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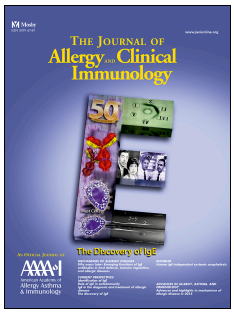

IL-33 in clinical practice: size matters?

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IL-33 in clinical practice: size matters?

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IL-33 is a member of the IL-1 family cytokines and is now recognized to be a key player in allergic diseases, especially asthma.[1,2] A recent genome-wide association study and the discovery of IL-33's major target cells, group 2 innate lymphoid cells (ILC2),[3] have shed light on its roles in allergic diseases. IL-33 has been reported to have several different forms as a result of post-translational processes, including intracellular and extracellular modification. Importantly, the biological activity of each form of IL-33 differs depending on the size of the protein. However, little is known about the size of IL-33 detected in clinical samples from patients with allergic diseases. This editorial summarizes the known mechanisms of intracellular and extracellular modification of IL-33's size and postulates future studies needed to understand the precise roles of IL-33 in clinical biology.

IL-33 was first identified as a nuclear protein expressed in endothelial cell nuclei, and it was named nuclear factor from high endothelial venules (NF-HEV). It was subsequently shown to be constitutively expressed in the nuclei of various cell types, such as endothelial and epithelial cells. IL-33 was recently shown to also be constitutively expressed in the cytoplasm of other cells, including platelets and megakaryocytes.[4] IL-33 has two major domains, an N-terminal nuclear domain and an IL-1-like cytokine domain, that are connected by a central domain (Fig 1). The N-terminal nuclear domain (amino acids 1-65) is critical for nuclear localization and chromatin association of IL-33, while the IL-1-like cytokine domain is critical for binding to the IL-33 receptor, ST2. Unlike many other conventional cytokines, IL-33 lacks a signal peptide that brings the proteins to the cytoplasmic reticulum and Golgi pathway, and it therefore cannot be secreted through the conventional mechanism. Instead,

full-length, biologically active IL-33 is immediately released into the extracellular space upon cell damage such as necrosis.[5] In contrast, during apoptosis (Fig 2),[5] full-length IL-33 can be cleaved by caspase-3 and caspase-7 into inactive forms. These facts suggest that IL-33 plays a role in induction of inflammation as an alarmin that is released from necrotic cells after tissue injury to alert the immune system, but in apoptotic cells it is degraded by caspases to inactive forms.

Recent studies also demonstrated that IL-33 can be induced in various types of cells during inflammation and then secreted into the extracellular space via unconventional mechanisms, without involving cell death. For instance, the levels of nuclear IL-33 were increased in the nuclei of airway epithelial cells from patients with asthma and chronic obstructive pulmonary disease (COPD) and in the nuclei of keratinocytes from patients with atopic dermatitis. IL-33 was increased in the cytoplasm of monocytes after stimulation with lipopolysaccharide.[6] In addition, in human bronchial epithelial cells, ATP and purinergic receptor stimulation, which results in calcium influx, induced translocation of IL-33 into the cytoplasm and release of full-length IL-33 into the extracellular space without cell death (Fig 2).[7] These findings indicate that IL-33 acts not just as an alarmin, but also as a cytokine in the presence of two stimulatory signals: one increases expression of IL-33 in the nucleus or cytoplasm, and the other induces secretion of IL-33 into the extracellular space without cell death (Fig 2). However, the exact mechanisms of increased expression and secretion in tissues with type-2 inflammation remain unclear.

In addition to intracellular modification as described above, IL-33 can also be modified extracellularly. For instance, proteases from inflammatory cells such as mast cells

and neutrophils recruited to sites of inflammation cleave the full-length IL-33 into shorter forms (18-21 kDa) and liberate the IL-1-like cytokine domain. These shorter forms possess approximately 10 times greater biological activity than full-length IL-33 and have been called “mature” forms (Fig 2).[8, 9] These findings indicate that inflammatory environments rich in immune cells provide an amplification system for IL-33-mediated immune responses. After its release into the extracellular space, IL-33 can be inactivated within a few hours by extensive conformational change caused by oxidation (Fig 2).[10] This mechanism contributes to limiting the lifespan of IL-33 molecules and their biological activity.

Clarification of the sizes and biological activities of IL-33 in extracellular spaces, as well as characterization of the downstream effecter cells, is important to understand the involvement of IL-33 in allergic diseases. However, most papers have shown only upregulation of IL-33 protein in tissues or cell nuclei, but not increased IL-33 in extracellular spaces such as by analyzing bronchoalveolar and nasal lavage fluids. In addition, although it is now clear that the size of IL-33 molecules strongly influences the biological activity, information about the size of IL-33 in human samples from different diseases remains unclear. Although ELISA and multiplex assay are the methods normally used to detect IL-33 proteins, they are unable to distinguish the forms of IL-33. The only way to identify the form of IL-33 is western blot (WB) using antibodies that recognize the C-terminal IL-1-like cytokine domain such as Nessy-1.[9] To understand the biological activity of IL-33, it is important to use highly sensitive WB to clarify the size of IL-33 molecules released into extracellular spaces.

Recently, several clinical trials using anti-IL-33 antibodies have been planned for

allergic diseases such as asthma and chronic rhinosinusitis with nasal polyps. However, each allergic disease such as asthma is not a single disease, but a complex and heterogeneous disease, meaning that each subtype of disease has different pathological mechanisms. Therefore, it will be very important to understand the precise mechanisms and involvement of IL-33 by determining the size in each disease subtype. Knowing the size of IL-33 in each subtype of allergic disease would enable us to select appropriate patients and decide the timing of treatment for clinical trials using anti-IL-33 antibodies.

Benchside research discovered and elucidated the importance of IL-33 in allergic diseases, and now it is time for bedside applications of IL-33 as a biomarker and/or therapeutic target. Before we take further steps, we need to know the actual size of IL-33 molecules we detect, measure, try to inhibit and discuss.

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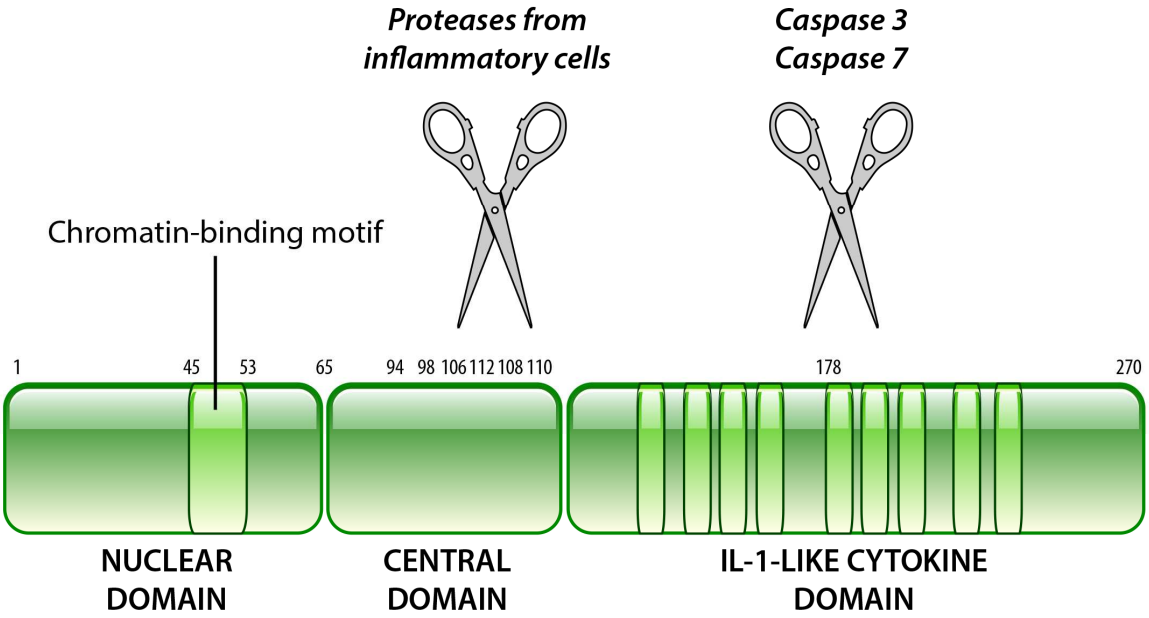
Figure 1. The structure of IL-33 protein

IL-33 protein is composed of two major domains, i.e., an N-terminal nuclear domain and an IL-1-like cytokine domain, which are connected by a central domain. The N-terminal nuclear domain is critical for nuclear localization and chromatin association, while the IL-1-like cytokine domain is critical for binding to the IL-33 receptor, ST2. The IL-1-like cytokine domain can be cleaved by caspase-3 and caspase-7. The central domain can be cleaved by proteases from inflammatory cells such as mast cells and neutrophils.

Figure 2. Intracellular and extracellular modification of IL-33

IL-33 is constitutively expressed in the nuclei of endothelial and epithelial cells. Bioactive full-length IL-33 is released during necrosis, whereas IL-33 is cleaved by caspase-3 and caspase-7 into inactive forms during apoptosis. IL-33 is also localized in the cytoplasm of other cells such as megakaryocytes and platelets and released into the extracellular space through unknown mechanisms. IL-33 protein increases in both nuclei and cytoplasm in response to some yet-unknown stimuli and is then secreted into the extracellular space

149 through unconventional mechanisms not involving cell death. Full-length IL-33 is cleaved
150 into “mature” forms by proteases from such inflammatory cells as mast cells and neutrophils.
151



	Form	Modified by	Activity
Full length	1-270	-	+
Cleaved	112-178,179-270	Caspase 3, 7	-
Cleaved	95-270, 109-270	Chymase from mast cells	30-fold greater than full length
Cleaved	107-270	Tryptase from mast cells	30-fold greater than full length
Cleaved	72-270, 79-270	Tryptase from mast cells	2-fold greater than full length
Cleaved	95-270, 109-270	Cathepsin G from neutrophils	10-fold greater than full length
Cleaved	99-270	Elastase from neutrophils	10-fold greater than full length
Disulfide bridge	S - S	Oxidation	-