



Mini Review

Emerging roles of IL-33 in inflammation and immune regulation

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Interleukin-33 (IL-33) belongs to the IL-1 family of cytokines and has been reported to play multiple roles in host defense, allergies and chronic inflammation. Constitutive expression of IL-33 in epithelial cells ensures rapid immune responses against invading pathogens such as parasites and viruses. Tissue damage caused by pathogens results in the release of extracellular IL-33 that in turn alerts a variety of immune cells such as group 2 innate lymphoid cells (ILC2s), eosinophils, basophils and mast cells. These cells mediate T helper type 2 (Th2) immune responses to destroy pathogens. IL-33 also plays central roles in mediating allergic diseases including asthma and atopic rhinitis. Although the functions of IL-33 in host defense and allergies initially received most attention, focus is turning to its roles in chronic inflammatory diseases. Recent advances, however, have led to problematic results. In addition to Th2 responses, IL-33 also promotes Th1 responses and there have been positive and negative roles reported for IL-33 in inflammatory diseases. This mini-review will summarize IL-33 biology and discuss issues regarding previously unrecognized roles of IL-33.

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Introduction

Interleukin-33 (IL-33) belongs to the IL-1 cytokine family, together with IL-1 α , IL-1 β and IL-18, and it plays central

roles in parasitic infection and allergies¹⁻⁴⁾. IL-33 was initially reported as a nuclear protein because of its constitutive expression in the nuclei of endothelial cells from high endo-

thelial venules⁵). A series of studies has revealed that IL-33 is preferentially expressed by cells at the mucosal surface, including epithelial cells and smooth muscle cells^{2, 4, 6}. Although IL-33 is localized within nuclei and possesses transcriptional activities *in vitro*^{7, 8}, it demonstrates powerful and broad effects when released into the extracellular environment. Release of IL-33 is caused by necrosis of damaged or infected tissue⁹ or by extracellular danger signals such as ATP¹⁰ and uric acid¹¹. The IL-33 receptor consists of ST2, also called IL-1 receptor-like 1 (IL-1RL1), T1, Der4 and fit-1, and IL-1 receptor accessory protein (IL-1RAcP)^{12, 13}. Binding of IL-33 to the receptor leads to rapid induction of T helper type 2 (Th2) immune responses¹⁴. This system alerts the host to danger, for example tissue damage caused by pathogens. Therefore, IL-33 is often referred to as an “alarmin”, together with IL-1 α and high mobility group box chromosomal protein 1 (HMGB-1)¹⁵. Accordingly, dysregulation of IL-33 localization within cells causes severe inflammation throughout the body¹⁶. Appreciating the regulatory mechanisms underlying IL-33 biology is thus important for understanding inflammation.

Molecular regulation of IL-33 expression

Members of the IL-1 cytokine family mediate strong inflammatory immune responses. Moreover, dysregulation of the members is often associated with severe inflammatory diseases. For these reasons, it is necessary for those cytokines to be maintained under tight regulation. Proteolytic processing by caspases has pivotal roles in regulating the IL-1 family of cytokines. Among the cytokines in the IL-1 family, IL-33 seems to be regulated particularly tightly by several regulatory programs¹⁷. In order to become biologically active, the proactive forms of IL-1 β and IL-18 require caspase-1 to remove their N-terminal domains to liberate the residual C-terminal domains, including the IL-1 domain¹⁸. However, IL-33 is active independent of caspase-1¹⁹. Interestingly, caspases-3 and -7 inactivate IL-33 by cleaving its C-terminal IL-1 domain^{9, 20, 21}. This inactivation event is believed to ensure specific roles of IL-33 in pathogenic situations such as parasitic infection, but not in non-inflammatory physiological situations, such as apoptosis. Invading pathogens mediate tissue damage that leads to necrosis of epithelial cells and this non-programmed cell death results in IL-33 release in the absence of inactivation by caspases-3 and -7.

Recent interesting findings suggest that the full-length form of IL-33, called pro-IL-33, is processed by an additional

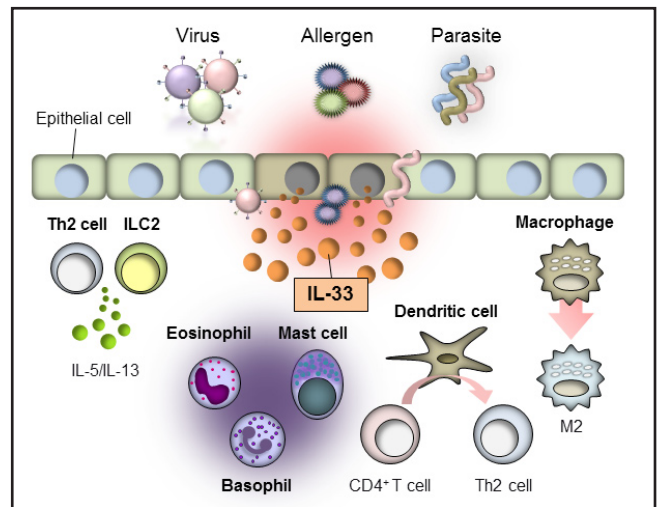


Fig. 1 Target cells of IL-33 in Th2 responses

Tissue damage caused by allergen or pathogens results in the release of IL-33. Release of IL-33 mediates Th2 cytokine production from ILC2 and Th2 cells, and activates key cells for Th2 responses such as eosinophils, basophils and mast cells. IL-33 also helps dendritic cells to promote T cell differentiation to Th2 cells. IL-33 is known to induce M2 polarization of macrophages.

regulatory step in the extracellular space. In this case, proteases mainly secreted by neutrophils play an active role. These proteases include calpain²², cathepsin G, elastase²³ and proteinase 3²⁴. They cleave pro-IL-33 and remove the N-terminal domain, leaving the mature form of IL-33 with a ten-fold higher affinity to its receptor. The innate immune system therefore employs neutrophils to amplify local inflammation through extracellular regulation of IL-33.

Target cells of IL-33 in Th2 responses

IL-33 acts on a variety of cells including immune and non-immune cells^{2, 6, 25}. One of the most well-characterized functions of IL-33 is to induce Th2 immune responses (Fig. 1). A series of studies has revealed that IL-33 accelerates Th2 cytokine production from group 2 innate lymphoid cells (ILC2s)²⁶⁻²⁹, Th2 cells^{30, 31} and invariant natural killer (NK) T cells³². In addition, IL-33 directly activates other key players in Th2 responses, such as mast cells, basophils, eosinophils and macrophages. IL-33-activated mast cells demonstrate prolonged survival and produce various kinds of cytokines and chemokines in humans^{33, 34} and in mice^{13, 35, 36}. IL-33 and IL-3 synergistically activate basophils to promote cytokine and chemokine production^{31, 32, 37-40}. Eosinophils isolated from human blood are also activated by IL-33 and show increased survival and function^{37, 41-43}. Polarization



of macrophages towards M2 (alternatively activated) macrophages is a critical determinant for induction and maintenance of the Th2 environment. IL-33 was reported to directly induce M2 polarization of macrophages^{44, 45}. In addition, IL-33 indirectly mediates Th2 responses through regulation of dendritic cells (DCs). DCs treated with IL-33 gain the ability to induce differentiation of naïve CD4⁺ T cells to Th2 cells^{46, 47}. Moreover, IL-33 alters properties of tissue cells such as endothelial and epithelial cells, augmenting local inflammation. IL-33 stimulates endothelial and epithelial cells to produce IL-6 and IL-8^{48, 49} and increases vascular permeability⁵⁰, which helps to recruit effector inflammatory cells. IL-33 therefore initiates Th2 responses in a variety of ways.

Role of IL-33 in allergy

A large number of investigations into IL-33 have focused on allergies and revealed that IL-33 is one of the most important cytokines associated with asthma^{51, 52}, allergic rhinitis⁵³ and atopic dermatitis⁵⁴. Upon allergen exposure, IL-33 is released by airway epithelial cells and activates a variety of cells as discussed above. Among them, ILC2s have pivotal roles during the early onset of allergic diseases⁵⁵⁻⁵⁷. In response to IL-33, regional ILC2s located proximal to airway epithelial layers rapidly produce IL-5 and IL-13, resulting in eosinophil accumulation and mucus secretion, respectively^{26, 28, 29}. In humans, ILC2s are also evident^{58, 59}, and targeting IL-33 is thus of great interest in therapies for human allergic diseases. Several animal studies of asthma development examined the effects of blocking IL-33 signaling. Consistent with the study using IL-33-deficient mice⁶⁰, both polyclonal anti-IL-33 antibodies⁶¹ and anti-ST2 monoclonal antibodies⁶² successfully reduced asthmatic symptoms in the animal models.

Emerging roles of IL-33

Recent advances in IL-33 research have led to puzzling results. Until recently, it had been believed that the major roles of IL-33 were to mediate Th2 responses in allergic reactions or against parasitic infection. Surprisingly, it has been found that IL-33 also plays roles in promoting Th1 immune responses⁶³. IL-33 directly activates NK, NKT and CD8⁺ T cells and enhances the secretion of a Th1 cytokine, IFN- γ ^{32, 64, 65}. Moreover, IL-33/ST2 signaling is necessary to elicit cytotoxic CD8⁺ T cell responses for viral clearance⁶⁶. Taking advantage of the ability of IL-33 to promote the Th1 response, one report demonstrated its potential usage in a

clinical application⁶⁷. Immunization with an IL-33-expressing plasmid was able to enhance anti-tumor immunity by mounting IFN- γ -producing CD4⁺ and CD8⁺ T cells and inducing antigen-specific IgG responses. These responses were not accompanied with Th2 responses such as IL-4 and IgE elevation, which may cause unwanted allergic conditions.

Accumulating evidence indicates that IL-33 is involved in a much broader range of inflammatory processes than previously believed^{6, 68, 69}. There have been problematic observations reported for protective as well as etiologic roles of IL-33 in inflammatory diseases. Administration of IL-33 helps to inhibit the development of atherosclerosis, possibly by elevating production of anti-ox-LDL specific IgM⁷⁰. IL-33 administration also prevents inflammation in adipose tissues⁴⁴. IL-33 employs ILC2s which provide Th2 environment for differentiation of macrophages into M2 macrophages⁷¹. They are known to reduce chronic inflammation which is thought to be one of the triggers for the development of obesity. Furthermore, IL-33 was shown to play a protective role in sepsis⁷². IL-33 treatment was demonstrated to contribute to bacterial clearance by enhancing neutrophil migration.

In contrast to the protective roles of IL-33, other studies suggest pathologic roles for IL-33 in inflammatory diseases. IL-33 expression was increased in patients with rheumatoid arthritis (RA)⁷³⁻⁷⁶ and animal models of RA demonstrated etiologic roles of IL-33 in its development^{73, 74, 77-79}. Expression of IL-33 and ST2 was also upregulated in patients with systemic sclerosis⁸⁰ and subcutaneous administration of IL-33 in animals induced skin fibrosis⁸¹. Although the contribution of IL-33 to pathogenesis remains to be clarified, the IL-33/ST2 pathway appears to be associated with systemic lupus erythematosus^{82, 83} and inflammatory bowel diseases^{7, 84-86} in humans.

In addition, an intractable disease, pulmonary arterial hypertension (PAH), characterized by severe obstruction of small pulmonary arteries and concomitant high pulmonary artery pressure, seems to be associated with IL-33 because serum soluble ST2 levels are increased in PAH patients⁸⁷. Our data support the idea that IL-33 is an etiological factor for development of PAH. Previously, we generated an IL-5 reporter mouse and demonstrated that IL-33 enhanced IL-5 production from ILC2s, which lead to severe lung eosinophilia²⁹. In unpublished experiments, IL-33 induced severe arterial hypertrophy in the lung, and the occurrence of the hypertrophy was largely dependent on IL-5 production from ILC2s. In line with this concept, IL-5 was reported to

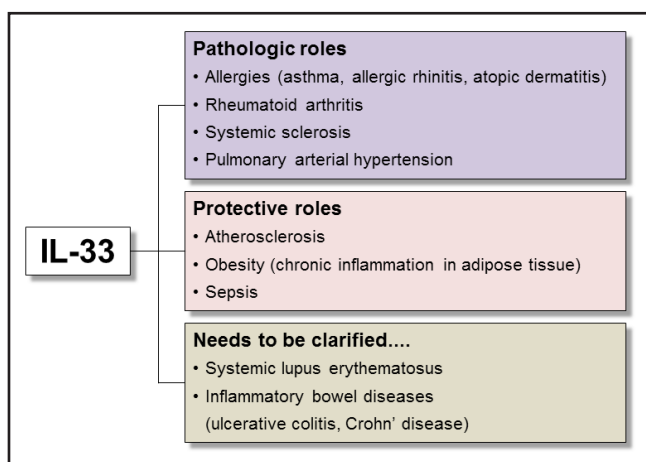


Fig. 2 Roles of IL-33 in inflammatory diseases

IL-33 plays pathologic roles in allergies, rheumatoid arthritis, systemic sclerosis and pulmonary arterial hypertension. In contrast, IL-33 protects from the development of atherosclerosis, obesity and sepsis. Although the IL-33/ST2 system is suggested to be involved in systemic lupus erythematosus and inflammatory bowel diseases, the precise roles of IL-33 in these diseases have yet to be elucidated.

be involved in arterial hypertrophy in an animal model of PAH⁸⁸). Other studies also indicated that Th2 responses trigger the development of PAH in ovalbumin- or house dust mite extract-induced PAH⁸⁹⁻⁹¹). Furthermore, PAH is considered to be associated with schistosomiasis and experimental *Schistosoma mansoni* infection can induce arterial hypertrophy^{92, 93}). In the schistosomiasis studies, Th2 cytokines, such as IL-4 and IL-13, or eosinophils, were suggested to be involved in PAH induction.

Taken many published results together, IL-33 is now known to be involved in a broad range of inflammatory diseases (Fig. 2) and therefore a large number of investigations aim to block IL-33 signaling for therapies.

Concluding remarks

IL-33 is a multifunctional molecule that acts as a transcription factor, alarmin, Th1/Th2 promoter and chronic inflammatory factor. As there have been a number of reports that describe etiological roles of IL-33 in allergies and inflammatory diseases, blockade of IL-33/ST2 signaling is a promising new approach to therapeutic intervention^{94, 95}). However, we have to consider carefully how we manage IL-33 because beneficial roles for IL-33 in diseases are also reported. The effects of IL-33 seem to be different among organs and dependent on disease state. It is important to elucidate the precise roles and the underlying mechanisms

of IL-33 in the development of diseases.

Potential conflict of interests

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