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The Inflammasome as the Gate Keeper of "Danger Signals"

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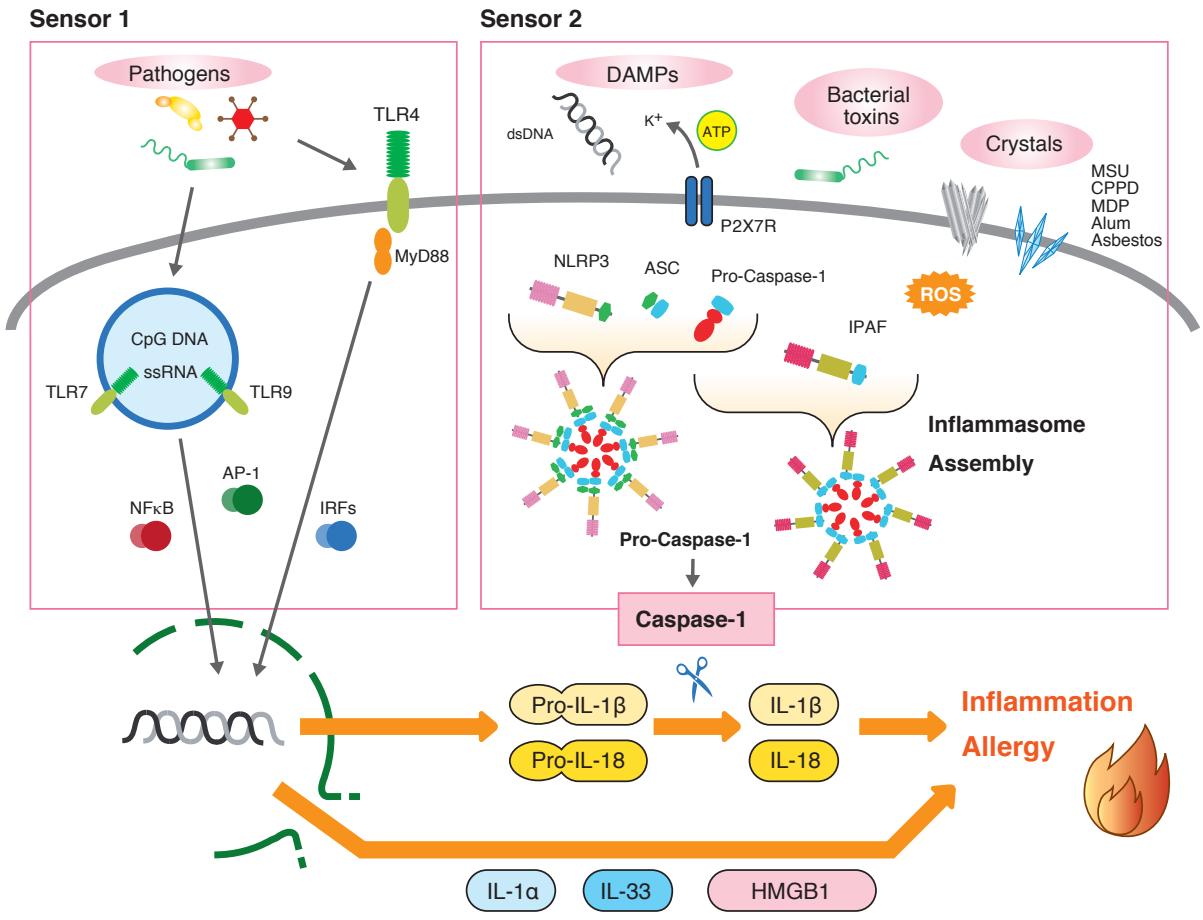
Inflammasomes

The living body is always exposed to various pathogens, but its health is maintained through defense mechanisms that sense and eliminate pathogens such as the immune system or through coexistence mechanisms in the case of some pathogens. Pathogens act as a danger signal to the host. Pattern recognition receptors (PRRs) expressed in some immune cells immediately recognize the danger signal and trigger activation of the immune system.[1] PRRs are generally divided into two types according to the mode of existence.

One type is toll-like receptors (TLRs) that perceive the structures common to microbes outside the cell (Figure 1, Sensor 1).[2] Examples of TLR ligands are peptidoglycan and lipopolysaccharide (LPS) derived from bacterial cell walls. TLR4, an LPS sensor, recognizes LPS on the surface of cells in cooperation with CD14 and MD2 to transmit signals into the cells. TLR7 and TLR9 are located in intracellular endosomes. TLR7 recognizes viral single-stranded RNA (ssRNA) while TLR9 recognizes bacterial, viral CpG DNA.

The other type of PRRs, which functions in the cytoplasm, includes the RIG (retinoic acid-inducible gene)-like helicase (RLH) family and NOD (nucleotide-binding oligomerization domain)-like receptor (NLR) family.[3] RLH recognizes viral RNA and induces production of type I interferon (IFN) via the activation of nuclear factor kappa B (NF-κB) and interferon regulatory factor (IRF) 3/7 (Figure 1, Sensor 2).[3]



Fifure 1 The recognition pathway and inflammatory response of danger signals

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The NLR family recognizes not only microbial components, but also double-stranded DNA (dsDNA), ATP, and uric acid crystals that are danger-associated molecular patterns (DAMPs) released from damaged cells; asbestos and silica that are inflammation-causing environmental factors; and aluminum hydroxide for adjuvant use. The activated NLR recruits procaspase-1 via the adaptor protein ASC (apoptotic-associated speck-like protein containing a caspase recruitment domain) into a supramolecular complex called inflammasome. Procaspase-1, approaching each other during the formation of the inflammasome and undergoing autodigestion, becomes activated caspase-1. Its enzymatic activity cleaves the precursors of IL-1 family cytokines, IL-1β and IL-18, for maturation.

One of the DAMPs is high-mobility group box-1 (HMGB1).[4] HMGB1, a nuclear protein associated with nonhistone chromatin, is released from the nucleus to the cell exterior upon necrosis and induces inflammatory reactions, as do IL-1α and IL-33 (described later). HMGB1 acts on dendritic cells and monocytes and promotes their maturation, infiltration, and release of cytokines and inflammation mediators. In addition, it has been reported that NADPH oxidase is activated via recognition of HMGB1 by TLR4 on neutrophils. Regarding the association with diseases, HMGB1 has been suggested as a contributing factor to the inflammation associated with arthritis and Sjögren's syndrome, and its high expression in cancers also has been indicated to enhance survival and growth of cells via activation of NF-ϰB.

IL-1 Family Cytokines

The IL-1 family consists of 11 structurally-related molecules, and their physiological activities are changed by enzymatic processing.[5),6)] Both IL-1β and IL-18 are usually present in precursor forms within the cells, and are cleaved by activated caspase-1, which is activated by inflammasomes, to mature forms. The mature IL-1β and IL-18 are then secreted extracellularly, and act on various cells to induce mainly Th1 (type 1 helper T cell) type inflammatory reactions and migration of neutrophils to infected locations. Since the IL-1 family has no signal sequence, the mechanism by which the cytokines are secreted from the cells remains unclear.

IL-18 and Related Diseases

IL-18 is a cytokine produced by dendritic cells, monocytes, macrophages, neutrophils, and epithelial cells. It facilitates primarily Th1-type immunoreactions by acting on T cells. Stimulation of Th1 cells with IL-18 in the presence of anti-CD3 antibody triggers production of IL-13 (a Th2 (type 2 helper T cell) cytokine) as well as IFN-γ. Furthermore, action of IL-18 on basophils and mast cells causes complex physiological actions, including allergic reactions. IL-18 binding protein (IL-18BP) is a serum inhibitor of IL-18 activity. Under normal conditions, the abundance of IL-18BP exceeding that of IL-18 suppresses IL-18 activity, whereas up-regulated expression of IL-18 at the onset of an inflammatory reaction seems to intensify the reaction.[7)]

In regard to diseases, high levels of IL-18 are detected in the blood of patients with allergic diseases (bronchial asthma, atopic dermatitis, allergic rhinitis, *etc*.) and those with autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus [SLE], adult Still's disease, multiple sclerosis, Crohn's disease, ulcerative colitis, *etc*.). IL-18 is also found in the urine of patients with acute renal disorder[8)] and appears to mediate the progression of type 2 diabetes.[9)]

IL-33 and Related Diseases

IL-33 is also a member of the IL-1 family, but differs from IL-1β and IL-18 in its control mechanism of activation (Figure 2). Like IL-1α, IL-33 is present in the nucleus under normal conditions. Although the function of IL-33 as a nuclear factor remains elusive, its binding to the nucleosomal surface to suppress transcription has been demonstrated by *in vitro* experiments. In association with necrosis, the full-length IL-33 is released extracellularly and activates the immune cells expressing IL-33 receptors. At the inflammation site, IL-33 undergoes limited proteolysis by the proteases released from neutrophils and other cells, and limited proteolysis results in its enhanced activity. In the course of apoptosis, IL-33 is cleaved by activated caspase-3 and caspase-7, which terminates its inflammation-inducing ability.

It has been confirmed in human that IL-33 is expressed in a variety of cells (including endothelial, epithelial, and fat cells) and tissues (such as the stomach, lungs, skin, lymph nodes, and kidneys). Its up-regulated expression is reported in the mouse brain and spinal cord. Full-length IL-33 released extracellularly acts on diverse leucocytes to induce production of primarily Th2-type cytokines[10)] and plays a role mainly in defense mechanisms against parasite infection. Most recently, a Keio University team including Moro and Koyasu discovered the presence in the mouse visceral fat tissues of natural helper (NH) cells that secrete a large amount of Th2-type cytokines through IL-33 action. This became a hot topic because part of the system that protects from parasite infection has been elucidated.[11)]

IL-33 is also considered to participate at various stages of a wide variety of diseases ranging from parasitic infection and allergic diseases (asthma, rhinitis, sinusitis, *etc*.) to arthritis, diabetes, inflammatory bowel disease, SLE, Alzheimer's disease, and heart diseases.[12)]

T1/ST2 and Related Diseases

T1/ST2 was initially detected in the supernatant of myocardial cell culture and thereafter identified as a receptor of IL-33.[13)] T1/ST2 is expressed in a variety of cells and tissues and has three isoforms: transmembrane ST2L; Soluble T1/ST2 (ST2S) lacking the transmembrane domain; and ST2V having a shorter *C*-terminal domain than that of ST2S. ST2S could interact with IL-33 to block IL-33 activity.[14)] ST2V is expressed at a very low level because of the decomposition of the mRNA.

Like IL-33, T1/ST2 is known to affect various diseases; an increase in ST2S in serum has been observed in heart diseases, ulcerative colitis, SLE, pneumonia, sepsis, and other diseases. However, the association between ST2S and the onset of diseases remains to be clarified.

In Conclusion

As mentioned above, inflammasomes play a critical role in the defense system against pathogens. Therefore, breakdown of this system will directly lead to the onset of disease. Mutations in NLRP3/cryopyrin (a member of the NLR family) cause excessive

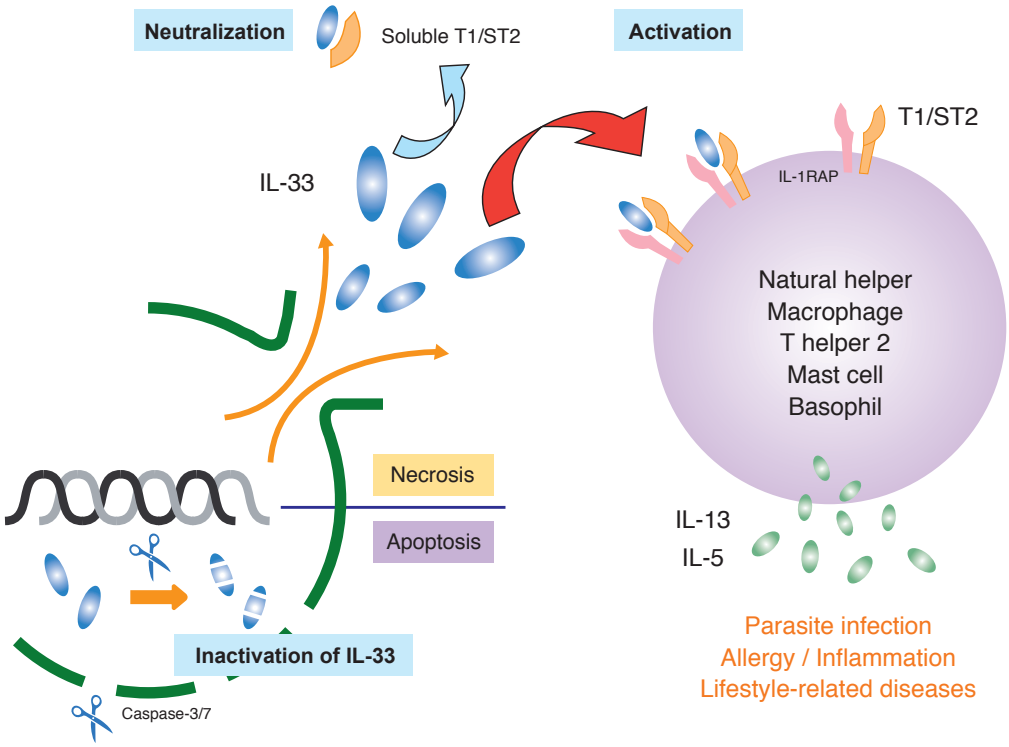

Figure 2 The activity control mechanism of IL-33

production of mature IL-1β in association with constitutive activation of inflammasomes and may result in the development of cryopyrin-associated periodic syndrome (CAPS), an autoinflammatory disease.[15] Mutations in the pyrin domain of ASC or the NOD2 gene are detected in patients with familial Mediterranean fever or inflammatory colitis-associated Crohn's disease.

Many IL-1 family members function as inflammatory cytokines and their immunoreaction-inducing ability varies widely. As a matter of fact, the immunosuppressive activity of IL-37 has been reported.[16] No reports are available on the physiological functions of other IL-1 family members. Future analysis will reveal their functions and significance.

Further, inflammasomes modulate immunoreactions by (indirectly) recognizing substances (such as inorganic substance and metal salts) via unknown receptors. It is thus expected that use of this characteristic feature will enable the application not only to treatment of cancer and autoimmune diseases, but also to improvement of vaccine efficiency, via artificial compounds controlling inflammasome activity.

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Antibodies

	Code No.	Product	Clone	Isotype	Application	Reactivity	Quantity
IL-18	D044-3	Anti-IL-18 (Human)	125-2H	Mo IgG1 κ	IP, ELISA, NT	Hu, Mo(-)	100 μg
	D048-3	Anti-IL-18 (Mouse)	93-10C	Rat IgG1	IP, NT	Mo	100 μg
	D304-3	Anti-IL-18 BP (Human)	#36	Mo IgG1 κ	WB	Hu, Mo(-)	100 μg
	D306-3	Anti-IL-18 BP (Mouse)	#36	Rat IgG2a	WB	Hu(-), Mo	100 μg
	M156-3	Anti-pro-IL-18 (Human)	43A11	Mo IgG1 κ	WB	Hu, Mo	100 μg
	M158-3	Anti-IL-18 (Rat)	91D8	Mo IgG2b κ	IP	Rat	100 μg
	M159-3	Anti-IL-18 receptor 1 (Human)	44G6	Mo IgG1 κ	FCM	Hu, Mo(-)	100 μg
	M163-3	Anti-IL-18 receptor 1 (Mouse)	33A11	Rat IgG1 κ	FCM	Hu, Mo	100 μg
IL-33	M138-3	Anti-IL-33 (Human)	5H1	Mo IgG1	WB, IP	Hu, Mo(-)	100 μg
	M187-3	Anti-IL-33 (Mouse)	1F11	Mo IgG1 κ	WB, NT*	Hu(-), Mo	200 μg
	M188-3	Anti-IL-33 (Mouse)	2C7	Mo IgG2a κ	WB, NT*	Hu(-), Mo	200 μg
	PM033	Anti-IL-33 (Human)	Polyclonal	Rab Ig(aff.)	WB, IH	Hu	100 μL
ST2	D067-3	Anti-ST2 (Human)	2A5	Mo IgG1	WB, IP, FCM	Hu	100 μg
HMGB1	M137-3	Anti-HMGB1 (HMG1) (Human)	4C9	Mo IgG1	WB	Hu, Mo, Rat	100 μg
TLR	D077-3	Anti-TLR4 (CD284) (Human)	HTA125	Mo IgG2a	FCM	Hu	100 μg
	D079-3	Anti-TLR4-MD-2 Complex (Mouse)	MTS510	Rat IgG2a κ	IP*, FCM, NT*	Mo	100 μg
	D205-3	Anti-TLR4 (CD284) (Mouse)	UT49	Mo IgG2b	IP, FCM	Mo	100 μg
	D206-3	Anti-TLR4-MD-2 Complex (Mouse)	UT15	Mo IgG1	IP, FCM	Mo	100 μg
	K0210-3	Anti-TLR1 (CD281) (Human)	GD2.F4	Mo IgG1	FCM	Hu	100 μg
	K0212-3	Anti-TLR2 (CD282) (Mouse)	T2.5	Mo IgG1	FCM	Hu, Mo	100 μg
	K0213-3	Anti-TLR9 (CD289) (Human)	5G5	Mo IgG2a	FCM	Hu, Mo	100 μg
ASC	D086-3	Anti-ASC (TMS1) (Human)	23-4	Mo IgG1	WB, IP, IC, IH	Hu, Mo(-)	100 μg

Hu: Human, Mo: Mouse, Rab: Rabbit, WB: Western Blotting, IP: Immunoprecipitation, FCM: Flow Cytometry, IH: Immuohistochemistry, NT: Neutralization, (aff.): affinity purified *: reported in journals

Kits and Proteins

Code No.	Product	Quantity
7620	Human IL-18 ELISA Kit	96 wells
7625	Mouse IL-18 ELISA Kit	96 wells
5332	Ab-Match ASSEMBLY Mouse IL-33 Kit	96 wells
7650	Human IL-33 Cytokine Domain Detection Kit	96 wells
7638	ST2 ELISA Kit	96 wells
B001-5	Recombinant Human IL-18	25 μg
B002-5	Recombinant Mouse IL-18	25 μg
B005-10	Recombinant Human IL-33	10 μg
B006-10	Recombinant Mouse IL-33	10 μg

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