of mice with sST2 prior to induction of an allergic asthma model suppressed IL-33 induced NF- κ B activity and IL-4, IL-5 and IL-13 expression[34]. Finally a less well-characterised variant known as ST2V, with as yet undefined function, has been identified within the gut[35].

Regulation and Release of IL-33

Originally IL-33, synthesised as a 30 kDa peptide, was thought to be cleaved at a caspase-1 site in the IL-1-like domain to form an 18 kDa mature peptide[36]. Indeed typical IL-1 family members require caspase-1 cleavage to form biologically active cytokine. However, more recently it was determined that caspase cleavage of IL-33 is not required for biological activity *in vivo*[37]. Interestingly, although cleavage of IL-33 is not necessary for activity, protease processing of IL-33 can produce highly activated isoforms. Such proteases have been observed within fungal[38], pollen, house-dust mite and cockroach allergens, and pathogens. Protease action may aid delivery across epithelial barriers or directly activate the allergic immune response via protease-activated receptors (PARs)[39]. In addition, self-enzymes produced by neutrophils and mast cells[39-41] can enhance IL-33 activity. During inflammation, neutrophils secrete proteases including elastase and cathepsin, which cleave IL-33 within the central domain and generate mature isoforms of IL-33 with 10-fold increased bioactivity[39]. Mast cell derived serine proteases, chymase and tryptase can cleave IL-33 in the central domain releasing five hyperactive isoforms, with up to 30 times increased potency in the activation of ILC2 and eosinophils[40]. Indeed granzyme-B[42], can also be released from cells including mast cells and processes IL-33 into mature peptides. The enhanced activity of these isoforms of IL-33, may stem from release of the C-terminal cytokine domain from an inhibitory effect of the N-terminus, as occurs with other IL-1 family members, potentially due to altered steric hindrance or polarity[40]. The effects of various proteases on IL-33 activity may be regulated spatially, with mast cell proteases enhancing activation of IL-33 in the lung and during allergic inflammation, and neutrophil derived proteases being important for viral infections.

As with all alarmins, a tight mechanism of control is required to regulate IL-33 signalling and prevent excessive damage-causing inflammation. This is particularly necessary as there is evidence that IL-33 signalling is self-potentiated, in a positive feedback loop downstream of ST2[20] and by inhibiting sST2 expression[5]. Full-length active IL-33 is released upon cellular necrosis, as inflammatory

caspases 1, 4 and 5 cannot degrade IL-33. However, upon programmed cell death, IL-33 is cleaved, within the cytokine domain, by caspases 3 and 7 and IL-33 signalling is abrogated[43]. Therefore specific caspases regulate IL-33 signalling, suppressing its alarmin function during apoptosis. Additionally, upon stimulation with HDM allergen, mast cells release chymase, which has been shown to be capable of cleaving IL-33 and abrogating binding to ST2, thus controlling alarmin function[41,44]. Furthermore an intriguing recent report proposed that oxidation rapidly inactivates IL-33 and could act as a switch to regulate inflammation in the lung. IL-33 signalling is ceased upon oxidation, when two disulphide bridges are formed between four free cysteines. These covalent bonds induce a conformational change that distorts the ST2 binding site. Oxidised IL-33 was observed in the lungs of mice challenged with fungal allergen and in sputum of asthmatic patients. Disturbance of this oxidative regulatory mechanism augmented lung inflammation *in vivo*[8]. Other mechanisms which regulate IL-33 subcellular location and release are likely to emerge, and may represent novel therapeutic targets.

Dichotomous Functions of IL-33 in Health and Disease

The IL-33:ST2 axis is involved in the immunopathology of disorders including atopic dermatitis (AD), asthma[45], IBD[46], and arthritis[47]. Furthermore IL-33 signalling is suggested to have protective effects in cardiac disease[48]. The best-characterised effect of IL-33, upon activating the diverse array of cells bearing ST2, is the initiation of innate and adaptive type-2 immune responses, characterised by the production of IL-4, IL-5, and IL-13. Exogenous administration of IL-33 to mice induces type-2 cytokines, serum IgE, eosinophilia, Th2 and ILC2 accumulation and activation, epithelial cell hyperplasia, excessive mucus production and smooth muscle contraction[49,50] (Figure 1). In response to IL-33, mast cells and basophils are activated, and exhibit enhanced type-2 cytokine production. In addition IL-33 can regulate Th17 and Th1-coordinated inflammation, including neutrophil and anti-viral T-cell responses. The effects of IL-33 in health and disease are highly tissue specific and influenced by the microenvironment, chemically and temporally.

Role of IL-33 in the Gut

The predominant source of IL-33 in the gut is thought to be intestinal epithelial cells. IL-33 has been shown to be important in the expulsion of parasites, and along with ST2, is markedly upregulated in IBD, specifically ulcerative colitis (UC). Interestingly, in the gut IL-33 drives both pathogenic Th2 and Th17 responses, while also promoting wound healing of mucosa damaged during the inflammatory response.

Type-2 immune responses evolved in part to protect the host from parasitic infections. IL-33 is a strong driving force in the expulsion of helminth through the instigation of this response. IL-33 promotes barrier defence through enhanced epithelial cell hyperplasia and by a process known as “weep-and-sweep”, inducing mucus secretion and smooth muscle contractions. Critically IL-33 activates gut ILC2 that are essential for efficient helminth clearance, through IL-13 secretion[30]. Intestinal IL-33 expression and release may be induced by epithelial damage caused by the physical invasion of parasitic worms[51].

Recently high expression of IL-33 and ST2 have been observed in inflamed lesions and blood of IBD patients during active disease, with IL-33 level correlating with severity[46]. Epithelial cells of non-inflamed colon express high levels of ST2. During chronic inflammation epithelial ST2 expression is diminished, and ST2⁺ haematopoietic cells, including antigen-presenting cells, Th cells, and possibly ILC2, infiltrate the lamina propria[52]. Notably, induction of IL-33 can occur downstream of TNF α , a pathogenic cytokine in IBD. Indeed anti-TNF α therapy increases sST2 levels, reducing circulating IL-33[46]. Two IL-33 polymorphisms have highlighted different aspects of IL-33 signalling in IBD. The

rs3939286 SNP in IL-33 is associated with severe colitis and has been linked to type-2 inflammation, including eosinophilia, nasal polyposis and asthma[53]. Although during early stages of UC, the rs3939286 variant favours a pathogenic type-2 response, the SNP may also provide an effective therapeutic avenue, conferring steroid responsiveness. Steroid treatment inhibits secretion of pro-inflammatory cytokines, such as TNF α , and the resulting increase of IL-33 in rs3939286 individuals promotes healing of the epithelial barrier and disease amelioration[13]. A second polymorphism, rs13015714, polarises the effects of IL-33 towards inducing type-1 inflammatory Crohn's disease. Indeed in arthritis, administration of anti-ST2 antibody reduced interferon-gamma and IL-17 expression, ameliorating disease severity[54]. Alternatively rs13015714 may impair IL-33 binding to ST2, biasing the response to type-1 immunity, promoting IL-33 driven ST2-independent Th1/Th17 immunity[55].

In the intestine, both protective and pathologic roles of IL-33 have been described in murine models of acute colitis[56-58]. Different animal models produce conflicting results, confounding the complexity of the roles of IL-33 in IBD. IL-33 potently induces production of inflammatory IL-5, IL-6 and IL-17, in SAMP1/YitFc mice, a spontaneous model of IBD with aspects of Th1 and Th2 immunity[59]. Furthermore, exogenous administration of IL-33 showed a protective effect in trinitrobenzene sulfonic acid chemically-induced colitis, which is a type-1 immune model[56]. Even within one model, IL-33 can have apparently contradictory effects. Following epithelial barrier disruption with DSS, IL-33 worsens colitis increasing neutrophilia and inflammation. However, during resolution, IL-33 promoted epithelial barrier function by wound healing and goblet cell proliferation[57].

IL-33 Promotes Response Resolution and Wound Healing

An exciting recent study has implicated IL-33 in wound resolution within in the gut. Colonic T_{reg} cells express ST2, where it promotes T_{reg} function and identity. IL-33 acts as a co-factor enhancing TGF- β ₁ mediated differentiation of T_{reg} cells, promoting accumulation in inflamed tissues. During murine helicobacter infection sST2 was significantly increased, as in patients with active IBD, suggesting pathogenic inflammation may regulate IL-33 activity to limit T_{reg} responses. Indeed, *Il1rl1*^{-/-} T_{reg} cells showed diminished ability to prevent colonic inflammation, and IL-23, an important mediator of IBD, abrogated T_{reg} responses by inhibiting ST2 expression[32]. Furthermore, another study suggested that IL-33 could additionally promote T_{reg} responses through activation of ILC2 in adipose tissue during homeostasis and helminth infection[60].

An accumulation of evidence points to IL-33 as an important regulator of wound healing, in the lung, skin and gut, as discussed above. The level of IL-33 is significantly increased at wound sites, and exogenous application of IL-33 was shown to enhance healing, promoting deposition of extracellular matrix components including collagen and fibronectin. IL-33 induced accumulation of alternatively activated macrophages in the wounded tissue. In addition to epithelial hyperplasia, IL-33 administration has an anti-bacterial effect in healing infectious wounds, potentially through proliferation and recruitment of neutrophils[61]. In addition to direct wound healing effects, upon cutaneous injury IL-33 initiates an ILC2 response, which induces re-epithelialisation, closing the wound. ILC2 have also been observed to contribute to re-epithelialisation after influenza infection, in part through the secretion of the epidermal growth factor amphiregulin[62] (Figure 1).

IL-33 and Allergy

IL-33 has been shown to be influential in the immunopathology of many allergic diseases. Aberrant IL-33 signalling, among other effects, results in uncontrolled inflammation, mucus production,

epithelial hyperplasia and immune cell infiltration characteristic of atopic triad diseases. IL-33 is necessary for the development of murine ragweed pollen models of allergic rhinitis. Nasal epithelial cell-derived IL-33 activates mast cell histamine release and induces basophil cytokine and chemokine production, resulting in eosinophilia and nasal inflammation[63]. Genetic studies originally linked IL-33 to atopic dermatitis, and IL-33 and ST2 upregulation is observed in skin lesions of AD patients[64,65]. Furthermore transgenic mice over-expressing IL-33 under the control of the keratin14 promoter showed symptoms of AD including infiltration and activation of ILC2 and mast cells, and release of type-2 cytokines, IgE and histamine[66]. ILC2 expressing ST2 are enriched in lesional skin of AD patients, and express IL-5, IL-6, IL-13 and amphiregulin upon stimulation with IL-33, which also acts as a chemoattractant for ILC2[67].

The role of IL-33 in allergic asthma has been relatively well studied. ST2^{-/-} knock out mice[68] demonstrate abrogated type-2 immune responses, reducing eosinophilia, antigen specific Th2 cytokine production, airways hyperresponsiveness (AHR), mucus production, and smooth muscle contraction[69]. IL-33 protein is increased in bronchoalveolar lavage, airway epithelial cells and smooth muscle cells of asthmatic patients, positively correlating with disease severity[23]. In murine models IL-33 is rapidly released following activation or damage of alveolar epithelial cells by, for example, protease action of fungal allergens[38]. Upon release IL-33 rapidly and potently activates ILC2 as a major source of IL-5 and IL-13[70]. Indeed ILC2 are sufficient in experimental models of allergic asthma to potentiate type-2 immune responses and promote AHR[49].

IL-33 and Viral Responses

Anti-viral immunity comprises both Th1 and Th2 responses and IL-33 may play a role in the balance of these responses. IL-33 has been shown to be necessary for potent anti-viral CD8⁺ T-cell responses. Indeed IL-33 deficient mice have diminished IFN γ ⁺ $\gamma\delta$ T cell, NK cell and DC responses, but increased IL-17⁺ $\gamma\delta$ T cells following lymphocytic choriomeningitis virus infection[71]. Furthermore, influenza infection up-regulates IL-33 in the alveolar epithelium, endothelial cells and macrophages, and correlated with a significant increase in TNF α , IFN γ / β , IL-1 β , and IL-6[9]. Virally induced IL-33 activates ILC2 to secrete IL-13, which along with IL-33, directly induces AHR through mucus secretion and muscle contraction. Additionally rhinovirus induces IL-33, promoting ILC2 and type-2 cytokine production in an asthma exacerbation model[72], thus providing a mechanism by which viral infection promotes type-2 allergic immunity in asthmatic patients. Conversely, during a state of vaginal microbiota dysbiosis, induced by oral antibiotics, IL-33 is secreted from damaged mucosal epithelia and the resulting infiltrating ILC2 and eosinophils contribute to suppression of anti-viral T cell IFN γ production, delaying clearance of HSV2 infection[73].

Therapeutic Potential of Targeting IL-33

It is important to consider IL-33 as part of a triad of cytokines that induce type-2 immune responses. Considerable functional redundancy is observed between IL-33 and IL-25 and TSLP and all three are expressed by epithelial cells at mucosal barrier tissues. Akin to ST2, IL-25 and TSLP receptors are expressed on Th2 cells, ILC2, mast cells and basophils, with ligand binding inducing proliferation and type-2 cytokine production. However, of the three cytokine genes only *Il33* has been linked to asthma by GWAS[74]. Indeed during AD, IL-33 induces TSLP expression by keratinocytes, via an Egr-1-dependent mechanism, making it difficult to distinguish the individual roles of these cytokines[75].

Interestingly, it has been proposed that two ILC2 subsets exist, natural (n)ILC2 and inflammatory (i)ILC2. IL-33-responsive 'nILC2' are mucosal tissue-resident and secrete IL-13 rapidly upon helminth

infection, whereas IL-25-responsive 'iILC2' were only detected upon stimulation. Of note, over the course of the anti-helminth response, iILC2 became more nILC2-like, thus whether distinct developmental pathways or temporal phases of ILC2 biology, the data highlight the complexity within IL-25/IL-33 redundancy[76]. Further studies comparing IL-25 and IL-33 receptor deficient mice demonstrate that IL-33 more rapidly and potently induces AHR and ILC2-derived IL-13, than IL-25[70]. Indeed IL-33, unlike IL-25, can directly induce smooth muscle cell contraction, contributing profoundly to AHR and can bind directly to the *Il13* gene locus inducing expression[6] and further amplifying the response.

Such data suggest that IL-33 is a promising therapeutic target for asthma, dermatitis, rhinitis, allergy and other type-2 disorders. However, as discussed IL-33 plays a wide range of roles in many beneficial settings including wound healing, anti-viral immunity, response resolution and appears to be protective for cardiovascular disease. Whilst it is difficult to predict how these will balance in interventional studies in humans, there is sufficient support for further evaluation, particularly as risks can be mitigated through better understanding of differential functions for post-translationally modified forms of IL-33 and ST2. Indeed monoclonal antibodies raised against IL-33 have proved successful in mice, ameliorating symptoms of experimental allergic asthma and allergic rhinitis models[77,78]. Alternatively, blocking IL-33 signalling with anti-T1/ST2 antibodies in an experimental allergic asthma model inhibited Th2 responses[79]; such an approach may be an alternative for inhibiting IL-33-induced inflammation. Anti-T1/ST2 antibodies would bypass adverse side effects of interfering with the transcription modulation roles of IL-33, which are as of yet not fully characterised. However it may be possible that ST2 has alternative ligands which are yet to be identified and understood. In addition selectively targeting the enhanced forms of IL-33, generated by protease action, using protease inhibitors within the lung may provide an interesting potential therapeutic route in the treatment of allergy.

Conclusions

The role of IL-33 in inflammatory immune responses is complex. In the lung IL-33 induces ILC2, Th2 cells, IL-5, IL-13 and eosinophilia[68], yet in the skin IL-33 recruits neutrophils, ILC2, Th2 cells, mast cells and induces TNF α production[80], and intriguingly within the peritoneal cavity IL-33 induces B cells to produce the anti-inflammatory cytokine IL-10[81]. Furthermore SNPs within ST2 and IL-33 genes affect the way patients respond during inflammatory disorders. For example, IBD patients with rs3939286 SNP in the *Il33* gene respond more effectively to steroid treatment than patients with the wild-type allele. Further investigation into the spatial and temporal regulation of IL-33 signalling and genetic variation associated with type-2 immune disorders, may be the key to providing effective, safe, personalised treatment.

Acknowledgements

We are grateful for funding from the MRC and the NIHR Biomedical Research Centre Programme for funding. We also acknowledge the support of the National Institute for Health Research Clinical Research Network and Barrie Trust.

References:

1. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, et al.: **IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines.** *Immunity* 2005, **23**:479-490.

2. Lingel A, Weiss TM, Niebuhr M, Pan B, Appleton BA, Wiesmann C, Bazan JF, Fairbrother WJ: **Structure of IL-33 and its interaction with the ST2 and IL-1RAcP receptors--insight into heterotrimeric IL-1 signaling complexes.** *Structure* 2009, **17**:1398-1410.
3. Carriere V, Roussel L, Ortega N, Lacorre DA, Americh L, Aguilar L, Bouche G, Girard JP: **IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo.** *Proc Natl Acad Sci U S A* 2007, **104**:282-287.
4. Roussel L, Erard M, Cayrol C, Girard JP: **Molecular mimicry between IL-33 and KSHV for attachment to chromatin through the H2A-H2B acidic pocket.** *EMBO Rep* 2008, **9**:1006-1012.
- **5. Shao D, Perros F, Caramori G, Meng C, Dormuller P, Chou PC, Church C, Papi A, Casolari P, Welsh D, et al.: **Nuclear IL-33 regulates soluble ST2 receptor and IL-6 expression in primary human arterial endothelial cells and is decreased in idiopathic pulmonary arterial hypertension.** *Biochem Biophys Res Commun* 2014, **451**:8-14.
Using siRNA the authors knocked-down IL-33 expression in human endothelial cells and observed increased IL-6 and sST2 expression. IL-33 was found to bind to the promoter regions of sST2 by chromatin immunoprecipitation (ChIP).
- **6. Ni Y, Tao L, Chen C, Song H, Li Z, Gao Y, Nie J, Piccioni M, Shi G, Li B: **The Deubiquitinase USP17 Regulates the Stability and Nuclear Function of IL-33.** *Int J Mol Sci* 2015, **16**:27956-27966.
The authors identify *IL13* as a target gene of IL-33 acting as a transcription factor. Using a ChIP assay IL-33 was shown to bind to the *IL13* locus and was regulated by deubiquitination.
7. Moussion C, Ortega N, Girard JP: **The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'?** *PLoS One* 2008, **3**:e3331.
- **8. Cohen ES, Scott IC, Majithiya JB, Rapley L, Kemp BP, England E, Rees DG, Overed-Sayer CL, Woods J, Bond NJ, et al.: **Oxidation of the alarmin IL-33 regulates ST2-dependent inflammation.** *Nat Commun* 2015, **6**:8327.
The authors describe a novel mechanism for the regulation of IL-33 signalling through oxidation of free cysteine residues. Both oxidised and reduced IL-33 were detected in the lungs of allergen challenged mice and the authors demonstrated that oxidised IL-33 did not bind ST2.
9. Le Goffic R, Arshad MI, Rauch M, L'Helgoualc'h A, Delmas B, Piquet-Pellorce C, Samson M: **Infection with influenza virus induces IL-33 in murine lungs.** *Am J Respir Cell Mol Biol* 2011, **45**:1125-1132.
10. Kakkar R, Hei H, Dobner S, Lee RT: **Interleukin 33 as a mechanically responsive cytokine secreted by living cells.** *J Biol Chem* 2012, **287**:6941-6948.
11. Kouzaki H, Iijima K, Kobayashi T, O'Grady SM, Kita H: **The danger signal, extracellular ATP, is a sensor for an airborne allergen and triggers IL-33 release and innate Th2-type responses.** *J Immunol* 2011, **186**:4375-4387.
12. Holtzman MJ, Byers DE, Brett JA, Patel AC, Agapov E, Jin X, Wu K: **Linking acute infection to chronic lung disease. The role of IL-33-expressing epithelial progenitor cells.** *Ann Am Thorac Soc* 2014, **11 Suppl 5**:S287-291.
13. Latiano A, Palmieri O, Pastorelli L, Vecchi M, Pizarro TT, Bossa F, Merla G, Augello B, Latiano T, Corritore G, et al.: **Associations between genetic polymorphisms in IL-33, IL1R1 and risk for inflammatory bowel disease.** *PLoS One* 2013, **8**:e62144.
14. Chen WY, Hong J, Gannon J, Kakkar R, Lee RT: **Myocardial pressure overload induces systemic inflammation through endothelial cell IL-33.** *Proc Natl Acad Sci U S A* 2015, **112**:7249-7254.
15. Verri WA, Jr., Souto FO, Vieira SM, Almeida SC, Fukada SY, Xu D, Alves-Filho JC, Cunha TM, Guerrero AT, Mattos-Guimaraes RB, et al.: **IL-33 induces neutrophil migration in rheumatoid arthritis and is a target of anti-TNF therapy.** *Ann Rheum Dis* 2010, **69**:1697-1703.

16. Wen Q, Wu F, Yang J, Wu J, Zhang X, He M, Wu T, Cheng L: **A single-nucleotide polymorphism in the interleukin-1 receptor-associated protein gene is associated with impaired glucose regulation and type 2 diabetes in a case-controlled study.** *Biomed Rep* 2015, **3**:549-553.
17. Ho JE, Chen WY, Chen MH, Larson MG, McCabe EL, Cheng S, Ghorbani A, Coglianese E, Emilsson V, Johnson AD, et al.: **Common genetic variation at the IL1RL1 locus regulates IL-33/ST2 signaling.** *J Clin Invest* 2013, **123**:4208-4218.
18. Shimizu M, Matsuda A, Yanagisawa K, Hirota T, Akahoshi M, Inomata N, Ebe K, Tanaka K, Sugiura H, Nakashima K, et al.: **Functional SNPs in the distal promoter of the ST2 gene are associated with atopic dermatitis.** *Hum Mol Genet* 2005, **14**:2919-2927.
- *19. Grotenboer NS, Ketelaar ME, Koppelman GH, Nawijn MC: **Decoding asthma: translating genetic variation in IL33 and IL1RL1 into disease pathophysiology.** *J Allergy Clin Immunol* 2013, **131**:856-865.
This review provides a comprehensive summary of the polymorphisms of *Il33* and *Il1rl1* associated with asthma.
20. Hardman CS, Panova V, McKenzie AN: **IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation.** *Eur J Immunol* 2013, **43**:488-498.
21. Nomura K, Kojima T, Fuchimoto J, Obata K, Keira T, Himi T, Sawada N: **Regulation of interleukin-33 and thymic stromal lymphopoietin in human nasal fibroblasts by proinflammatory cytokines.** *Laryngoscope* 2012, **122**:1185-1192.
22. Byrne SN, Beaugie C, O'Sullivan C, Leighton S, Halliday GM: **The immune-modulating cytokine and endogenous Alarmin interleukin-33 is upregulated in skin exposed to inflammatory UVB radiation.** *Am J Pathol* 2011, **179**:211-222.
23. Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemiere C, Martin JG, Hamid Q: **Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells.** *J Immunol* 2009, **183**:5094-5103.
24. Pichery M, Mirey E, Mercier P, Lefrancais E, Dujardin A, Ortega N, Girard JP: **Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel IL-33-LacZ gene trap reporter strain.** *J Immunol* 2012, **188**:3488-3495.
25. Hsu CL, Bryce PJ: **Inducible IL-33 expression by mast cells is regulated by a calcium-dependent pathway.** *J Immunol* 2012, **189**:3421-3429.
26. Williams JW, Tjota MY, Clay BS, Vander Lugt B, Bandukwala HS, Hrusch CL, Decker DC, Blaine KM, Fixsen BR, Singh H, et al.: **Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation.** *Nat Commun* 2013, **4**:2990.
27. Polumuri SK, Jayakar GG, Shirey KA, Roberts ZJ, Perkins DJ, Pitha PM, Vogel SN: **Transcriptional regulation of murine IL-33 by TLR and non-TLR agonists.** *J Immunol* 2012, **189**:50-60.
28. Nile CJ, Barksby E, Jitprasertwong P, Preshaw PM, Taylor JJ: **Expression and regulation of interleukin-33 in human monocytes.** *Immunology* 2010, **130**:172-180.
29. Liu X, Hammel M, He Y, Tainer JA, Jeng US, Zhang L, Wang S, Wang X: **Structural insights into the interaction of IL-33 with its receptors.** *Proc Natl Acad Sci U S A* 2013, **110**:14918-14923.
30. Neill DR, Wong SH, Bellosi A, Flynn RJ, Daly M, Langford TK, Bucks C, Kane CM, Fallon PG, Pannell R, et al.: **Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity.** *Nature* 2010, **464**:1367-1370.
31. Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, Pitman N, Mirchandani A, Rana B, van Rooijen N, et al.: **IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation.** *J Immunol* 2009, **183**:6469-6477.
- **32. Schiering C, Krausgruber T, Chomka A, Fröhlich A, Adelmann K, Wohlfert EA, Pott J, Griseri T, Bollrath J, Hegazy AN, et al.: **The alarmin IL-33 promotes regulatory T-cell function in the intestine.** *Nature* 2014, **513**:564-568.

The authors describe expression of ST2 by colonic regulatory T cells, and report that IL-33 is a novel co-factor for TGF β -dependent Treg differentiation. Using a ChIP assay IL-33 was shown to direct GATA3 binding to the *FoxP3* promoter inducing Treg *Foxp3* expression.

33. Smithgall MD, Comeau MR, Yoon BR, Kaufman D, Armitage R, Smith DE: **IL-33 amplifies both Th1- and Th2-type responses through its activity on human basophils, allergen-reactive Th2 cells, iNKT and NK cells.** *Int Immunol* 2008, **20**:1019-1030.
34. Hayakawa H, Hayakawa M, Kume A, Tominaga S: **Soluble ST2 blocks interleukin-33 signaling in allergic airway inflammation.** *J Biol Chem* 2007, **282**:26369-26380.
35. Tago K, Noda T, Hayakawa M, Iwahana H, Yanagisawa K, Yashiro T, Tominaga S: **Tissue distribution and subcellular localization of a variant form of the human ST2 gene product, ST2V.** *Biochem Biophys Res Commun* 2001, **285**:1377-1383.
36. Cayrol C, Girard JP: **The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1.** *Proc Natl Acad Sci U S A* 2009, **106**:9021-9026.
37. Talabot-Ayer D, Lamacchia C, Gabay C, Palmer G: **Interleukin-33 is biologically active independently of caspase-1 cleavage.** *J Biol Chem* 2009, **284**:19420-19426.
38. Snelgrove RJ, Gregory LG, Peiro T, Akthar S, Campbell GA, Walker SA, Lloyd CM: **Alternaria-derived serine protease activity drives IL-33-mediated asthma exacerbations.** *J Allergy Clin Immunol* 2014, **134**:583-592 e586.
39. Lefrancais E, Roga S, Gautier V, Gonzalez-de-Peredo A, Monsarrat B, Girard JP, Cayrol C: **IL-33 is processed into mature bioactive forms by neutrophil elastase and cathepsin G.** *Proc Natl Acad Sci U S A* 2012, **109**:1673-1678.
- **40. Lefrancais E, Duval A, Mirey E, Roga S, Espinosa E, Cayrol C, Girard JP: **Central domain of IL-33 is cleaved by mast cell proteases for potent activation of group-2 innate lymphoid cells.** *Proc Natl Acad Sci U S A* 2014, **111**:15502-15507.
The authors identified and characterised proteases within activated mast cell supernatants that could cleave IL-33 into smaller isoforms. Full length and cleaved forms of IL-33 were administered to mice and a significantly more potent type-2 response was observed in the lungs of mice treated with processed IL-33.
41. Roy A, Ganesh G, Sippola H, Bolin S, Sawesi O, Dagalv A, Schlenner SM, Feyerabend T, Rodewald HR, Kjellen L, et al.: **Mast cell chymase degrades the alarmins heat shock protein 70, biglycan, HMGB1, and interleukin-33 (IL-33) and limits danger-induced inflammation.** *J Biol Chem* 2014, **289**:237-250.
42. Strik MC, de Koning PJ, Kleijmeer MJ, Bladergroen BA, Wolbink AM, Griffith JM, Wouters D, Fukuoka Y, Schwartz LB, Hack CE, et al.: **Human mast cells produce and release the cytotoxic lymphocyte associated protease granzyme B upon activation.** *Mol Immunol* 2007, **44**:3462-3472.
43. Luthi AU, Cullen SP, McNeela EA, Duriez PJ, Afonina IS, Sheridan C, Brumatti G, Taylor RC, Kersse K, Vandenabeele P, et al.: **Suppression of interleukin-33 bioactivity through proteolysis by apoptotic caspases.** *Immunity* 2009, **31**:84-98.
44. Waern I, Lundequist A, Pejler G, Wernersson S: **Mast cell chymase modulates IL-33 levels and controls allergic sensitization in dust-mite induced airway inflammation.** *Mucosal Immunol* 2013, **6**:911-920.
45. Kurowska-Stolarska M, Kewin P, Murphy G, Russo RC, Stolarski B, Garcia CC, Komai-Koma M, Pitman N, Li Y, Niedbala W, et al.: **IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4.** *J Immunol* 2008, **181**:4780-4790.
46. Pastorelli L, Garg RR, Hoang SB, Spina L, Mattioli B, Scarpa M, Fiocchi C, Vecchi M, Pizarro TT: **Epithelial-derived IL-33 and its receptor ST2 are dysregulated in ulcerative colitis and in experimental Th1/Th2 driven enteritis.** *Proc Natl Acad Sci U S A* 2010, **107**:8017-8022.
47. Xu D, Jiang HR, Kewin P, Li Y, Mu R, Fraser AR, Pitman N, Kurowska-Stolarska M, McKenzie AN, McInnes IB, et al.: **IL-33 exacerbates antigen-induced arthritis by activating mast cells.** *Proc Natl Acad Sci U S A* 2008, **105**:10913-10918.

48. Weinberg EO, Shimp M, De Keulenaer GW, MacGillivray C, Tominaga S, Solomon SD, Rouleau JL, Lee RT: **Expression and regulation of ST2, an interleukin-1 receptor family member, in cardiomyocytes and myocardial infarction.** *Circulation* 2002, **106**:2961-2966.
49. Barlow JL, Bellosi A, Hardman CS, Drynan LF, Wong SH, Cruickshank JP, McKenzie AN: **Innate IL-13-producing nuocytes arise during allergic lung inflammation and contribute to airways hyperreactivity.** *J Allergy Clin Immunol* 2012, **129**:191-198 e191-194.
50. Kondo Y, Yoshimoto T, Yasuda K, Futatsugi-Yumikura S, Morimoto M, Hayashi N, Hoshino T, Fujimoto J, Nakanishi K: **Administration of IL-33 induces airway hyperresponsiveness and goblet cell hyperplasia in the lungs in the absence of adaptive immune system.** *Int Immunol* 2008, **20**:791-800.
51. Andronikos NM, McNally J, Kotze AC, Hunt PW, Ingham A: **Trichostrongylus colubriformis larvae induce necrosis and release of IL33 from intestinal epithelial cells in vitro: implications for gastrointestinal nematode vaccine design.** *Int J Parasitol* 2012, **42**:295-304.
52. Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, Saito Y, Fujiyama Y, Andoh A: **Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis.** *J Gastroenterol* 2010, **45**:999-1007.
53. Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadóttir A, Sulem P, Jonsdóttir GM, Thorleifsson G, Helgadóttir H, Steinthorsdóttir V, Stefansson H, et al.: **Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction.** *Nat Genet* 2009, **41**:342-347.
54. Palmer G, Talabot-Ayer D, Lamacchia C, Toy D, Seemayer CA, Viatte S, Finckh A, Smith DE, Gabay C: **Inhibition of interleukin-33 signaling attenuates the severity of experimental arthritis.** *Arthritis Rheum* 2009, **60**:738-749.
55. Luzina IG, Pickering EM, Kopach P, Kang PH, Lockatell V, Todd NW, Papadimitriou JC, McKenzie AN, Atamas SP: **Full-length IL-33 promotes inflammation but not Th2 response in vivo in an ST2-independent fashion.** *J Immunol* 2012, **189**:403-410.
56. Duan L, Chen J, Zhang H, Yang H, Zhu P, Xiong A, Xia Q, Zheng F, Tan Z, Gong F, et al.: **Interleukin-33 ameliorates experimental colitis through promoting Th2/Foxp3(+) regulatory T-cell responses in mice.** *Mol Med* 2012, **18**:753-761.
57. Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto K, et al.: **IL-33 is a crucial amplifier of innate rather than acquired immunity.** *Proc Natl Acad Sci U S A* 2010, **107**:18581-18586.
58. Sedhom MA, Pichery M, Murdoch JR, Foligne B, Ortega N, Normand S, Mertz K, Sanmugalingam D, Braut L, Grandjean T, et al.: **Neutralisation of the interleukin-33/ST2 pathway ameliorates experimental colitis through enhancement of mucosal healing in mice.** *Gut* 2013, **62**:1714-1723.
59. Bamias G, Martin C, Mishina M, Ross WG, Rivera-Nieves J, Marini M, Cominelli F: **Proinflammatory effects of TH2 cytokines in a murine model of chronic small intestinal inflammation.** *Gastroenterology* 2005, **128**:654-666.
- *60. Molofsky AB, Van Gool F, Liang HE, Van Dyken SJ, Nussbaum JC, Lee J, Bluestone JA, Locksley RM: **Interleukin-33 and Interferon-gamma Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation.** *Immunity* 2015, **43**:161-174.
The authors administered IL-33 to ILC2 deficient mice and demonstrated that in addition to direct effects of IL-33 in activating ST2⁺ regulatory T cells, ILC2 are important for enhancement of Treg responses in the gut.
61. Yin H, Li X, Hu S, Liu T, Yuan B, Ni Q, Lan F, Luo X, Gu H, Zheng F: **IL-33 promotes Staphylococcus aureus-infected wound healing in mice.** *Int Immunopharmacol* 2013, **17**:432-438.
62. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, Angelosanto JM, Laidlaw BJ, Yang CY, Sathaliyawala T, et al.: **Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus.** *Nat Immunol* 2011, **12**:1045-1054.

63. Haenuki Y, Matsushita K, Futatsugi-Yumikura S, Ishii KJ, Kawagoe T, Imoto Y, Fujieda S, Yasuda M, Hisa Y, Akira S, et al.: **A critical role of IL-33 in experimental allergic rhinitis.** *J Allergy Clin Immunol* 2012, **130**:184-194 e111.
64. Meeaphansan J, Tsuda H, Komine M, Tominaga S, Ohtsuki M: **Regulation of IL-33 expression by IFN-gamma and tumor necrosis factor-alpha in normal human epidermal keratinocytes.** *J Invest Dermatol* 2012, **132**:2593-2600.
65. Savinko T, Matikainen S, Saarialho-Kere U, Lehto M, Wang G, Lehtimäki S, Karisola P, Reunala T, Wolff H, Lauerma A, et al.: **IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors.** *J Invest Dermatol* 2012, **132**:1392-1400.
66. Imai Y, Yasuda K, Sakaguchi Y, Haneda T, Mizutani H, Yoshimoto T, Nakanishi K, Yamanishi K: **Skin-specific expression of IL-33 activates group 2 innate lymphoid cells and elicits atopic dermatitis-like inflammation in mice.** *Proc Natl Acad Sci U S A* 2013, **110**:13921-13926.
67. Salimi M, Barlow JL, Saunders SP, Xue L, Gutowska-Owsiak D, Wang X, Huang LC, Johnson D, Scanlon ST, McKenzie AN, et al.: **A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis.** *J Exp Med* 2013, **210**:2939-2950.
68. Townsend MJ, Fallon PG, Matthews DJ, Jolin HE, McKenzie AN: **T1/ST2-deficient mice demonstrate the importance of T1/ST2 in developing primary T helper cell type 2 responses.** *J Exp Med* 2000, **191**:1069-1076.
69. Chang YJ, Kim HY, Albacker LA, Baumgarth N, McKenzie AN, Smith DE, Dekrueff RH, Umetsu DT: **Innate lymphoid cells mediate influenza-induced airway hyper-reactivity independently of adaptive immunity.** *Nat Immunol* 2011, **12**:631-638.
- **70. Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, Bucks C, Wu X, Kane CM, Neill DR, et al.: **IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction.** *J Allergy Clin Immunol* 2013, **132**:933-941.
Through analysis of experimental allergic lung inflammation in *Il17rb* and *Il1rl1* deficient mice, and exogenous administration of IL-25 or IL-33, the authors demonstrate that IL-33 more rapidly and potently induces airway contraction, ILC2 infiltration and IL-13 production than IL-25.
- **71. Bonilla WV, Frohlich A, Senn K, Kallert S, Fernandez M, Johnson S, Kreutzfeldt M, Hegazy AN, Schrick C, Fallon PG, et al.: **The alarmin interleukin-33 drives protective antiviral CD8(+) T cell responses.** *Science* 2012, **335**:984-989.
The authors observed increased IL-33 expression upon viral infection. Utilising *Il33* and *Il1rl1* deficient mice, the authors demonstrated that IL-33 signalling is required for a robust CTL response to viral infection.
72. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J, Jerico D-R, Telcian AG, Nikonova A, Zhu J, et al.: **IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo.** *Am J Respir Crit Care Med* 2014, **190**:1373-1382.
- *73. Oh JE, Kim BC, Chang DH, Kwon M, Lee SY, Kang D, Kim JY, Hwang I, Yu JW, Nakae S, et al.: **Dysbiosis-induced IL-33 contributes to impaired antiviral immunity in the genital mucosa.** *Proc Natl Acad Sci U S A* 2016, **113**:E762-771.
The authors detected IL-33 secretion following anti-biotic treatment of mice, which recruited ILC2 and subsequently eosinophils to the vaginal mucosa. Exogenous administration of IL-33 prior to viral infection prevented effective IFN production and T cell recruitment to the mucosa. Highlighting the potentially opposing roles of IL-33 during different viral infections.
74. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, Moore CL, Seunghyun In T, Wasserman S, Coyle AJ, et al.: **IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization.** *J Allergy Clin Immunol* 2013, **131**:187-200 e181-188.
75. Ryu WI, Lee H, Kim JH, Bae HC, Ryu HJ, Son SW: **IL-33 induces Egr-1-dependent TSLP expression via the MAPK pathways in human keratinocytes.** *Exp Dermatol* 2015, **24**:857-863.

76. Huang Y, Guo L, Qiu J, Chen X, Hu-Li J, Siebenlist U, Williamson PR, Urban JF, Jr., Paul WE: **IL-25-responsive, lineage-negative KLRG1(hi) cells are multipotential 'inflammatory' type 2 innate lymphoid cells.** *Nat Immunol* 2015, **16**:161-169.
77. Kim YH, Yang TY, Park CS, Ahn SH, Son BK, Kim JH, Lim DH, Jang TY: **Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis.** *Allergy* 2012, **67**:183-190.
78. Mizutani N, Nabe T, Yoshino S: **Interleukin-33 and alveolar macrophages contribute to the mechanisms underlying the exacerbation of IgE-mediated airway inflammation and remodelling in mice.** *Immunology* 2013, **139**:205-218.
79. Kearley J, Buckland KF, Mathie SA, Lloyd CM: **Resolution of allergic inflammation and airway hyperreactivity is dependent upon disruption of the T1/ST2-IL-33 pathway.** *Am J Respir Crit Care Med* 2009, **179**:772-781.
80. Hueber AJ, Alves-Filho JC, Asquith DL, Michels C, Millar NL, Reilly JH, Graham GJ, Liew FY, Miller AM, McInnes IB: **IL-33 induces skin inflammation with mast cell and neutrophil activation.** *Eur J Immunol* 2011, **41**:2229-2237.
81. Sattler S, Ling GS, Xu D, Hussaarts L, Romaine A, Zhao H, Fossati-Jimack L, Malik T, Cook HT, Botto M, et al.: **IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut.** *J Autoimmun* 2014, **50**:107-122.