IL-33: an alarmin cytokine with crucial roles in innate immunity, inflammation and allergy
Corinne Cayrol1,2 and Jean-Philippe Girard1,2

IL-33 is a nuclear cytokine from the IL-1 family constitutively expressed in epithelial barrier tissues and lymphoid organs, which plays important roles in type-2 innate immunity and human asthma. Recent studies indicate that IL-33 induces production of large amounts of IL-5 and IL-13 by group 2 innate lymphoid cells (ILC2s), for initiation of allergic inflammation shortly after exposure to allergens or infection with parasites or viruses. IL-33 appears to function as an alarmin (alarm signal) rapidly released from producing cells upon cellular damage or cellular stress. In this review, we discuss the cellular sources, mode of action and regulation of IL-33, and we highlight its crucial roles in vivo with particular emphasis on results obtained using IL33-deficient mice.

Addresses
1 CNRS, IPBS (Institut de Pharmacologie et de Biologie Structurale), 205 route de Narbonne, F-31077 Toulouse, France
2 Université de Toulouse, UPS, IPBS, F-31077 Toulouse, France

Corresponding author: Girard, Jean-Philippe (Jean-Philippe.Girard@ipbs.fr)

Current Opinion in Immunology 2014, 31:31–37
This review comes from a themed issue on Allergy and hypersensitivity
Edited by Anne Sperling and Mark Ansel
For a complete overview see the Issue and the Editorial
Available online 29th September 2014
http://dx.doi.org/10.1016/j.coi.2014.09.004
0952-7915/© 2014 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Introduction
IL-33 is a nuclear cytokine, initially designated NF-HIEV [1,2], which exhibits structural similarities with IL-1 [3–6]. It activates Myd88-dependent signaling pathways in target cells expressing the ST2/IL-1RACP receptor complex [3,4,6], including group 2 innate lymphoid cells (ILC2s), natural helper cells, nuocytes, innate helper 2 cells), mast cells and their progenitors, basophils, eosinophils, Th2 cells, NKT and NK cells [3,5,6]. Studies performed over the past three years indicate that ILC2s, which secrete huge amounts of IL-5 and IL-13 in response to IL-33, and play crucial roles in type-2 immunity, allergic inflammation and eosinophil homeostasis, are major targets of IL-33 in vivo [7–12,13**]. The purpose of this review is to highlight the crucial role of IL-33 in innate immunity, inflammation and allergy, and to discuss its mode of action as an ‘alarmin’ and the mechanisms involved in its regulation, with particular emphasis on recent advances and studies focused on the analysis of endogenous IL-33.

IL-33: a crucial actor in innate immunity, inflammation and allergy
Role in innate immune responses following infection with parasites and viruses
IL-33 plays important roles in type-2 innate immunity. After infection with the helminth Nippostrongylus brasiliensis and in response to IL-33, ILC2s expanded robustly and produced large amounts of IL-13, which led to goblet cell hyperplasia in the intestine and worm expulsion, even in the absence of adaptive immunity [7–9]. IL-33-deficient mice failed to clear worms due to a selective defect in ILC2-derived IL-13 [14]. Responsiveness of ILC2s to IL-33 was found to be controlled by Gfi1, a transcription factor which regulates ST2 expression at the surface of ILC2s [15**]. Endogenous IL-33 has also been shown to be important for lung eosinophilic inflammation and IL-5 production by ILC2s, after infection with the nematode Strongyloides venezuelensis or intranasal administration of chitin, a polysaccharide constituent of many parasites and allergens [16**,17].

IL-33 is involved in the response to viral infection. For instance, IL-33/ST2 signaling has been found to be required for ILC2-dependent restoration of airway epithelial integrity after infection with influenza virus [18]. Activation of lung ILC2s by IL-33 was also shown to mediate influenza-induced airway hyper-reactivity independently of adaptive immunity [19]. In addition, analysis of parainfluenza virus infection in IL-33-deficient mice revealed an essential role of IL-33 in induction of IL-13, mucus overproduction and chronic lung disease following viral infection [20**]. Finally, endogenous IL-33 has been found to be necessary for induction of potent CD8+ T cell responses to replicating, prototypic RNA and DNA viruses in mice [21], indicating that IL-33 may play a role in type-1 immune responses under certain conditions.

Activation of ILC2s in allergic inflammation
The crucial role of endogenous IL-33 in allergic inflammation was first demonstrated using IL-33-deficient mice [22]. IL-33 was found to be required for ovalbumin-induced and protease allergen (papain)-induced airway inflammation [22,23]. Further analyses revealed that IL-33 induces allergic airway inflammation by stimulating lung ILC2s [24–26,27*]. Indeed, papain-driven IL-5
and IL-13 production from ILC2s, eosinophilic lung inflammation and Th2 cell differentiation were all found to be impaired in intranasally challenged IL-33-deficient mice [26,27]. IL-33/ST2 signaling was also required for IL-5 and IL-13 production by lung ILC2s, and airway eosinophilia following exposure to the clinically relevant fungal allergen *Alternaria alternata* [24] or the danger signal uric acid [28*].

IL-33 also appears to be important for allergic inflammation in other tissues (nasopharynx, skin). For instance, studies using IL-33-deficient mice have revealed the crucial role of IL-33 in the development of experimental allergic rhinitis induced by ragweed pollen [29**]. IL-33 is a potent stimulator for skin ILC2s, and the absence of IL-33 signaling resulted in decreased skin inflammation in a mouse model of atop dermatitis [30**]. In humans, IL-33-responsive ILC2s have been shown to be enriched in nasal polyps of patients with chronic rhinosinusitis [10], and in lesional skin biopsies of atop dermatitis patients [30**].

### Susceptibility to human asthma

The genes encoding IL-33 and ST2/IL1RL1 have been identified as major susceptibility loci for human asthma in several genome-wide association studies, which included thousands of patients from diverse ethnic groups and different forms of asthma (asthma associated with blood eosinophils, early childhood asthma with severe exacerbations, etc.). Interestingly, *IL33* and *ST2/IL1RL1* were the only two genes reproducibly found to be associated with asthma in all these studies [31–34,35*]. Several other genes important for ILC2 differentiation (*RORA*, transcription factor RORa), proliferation (*IL2RB*, IL-2 receptor subunit), activation (*TSLP*, cytokine TSLP) and function (*IL13*, type-2 cytokine IL-13) have been identified as susceptibility loci in some of these studies [32–34]. The IL-33/ST2-ILC2 axis is thus likely to play a crucial role in human asthma (Figure 1).

### IL-33: a tissue-derived nuclear cytokine

**Constitutive expression in epithelial barrier tissues and lymphoid organs**

An important characteristic of IL-33 is the fact that it is constitutively expressed to high levels in human and mouse tissues during homeostasis [36,37*]. Indeed, abundant expression of the endogenous IL-33 protein has been observed in epithelial cells from tissues exposed to the environment, and in fibroblastic reticular cells (FRCs) of lymphoid organs (Table 1) [36,37*]. High levels of IL-33 were also detected in endothelial cells from blood vessels in human tissues [2,36], but not in mouse [37*].

Strikingly, the endogenous IL-33 protein was always localized in the nucleus of producing cells in both human and mouse tissues [36,37*], with no evidence for cytoplasmic or extracellular localization, indicating that IL-33 is a nuclear cytokine *in vivo*. Although its nuclear roles remain unclear, IL-33 can associate with chromatin by tethering to histones H2A/H2B, via a short chromatin-binding motif, located in its N-terminal nuclear domain...
Although [2,38]. Deletion of this chromatin-binding nuclear domain has recently been shown to result in constitutive extracellular release of the protein, ST2-dependent multi-organ inflammation and death of the organism [39**]. Nuclear localization (retention) is thus a fundamental property of IL-33, which is crucial for regulation of its cytokine activity.

**Inducible expression during inflammation**

Although IL-33 is constitutively expressed in tissues under basal conditions, its expression can be further increased during inflammation. For instance, induction of IL-33 promoter activity and upregulation of IL-33 protein levels were observed in alveolar type II (ATII) pneumocytes upon allergic lung inflammation following exposure to ovalbumin, ragweed pollen or *Alternaria* [25,40*]. Upregulation of nuclear IL-33 in mouse ATII cells has also been detected upon lung eosinophilic inflammation induced by intestinal nematode infection, and after intranasal administration of chitin [16**]. In humans, increased expression of IL-33 in the nuclei of airway epithelial cells has been reported in patients with asthma [41] and chronic obstructive pulmonary disease (COPD) [20**]. Interestingly, IL-33 expression was traceable to a subset of airway epithelial cells with progenitor function [20**]. Inducible expression of IL-33 in mouse tissues has also been observed outside the lungs, for instance in hepatocytes during acute hepatitis [42], and in endothelial cells from the inflamed colon during colitis [37*].

IL-33 is generally not expressed in CD45* hematopoietic cells under basal conditions, but it can be induced in macrophages and dendritic cells during allergic inflammation and infection [19,40*,43]. However, IL-33 levels in CD45* cells appear to be at least 10 fold lower than those found in CD45* epithelial cells [20**,25,40*], and the protein was not detected in F4/80* alveolar macrophages in lung tissue sections during allergic inflammation [23] or infection [16**]. In addition, recent analyses in a mouse model of allergic rhinitis revealed that tissue-derived IL-33, rather than immune-cell derived IL-33, is crucial for induction of allergic inflammation [44].

### IL-33: an alarmin released upon cellular stress and injury

**Mode of action as an alarmin**

Biologically active full length IL-33 can be released in the extracellular space after cell damage (necrotic cell death) or mechanical injury [45,46]. IL-33 was thus proposed to function as a novel alarmin (intracellular alarm signal released upon cell injury) to alert the immune system of tissue damage following trauma or infection [36,37*,45,46]. IL-33 is likely to be a very good alarm signal because, due to its constitutive expression in normal tissues, it is ready to be released at any time, for ‘alarming’ ILC2s and other immune cells (Figure 2).

Environmental allergens, such as ragweed pollen and *A. alternata*, have been shown to induce the rapid (~1 hour) release of IL-33 in nasal and bronchoalveolar lavage (BAL) fluids, respectively [29**,47,48]. This increase of IL-33 protein in extracellular fluids was associated with reduced staining for IL-33 in the nuclei of nasal epithelial cells [29**] and ATII pneumocytes [48], suggesting extracellular release of preformed nuclear IL-33. Many airborne allergens have intrinsic protease activities [26,28*,48], and allergen proteases have been shown to play a role in the rapid increase of IL-33 levels in BAL fluids after intranasal administration [26,48]. Allergens and allergen proteases can cause breakdown of epithelial barriers in *vivo* and may thus induce the release of IL-33 through cellular necrosis. However, allergen exposure also leads to extracellular accumulation of danger signals, such as ATP and uric acid, which appear to induce the extracellular release of IL-33 without apparent cell death [20**,28*,47]. ATP is known to be released in various noncytolytic conditions, including membrane deformations, mechanical stress or osmotic stress [47]. Cellular stress, in addition to cellular necrosis, may thus turn out to be an important mechanism for IL-33 release in *vivo*.

### Regulation by proteases

Proteases have been shown to regulate IL-33 activity (Figure 2). IL-33 contains a consensus site of cleavage for caspase-3 (DGVD178G in human), and cleavage by caspases at this site generates two biologically inactive products [45,46]. Inactivation of IL-33 during apoptosis is likely to be important to avoid alerting the immune system unnecessarily after physiological programmed (apoptotic) cell death, as opposed to pathological (necrotic) cell death [45,46].
Figure 2

IL-33, a tissue-derived nuclear alarmin. During homeostasis, nuclear IL-33 is constitutively expressed to high levels in epithelial barrier tissues, such as the lung, skin and stomach. Full length bioactive IL-33 is released extracellularly upon tissue damage and cell death (or cellular stress), following exposure to allergens or infection with viruses or parasites. After release, IL-33 ‘raises the alarm’ in the immune system by activating various types of immune cells, including mast cells and, most importantly, ILC2s, which secrete large amounts of IL-5 and IL-13. After programmed cell death (apoptosis), IL-33 is inactivated by caspases to avoid alerting the immune system unnecessarily. Although full length IL-33 is active, it can be processed by inflammatory proteases (cathepsin G, elastase) into shorter ‘hyperactive’ mature forms, which may be the crucial bioactive forms in vivo.
By contrast to caspases which inactivate IL-33, proteases released during inflammation appear to increase IL-33 biological activity [49**]. Neutrophil serine proteases, cathepsin G and elastase, were found to process full length IL-33 into mature forms containing the IL-1-like cytokine domain (IL-3395–270, IL-3399–270 and IL-33109–270), that had greatly increased biological activity (~10 fold) compared to the full length protein [49**]. Both full length and mature endogenous IL-33 were detected in BAL fluids in a model of acute lung injury associated with high levels of neutrophil recruitment in the alveolar wall [49**]. Together, these results suggested that proteolytic processing of IL-33 may be required for the extracellular generation of highly active cytokine in vivo.

Conclusions and future directions
IL-33 is an alarmin cytokine from the IL-1 family, which plays a crucial role in the initiation of type-2 immune responses following infection with parasites or viruses, or exposure to allergens. IL-33 appears to act by activating ILC2s for production of large amounts of type-2 cytokines IL-5 and IL-13. The potent activity of IL-33 on ILC2s and the crucial role of these cells in the initiation of allergic airway inflammation are likely to explain the dominant role of the IL-33/ST2 pathway in genetic susceptibility to human asthma. Despite these important advances, many questions remain to be answered. For instance, the potential redundancy or synergy of IL-33 with other activators of ILC2s, that have been recently identified (Prostaglandin D2, Leukotriene D4, IL-9, etc.), needs to be studied. Although the functions of IL-33 in the activation of ILC2s and the initiation of allergic inflammation in the lungs have been well established, its roles in allergic and non-allergic inflammation in other tissues, exhibiting high expression levels of the endogenous protein, remain to be fully explored. A better understanding of IL-33 release, mode of action and regulation will be crucial for the development of therapeutics that target the IL-33/ST2 pathway to treat asthma and other inflammatory diseases.

Acknowledgements
We thank members of the Girard lab for fruitful discussions. Research in Girard lab is supported by grants from Agence Nationale pour la Recherche (ANR-12-BSV3-0005-01) and Fondation ARC (SL.22011063471).

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
● of special interest
◆ of outstanding interest
This study revealed that transcription factor Gfi1 controls responsiveness of ILC2s to IL-33 by regulating the expression of the ST2.
This study demonstrated the expression of endogenous IL-33 in subsets of airway epithelial cells with progenitor function, and its critical role in human COPD and parainfluenza virus-induced COPD-like disease in mouse.


29. This work revealed that uric acid, similar to other danger signals such as ATP, can induce the extracellular release of IL-33 from human bronchial epithelial cells in culture without apparent cell death.


31. This paper revealed that endogenous IL-33 is rapidly released from nasal epithelial cells following allergen exposure, and plays a critical role in allergic rhinitis.


33. This paper reported a key role for IL-33 in the activation of ILC2s in human atopic dermatitis and in a mouse model of the disease.


38. This study and previous reports revealed that the genes encoding IL-33 and ST2/IL1RL1 are major susceptibility loci for different forms of asthma, including early childhood severe asthma.


41. Using a novel IL-33 reporter line, this study reported the expression profile of endogenous IL-33 in mouse adult tissues and embryos and revealed that IL-33 is constitutively expressed to high levels in mouse epithelial barrier tissues and lymphoid organs.


44. This study revealed that genetic deletion of the N-terminal chlamydomin-binding nuclear domain of IL-33 results in constitutive release of the cytokine and ST2-dependent lethal inflammation, characterized by eosinophil infiltration of multiple organs.


46. This paper reported the generation of a fluorescent reporter mouse for analysis of IL-33 promoter activity and revealed the inducible expression of IL-33 upon allergic lung inflammation.


48. This paper revealed that endogenous IL-33 is rapidly released from nasal epithelial cells following allergen exposure, and plays a critical role in allergic rhinitis.


This study was the first to show that inflammatory proteases can generate mature forms of IL-33 that have greatly increased biological activity compared to the uncleaved protein. Endogenous full length and cleaved IL-33 were both detected in BAL fluids upon lung tissue damage.