The Inflammasome: An Antimicrobial against multi-drug-resistant bacterial infection in a clinical scenario

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Abstract

Inflammasomes are the sensors/receptors of the innate immune system and are responsible for the activation of caspase-1. It enhances the rate of inflammation in response to molecules originating from host proteins and infectious microbes. It has been involved in a host of inflammatory disorders. Inflammasomes are first-line immune pathways and regulate two defense responses to defense host cells: the proinflammatory cytokines synthesis and pyroptosis induction or programmed cell death. Assembly of the inflammasome complex requires a range of biochemical signals that come out during infections, metabolic variations, or tissue damage. Once the protein cascades have been made, caspase-1 is activated by inflammasomes, and caspase1 proteolytically converts inactivated proinflammatory cytokines IL-1β and IL-18 into activated form. Current studies on the mouse model have greatly enhanced our understanding of inflammasome molecular mechanisms and supporting human data on inflammasome in initiating or progressing diseases with a high impact on public health, including neurodegenerative diseases and metabolic disorders. Recent advancements looking forward to therapeutics that target inflammasome activity in inflammatory conditions have been reported. This review summarizes the current advances in understanding the mechanisms of inflammasome organization and activation, and will focus on this area of inflammasome research.

Keywords- NLR, TLR, PAMP, DAMP, HAMP, IL

1.0 Introduction

Inflammasomes are the innate immune sensors or pattern recognition receptors of pathogen-associated molecular patterns (PAMPs), danger-associated molecular patterns (DAMPs), and homeostasis-altering molecular patterns (HAMPs) (1) (2). They are assembled and found in

the cytosol of immune cells. PRR is generally classified into two categories: membrane-associated and cytosolic. Toll-like receptors (TLR) and C-Type lectin receptors (CLR) are examples of membrane-associated categories, while retinoic acid-inducible protease likes receptors (RLRs) and Nod-like receptors (NLR) are examples of cytosolic receptors (3). These all are receptors belonging to the class of Pattern Recognition Receptors family. They sense molecular patterns by different types of inflammasome sensors. PAMPs are secreted from microbial sources including viruses, fungi, bacteria, protozoa, and helminths (4). PAMPs specific to bacteria include all structural units that make up the structure of microbes like flagellin subunits, bacterial nucleic acids (DNA & RNA), LPS, and lipoproteins (5)(6). Bacterial types 3, 4, and 6 secretion systems are known for their impact on caspase-1 activating inflammasomes, necessary for producing bioactive inflammatory cytokines IL-1β and IL-18, key participants of anti-bacterial responses (7). Bacteria through secretion systems synthesize their own virulence factors to modify host cell functions and also have toxin components that kill host cells (8). They also enable the bacteria to rival other species. Inflammasome receptors can also sense danger-associated molecular signals in the form of danger-associated molecular patterns (DAMPs). DAMPs are derived by host cells during tissue injury and cell damage or inflammation. Host DNA and RNA, extracellular ATP, and uric acid are all examples of DAMPs released by damaged or dying cells (9). They also efficiently activate innate immune receptors to activate innate immunity (10). Another class of molecular patterns is homeostasis-altering molecular patterns (HAMPs), which are host-derived and are categorized by their mode of activation after sensing the inactivation of the host components due to toxins. HAMPs act as indirect molecular patterns and function like homeostasis modifying molecules (11).

2.0 Pattern Recognition Receptors' relation with inflammasomes

Pattern recognition receptors are protein sensors in and on cells that detect evidence of infection or tissue damage, which launch a signaling cascade designed to deal with the threat and they are important for inflammasome assembly and processing (12). The signals detected by PRR are called PAMPs, DAMPs, and HAMPs. PAMPs are specific molecular sequences that are only found in pathogens, for example, components of the bacterial cell wall or unique forms of nucleic acids include viral single-stranded DNA or ds-RNA (13)(14). PAMPs also tend to be molecules a pathogen needs in order to survive and prevent a pathogen from evolving away from innate immune recognition. PAMPs are highly conserved molecules of pathogens (15). DAMPs are host molecules that appear in the wrong place at the wrong time like ATP is all over the place inside cells but it is rarely found outside of cells, if a cell senses extracellular

ATP it suggests there is a damaged cell nearby and in this case, extracellular ATP would be acting as a DAMP **(Table 1)** (16).

Table. 1 Danger-associated molecular patterns, their intracellular localization, receptors, and references.

Receptors	DAMPs	Intracellular localization	References
TLR 2, 4, 9	Histones	Nucleus	(1)(12)
TLR 9	Genomic DNA	Nucleus	(1)(13)
TLR 2, 4, RAGE, TIM3	HMGB 1	Nucleus	(2)(14)
IL-1R	IL-1α	Nucleus	(15)
ST2	IL-33	Nucleus	(4)(16)
P2Y2, P2X7	ATP	Cytosol	(6)(16)
DNGR 1	F-actin	Cytosol	(16)
CD147	Cyclophilin A	Cytosol	(17)
CD91,TLR2,4, SREC 1, FEEL 1	HSPs	Cytosol	(18)
NLRP3	Uric acid crystals	Cytosol	(18)
TLR 2, 4, RAGE	Ferritin	Cytosol	(10)(19)
-	S100 s	Cytosol	(19)

TLR9, RAGE	Mitochondrial DNA	Mitochondria	(5)(19)
CD91	Calreticulin	Endoplasmic reticulum	(20)

Not only is the location of PAMPs and DAMPs important for immune signaling but the location of PRR is also extremely important. Some are on the surface of cells and can sense extracellular PAMPs & DAMPs (17). Others are cytosolic for intracellular sensing and still, others can be found in endosomes, an organelle formed during endocytosis. Endosomal pattern recognition receptors scent PAMPs or DAMPs that have been endocytosed from the extracellular space the distribution of membrane-bound PRR can be extremely polarized for example intestinal epithelial cells are constantly exposed to microbes in the intestinal microbiome (18)(19). If they were constantly sensing extracellular bacteria in the gut the intestine would always be inflamed instead many of these bacterial sensing PRRs are only located on the basolateral side of the cell, which is the side not facing inside of the gut (20). In this way, cells will only sense bacteria that have crossed an epithelial barrier and that need to be contained by the immune system.

2.1 Toll-Like Receptors (TLRs)

TLRs are well-studied families of pattern recognition receptors these receptors are homologs of a fruit fly protein called Toll. A toll is involved in the defense against bacterial and fungal pathogens (21). TLR homologous can be found in many mammals' other invertebrates even plants. This family of sensors is very ancient in an evolutionary context and TLRs are single-pass transmembrane proteins meaning that only pass through a lipid membrane one time **(Table 2)** (22)(23).

Table. 2 Toll-like receptors and their Pathogen associated molecular patterns with microbial sources

TLRs	PAMPs	Microbial sources	References
TLR 1	Triacyl lipopeptides	Mycobacteria	(21)
TLR 2	Peptidoglycans GPI-linked proteins Lipoproteins Zymosan	Gram-positive Trypanosomes Mycobacteria Yeasts and other fungi	(22)(23)

TLR3	Double-stranded RNA (dsRNA)	Viruses	(24)
TLR4	LPS F-Protein	Gram-negative bacteria Respiratory syncytial virus	(21)(24)
TLR5	Flagellin	Bacteria	(24)
TLR6	Diacyl lipoproteins Zymosan	Mycobacteria Yeast and fungi	(23)(25)
TLR7/TLR8	Single-stranded RNA	Virus	(25)
TLR9	cpG DNA		(26)
TLR10/TLR11	Unknown	Unknown	(26)

TLRs contain one extracellular and one transmembrane domain in structure and play a crucial role in inflammasome signaling and processing. The extracellular domain of a TLR has leucine-rich repeats from a horse shape or c shape and this is part of the protein that binds to ligands intracellular side has a distinct region called a TIR domain (24). The TIR region is special because it can interact with TIR domains on other proteins, that are essential for signal transduction. When a TLR binds ligand it either homodimerizes or forms a heterodimer with other TLRs dimerization brings together cytoplasmic tails of receptors, which allows for docking of TIR domain-containing adapter proteins MyD88, TRIF, Mal, and TRAM with each adapter having different endpoints to their signaling cascades (25)(26).

2.2 The difference between signaling cascades of two major adapter proteins MyD88 and TRIF

MyD88 pathway begins when cytoplasmic tails of dimerized TLRs are brought together allowing MyD88 to bind here MyD88 recruit's kinases IRAK-4, IRAK-1, and IRAK-2 (27). IRKs phosphorylate and activate TRAF-6 an E3 ubiquitin ligase is a type of enzyme that tags other proteins with ubiquitin groups. TRAF-6 poly ubiquities itself and the protein NEMO which recruits and activates TAK-1. TAK-1 then phosphorylates and activates the IKK complex, activated IKK complex phosphorylates Ikβ leading to the degradation of Ikβ (28). Normally Ikβ is bound to a protein called NFkβ in the cytosol, however, when Ikβ gets degraded NFkβ translocate to the nucleus where it acts as a transcription factor for inflammatory cytokines such as TNF-α and IL-6.

2.2.1 TRIF

TRIF is the other main adapter protein for TLRs and recruits TRIF-6 & TRIF-3 (29). TRIF-6 recruits RIP-1 which activates TAK-1, after activation of TAK-1 remaining pathway looks similar to the MyD88 pathway and results in NFkβ activation. TRIF-3 on the other hand recruits TBK-1 and IKKI together these phosphorylate and activate IRF-3. Activated IRF-3 forms a dimer and moves into the nucleus where it drives the expression of type-1 interferons are cytokines critical for antiviral responses (30)(31). Different TLRs use different adapters or combinations of adapters that allow the cell to tailor its response to the type of threat at hand.

2.3 TLRs working

TLR-2 heterodimerizes with TLR-1 or TLR-6 to bind bacterial lipoproteins and lipoteichoic acid a common component of gram-positive bacterial cell walls (32). TLR-3 is located on the inner surface of the endosome it recognizes double-stranded RNA which is a feature of many viral genomes because TLR-3 is located in the endosome and not the cytosol. It cannot directly sense intracellular viral infection however it can sense extracellular viruses that have been endocytosed or viruses that have infected a neighbouring cell gets phagocytosed. TLR-4 is one of the most well-studied mammalian TLRs and its senses lipopolysaccharide or LPS, which is found in the outer cell membrane of gram-negative bacteria (33)(34). It is the unique and only known TLR to use all four adapter proteins and uses an accessory protein called MD-2 to sense LPS. TLR-5 is expressed on the surface of myeloid cells, and intestinal epithelial cells and binds to bacterial flagellin (35). Flagellin is a protein and the main subunit of flagella whip-like structures of many bacteria use to move around. TLR-3 & TLR-7 are also endosomal PRRs and it binds single-stranded RNA (36). Mammalian cells have single RNA too and this is important to remember that TLR location is key mammalian cells do have single-stranded RNA in the nucleus and cytoplasm but not in the endosome. If there is SS-RNA in the endosome it usually results in phagocytosing SS-RNA viral particles. TLR-9 is another endosomal sensor and it recognizes unmethylated CpG DNA (37). CpG DNA is a sequence involving cytosine and guanine being adjacent to each other with cytosine being at the five prime ends. In mammals this sequence is heavily covered in methyl groups but not in bacteria or some viral DNA hence the term unmethylated (38). TLRs are not the only pattern recognition receptors used by mammalian cells, while TLRs are confined to lipid membranes there is another family of pattern recognition receptors that can sense microbial products in host cell cytoplasm these receptors are called Node like receptors.

2.4 Nod-like Receptors or NLRs

NLRs or cytosolic receptors are particularly good at sensing intracellular bacteria and are often expressed in epithelial cells & myeloid cells (39). These receptors are the main building blocks of inflammasome structure and assembly. TLRs and NLRs are also ancient pathogen sensors with leucine-rich repeats (40). However, instead of having a TIR domain, they have an amino-terminal card domain which is a docking site for other proteins with card domains. NLRs recognize components of peptidoglycan from both gram-positive and gram-negative bacteria, when NLRs sense its target a protein called RIPK-2 binds to NLRs card domain and leads to downstream activation of TAK-1 & IKK complex, then NFkβ similar to TRIF dependent pathway of TLR signaling (41). NLRs can sense intracellular bacteria and TLR-3,7 & 9 can sense endocytosed viruses. With the help of NLRs, cells do sense intracellular viruses and they use RIG-1-like helicases. RIG-1 like helicases sense viral nucleic acids, they have a helicase-like domain that can bind to viral RNA, and not one but two amino-terminal card domains are used for signal transduction . Some examples of RIG-1-like helicases include RIG-1 can especially sense viral SS-RNA and MDA-5 senses cytosolic ds-DNA activation of RIG-1 like helicases leads to the production of type-1 interferons, which are important antiviral cytokines (42). NLRs and RIG-1 helicase are important for the sensing of PAMPs but the P2X7 receptor senses extracellular ATP, RAGE is a receptor sense HMGB1 is a DNA binding protein. That should only be outside the nucleus but within the cell, once it is extracellular it becomes a DAMP. RAGE also senses a family of cytosolic calcium-binding proteins called S-100s. DAMP sensing leads to pro-inflammatory signaling cascades.

2.5 Activation of inflammasomes during bacterial infections

Specialised secretion systems of bacteria for example type 3 secretion system (T3SS), T4SS and T7SS in *Salmonella, S.aureus, Legionella* and *Mycobacterium tuberculosis* (Mtb) respectively are potent vacuolar pathogens for inflammasome activation. In the unique case of T3SS, structural needle and rod proteins of the injectisome are detected by the NAIP-NLRC4 pathway (43). In addition, flagellin can be translocated by both T3SS and T4SS and is detected by Naip5/6-Nlrc4 in mice. *Legionella* and *Salmonella* both lacking T3SS/T4SS/flagellins are detected through the non-canonical caspase-11 pathway presumably via their LPS. There are conflicting reports on how *Mycobacterium tuberculosis* initiates IL-1β maturation. However, the T7SS appears essential. *Mycobacterium tuberculosis* infected human monocytes, macrophages and dendritic cells synthesize IL-1β in a partially caspase-1 non dependent fashion. For example, Dectin-1 based detection of *Mycobacterium tuberculosis* in human dendritic cells can result in directly IL-1β synthesis through caspase-8 independently of caspase-1 (44). In the case of *Yersinia*, the T3SS effector YopJ decreases the rate of NF-$_k$B

and MAPK signalling and trigger the Ripk-1 (Receptor interacting kinase), Fadd (Fas associated death domain protein), and caspase-8 dependent pathway of caspase-1 activation, cytokine processing and cell death. *Yersinia pestis* is detected by Nlrc12 and Nlrp3 which together promote IL-18/caspase-1 dependent immunity in mice. The molecular determinants of Nlrp12 activation are unknown till date. Bacterial effectors may also subvert inflammasome signalling. For example, *Yersinia pestis* which lacking T3SS effectors but retain a functional injectisome are sensed solely by Nlrp3 (45). However, this pathway is naturally suppressed through YopK which bind to T3SS translocon and prevents its detection. Similarity, subversion of Nlrc4 and Nlrp3 dependent detection of *Salmonella* requires bacterial oxidative phosphorylation genes such as aconitase (acnB), isocitrate dehydrogenase (icdA) and isocitrate lyase (aceA). *Mycobacterium tuberculosis* subverts Aim2 and Nlrp3 inflammasomes in mouse cells in a T7SS dependent fashion. A large number of cytosolic bacterial toxins can be detected by Nlrp1b, Nlrp3 and Pyrin, which reveals the versatility of inflammasomes in detecting changes to host cytosolic components. Infection by wild type strains of Gram negative bacteria such as *Burkholderia, Vibrio, Proteus* and *Hemophilus* predominantly switch on the non-canonical caspase-11 pathway (46). Other bacteria, including *Salmonella, Legionella, Pseudomonas, Shigella* or pathogenic *E.coli,* also containing LPS that is detectable by caspase-11 when transfected directly into the cytosol. However, they express more scientific activators for the NAIP-NLRC4 axis, which overrides the caspase-11 pathway (47). This in the case of some Gram negative bacteria non-canonical activation by caspase-11 is triggered only in experimental situations using strains that lack other activators, including *Salmonella* or *Legionella* lacking flagellins and/or secretion systems.

3.0 Innate immunity relation with inflammasomes

During an immune response, host cells can respond to infectious threats and bacterial doubling times can range from the order of hours to minutes, they can rapidly overtake host tissues. The innate immune system needs to be tightly regulated mechanisms, which fight pathogens and don't start harming host tissues, but it can take hours to sense a threat and transcribe, translate and secrete effector proteins that are best suited to fight a threat (48). In order to counter pathogens, a cell needs a molecular mechanism that allows it to take decisive action and warn other cells quickly. Antimicrobials play important role in clearing infections from infected vacuoles but sometimes pathogens inside them secretory products that enable them to lyse the endosomal compartment reaching the cytoplasm and accessing sufficient nutrients for them to

replicate and take over the cells (49)(50). One way to do this is through an inflammasome, which is a large, multiprotein complex that senses threats and initiates inflammation. Inflammasomes are the sensors of innate immunity and they contain three important components in our structure including one sensor, card domain, and inflammatory caspases. The inflammasome sensors sense a wide range of pathogenic or damage-associated signals (51). Immunologists are still learning how sensing these signals triggers a response since the sensor doesn't necessarily need to bind a ligand in order for it to be sensed, which makes it different from a pattern recognition receptor (52)(53). The card domain can either be built directly into the sensor, or it can be found in adaptor protein ASC, which many inflammasomes use. The CARD domain is essential for inflammatory caspases to bind to inflammasome complexes. Inflammatory caspases, usually caspase-1 or caspase 4 and 5 in humans, are a kick-off signaling cascade (54). They are zymogens, meaning they need to be cleaved to be activated, but once they are activated, they are responsible for all of the effector functions of inflammasomes. Before Inflammasomes can assemble, it first needs to be primed. The priming step transcriptionally upregulates sensor components as well as IL-1β, which is one of the downstream cytokine effectors of inflammasome (55). This is the first signal in a two-signal system, priming occurs when pattern recognition receptors sense PAMPs or DAMPs. The next step is sensing, which occurs when sensor components sense additional PAMP or DAMP signals (56)(57). Inflammasome doesn't necessarily need to bind directly to these signals. The individual inflammasomes, but some of these signals include bacterial lipopolysaccharide, viral DNA, bacterial toxins, extracellular ATP, silica particles, reactive oxygen species, and changes in intracellular potassium levels (58). Once the sensor is activated, the inflammasome begins to assemble. Multiple copies of the active sensor get together, and each of them binds to the ASC adaptor. The ASC adaptor has a CARD domain that allows pro-caspase-1 to bind. The fully assembled inflammasome has multiple subunits of each of the components, meaning that when it's ready, it can execute downstream steps quickly (59). The way pro-caspase-1 binds to inflammasome enables autocatalytic cleavage of pro-caspase-1, meaning that it cleaves and activates itself. Activated caspase-1 can then cleave pro-IL-1β and pro-IL 18. The mature forms of these proteins, IL-1β and IL 18, are then potent stimulators of inflammation in other cells and also a part of innate immunity (60). IL-1β signals through the IL-1 receptor, which signals similar to a TLR, in that it has a TIR domain and activates NF-kB through MyD88, also inducing transcription of several pro-inflammatory genes, including cytokines IL-6 and TNF-α. IL-18 can induce vascular components of inflammation, such as increased expression of cell adhesion proteins with the production of chemokines (61). It can also induce IFN-y, which is

an important cytokine for dealing with viral infections. Caspase-1 also cleaves a host protein called Gasdermin-D and cleaved Gasdermin D moves to the cell membrane and forms pores, causing the cell to die in an inflammatory form of programmed cell death called pyroptosis (62)(63). Pyroptosis is an effective way to kill intracellular pathogens, and it can also recruit other immune cells to the site of damage. Current research is adding more nuance to these pathways, and a better understanding of what kind of signals lead cells to fully undergo pyroptosis. It is understood that IL-1β and IL 18 are released through Gasdermin-D pores (64). Inflammasomes have been most widely studied in myeloid cells like macrophages and dendritic cells, but there has been growing appreciation for inflammasome activation in epithelial cells, which line surfaces of the body that interface with the outside world.

4.0 Inflammasome sensors with specific ligands

Inflammasomes are formed when different sensors or receptors sense different types of ligands. NLRP3 is a global sensor of PAMPs and DAMPs, the sensor of NLRP3 recognizes, logical changes in the cell caused by PAMPs and DAMPs, rather than a specific ligand **(Table 3)** (65). AIM 2 is the sensor of ds-DNA containing a pyrin subunit for the recruitment of ASC adaptor through pyrin-pyrin interaction. ASC subunit has a CARD domain for the recruitment of caspase through CARD-CARD interaction (66). AIM is the principal member of the ALR family. In humans, there are only 4 members, but in mice, there are 13 of them.

Table. 3 Inflammasome sensors with specific ligands

Inflammasome sensor	Ligand	References
AIM 2	ds- DNA	(30)
NLRP2	Extracellular ATP	(31)(32)
NLRP7	Acylated lipoproteins of bacteria	(33)(34)(35)
NAIP and NLRC4	S.Typhimurium, flagellin,T3SS & T4SS components	(36)
NLRP1	Protease	(36)(37)
NLRP1b	B. anthracis	(37)
NLRP3 and NEK7	PAMPs, DAMPs	(65)
NLRP6	Metabolites and lipoproteins	(38)(65)
NLRP9	ds-RNA (Virus)	(39)

Pyrin	Toxin-induced modifications of Rho GTPase	(40)(66)
NLRP12	Klebsiella pneumonia and Mycobacterium Tuberculosis	(40)(66)
Caspase-11	Lipopolysaccharide (LPS)	(67)(68)
IFI-16	Double-stranded DNA	(68)

AIM 2 is the combination of the HIN 200 domain and a pyrin domain and this is normally inhibited in the cell, but once the DNA is introduced into the cytoplasm in the architecture of AIM 2, HIN 200 directly binds to ds DNA through electrostatic interaction indicating that AIM 2 can recognize DNA of are recognize irrespective of sequence specificity as long as its 80 base pair in length (67). AIM 2 does not always behave like an inflammasome. It also can function independently of the inflammasome as in the case of colitis and colon cancer, where it directly inhibits cell cycle regulation or proliferation separately of ASC and caspase 1 (68). But in reference to infectious diseases, there is no doubt that AIM 2 can function like an inflammasome. The NLRP1, NLRP2, NLRP3, NLRP6, NLRP7, NLRP12, and NAIP/NLRC4 are found in humans and these are all known as human NLRs. Nalp1b, Nlrp3, Nlrp6, Nlrp12, and Nlrc4 are reported NLRs in mice.

5.0 General principles of inflammasomes activation

Dr. Jurg Tschopp was the first man, who defined the concept of inflammasome in 2002 and two years later, Dr. Tschopp and co-workers discovered the NLRP3 inflammasome and reported its curial role in autoinflammatory disorders (69). The cytosolic pathogens interact with different cytosolic receptors that activate innate immune responses leading to the clearing of infections. Cytosolic receptors like AIM- 2 like receptors (ALRs), NOD-like receptors (NLRs), and RIG-1-like receptors (RLRs) are very crucial for cytosol-associated immune responses and they efficiently induce transcription regulation of inflammatory cytokines and type -1 interferons but some members of these NLRs and ALRs can also initiate the formation

of inflammasomes **(Figure 1).**

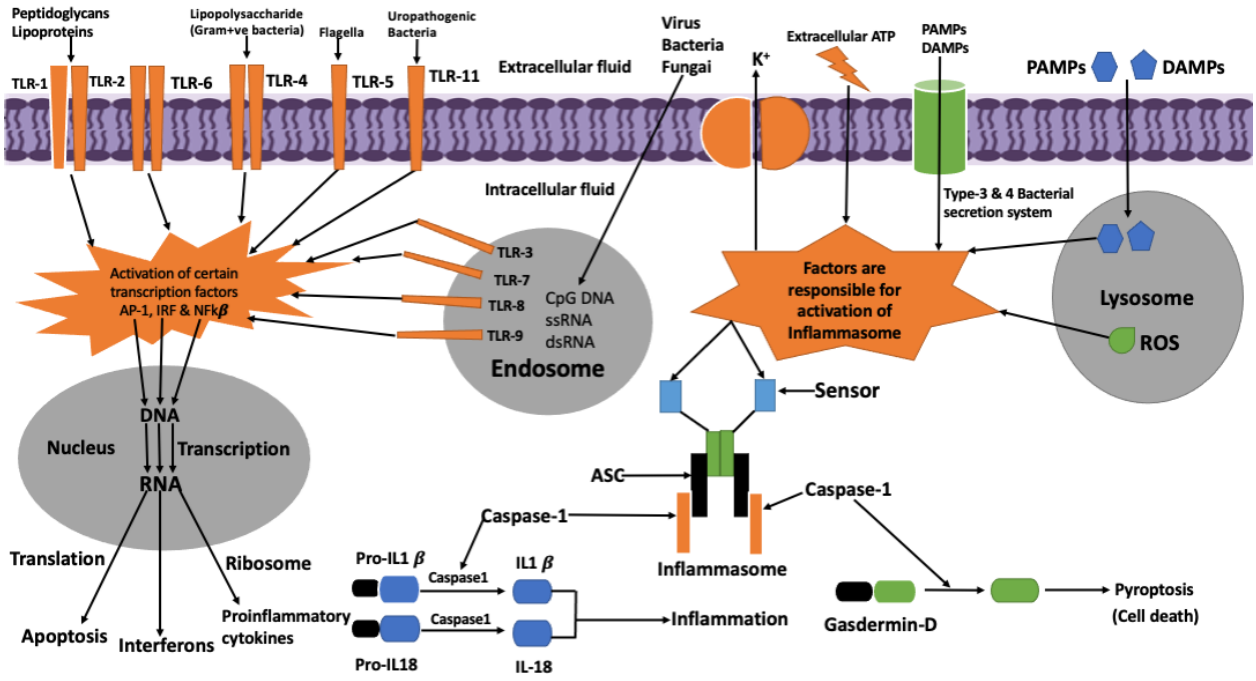

Figure 1. Inflammasomes are the part of the innate immune system responsible for the activation of inflammatory cell death and part of cytosolic multiprotein oligomers. Inflammasome activation and assembly initiates proteolytic cleavage, secretion, and maturation of pro-inflammatory cytokines interleukin-18 and interleukin-1β as well as cleavage of Gasdermin-D (69). The N-terminal fragment resulting from this cleavage induces a pro-inflammatory form of programmed cell death distinct from apoptosis, referred to as pyroptosis, and is responsible for the secretion of mature cytokines, presumably through the formation of pores in the plasma membrane (70).

Many inflammasomes belong to the NLR family of pattern recognition receptors. NLRP-1 seems to be found in more diverse cell types than some of the other inflammasomes, as it can be found in adaptive immune cells and even non-immune cells. In addition to innate immune cells (70). The NLRP-1 inflammasome is activated when the NLRP-1 sensor is cleaved by bacillus anthracis lethal toxin, which is one of the components of anthrax toxin. Certain alleles of NLRP-1 in rodents can also respond to infection with the protozoan parasite toxoplasma gondii (71). NLRP-3 is one of the best-studied inflammasomes and is primarily expressed in myeloid cells like macrophages and dendritic cells. It is also known as NALP-3 or cryopyrin. NLRP-3 can be activated in response to a huge range of bacterial, fungal, and viral infections, as well as many DAMPs. Some of these DAMPs include extracellular ATP, monosodium urate, cholesterol crystals, and amyloid beta, which is a peptide that makes up amyloid plaques in the brains of patients with Alzheimer's disease (72)(73). NLRP-3 also senses several environmental irritants, silica, and asbestos. NLRC-4 is another inflammasome that is primarily expressed in immune cells and tissues. Unlike the other NLRPs, NLRC-4 contains an

endogenous CARD domain, meaning that it can directly bind procaspase-1. NLRC-4 is involved in sensing several different bacterial infections, including Salmonella, Legionella, Shigella, and Pseudomonas (74). NLRC-4 has been shown to be activated in response to flagellin, the protein that makes up bacterial flagella. NLRP-6, also known as NALP-6, is a more recently discovered member of the NLR inflammasome family. It is unique among inflammasomes in that it can activate the transcription factor NF-kB, similar to a pattern recognition receptor (75). NLRP-6 senses bacterial infections such as Listeria and Staphylococcus aureus, as well as some viral infections. NLRP-6 is highly expressed in the intestinal epithelium and may be involved in controlling the composition of gut microbiota (76). In addition to NLR family inflammasomes, there are some inflammasome sensors that sense double-stranded DNA. The AIM-2 inflammasome senses cytosolic double-stranded DNA, meaning that it is especially important for sensing viral and intracellular bacterial infections (77). Unlike some sensors, which can distinguish between microbial and host DNA, AIM-2 can be activated by either, as long as it is in the cytosol. That host DNA should be safely contained in the nucleus under normal conditions meaning that it will be shielded from activating AIM-2 (78). This is similar to a recently discovered inflammasome called IFI16, which also senses double-stranded DNA.

5.1 Canonical and non-canonical inflammasome activation

In the non-canonical inflammasome, direct cytosolic sensing of intracellular LPS by caspase-11 in mice or caspases 4 and 5 in humans, can lead to the assembly of a macromolecular complex that cleaves Gasdermin-D, enabling pyroptosis (79)(80). In some cases, non-canonical inflammasome activation leads to canonical caspase activation that can cleave pro-IL-1β and pro-IL 18. Inflammasomes are an exciting phenomenon, and their study represents a relatively new branch of immunology. There has been quite a bit of recent research focusing on their importance in controlling various infections, as well as their ability to perpetuate inflammation and disease when they are not well regulated (80)(81). **Pyroptosis** is inflammatory programmed cell death and it takes place in immune cells and epithelial cells. Pyroptosis is triggered by different types of caspases after the cleavage of the Gasdermin-D protein in the case of both canonical and non-canonical inflammasomes (81).

6.0 NLRP-3 Inflammasome for drug targets in inflammatory diseases

NLRP-3 is a universal or global inflammasome and plays a major role in innate immunity to maintain homeostatic tissue function. NLRP-3 acts as an innate immunity signalling receptor and monitors extracellular space as well as many subcellular compartments for signs of

infection damage or other cellular stressors (82). The organization of NLRP3 is illustrated in **Figure 2.**

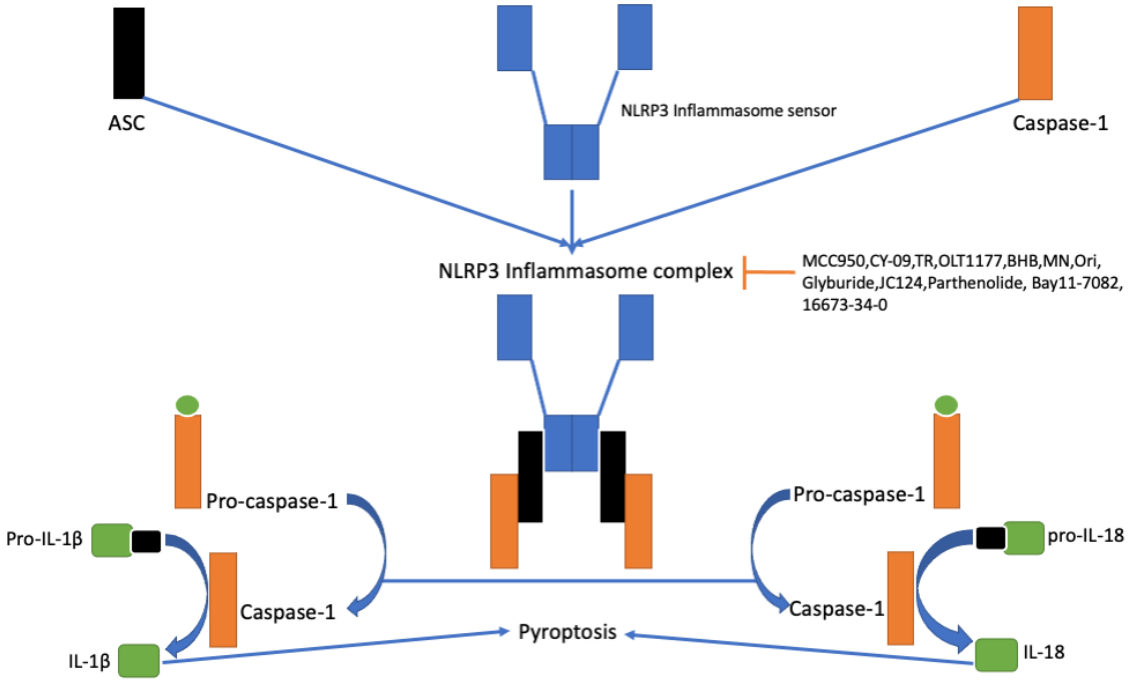

Figure 2. NLRP3 inflammasome is the complex of three constituent molecules (i.e. NLRP3, ASC, and caspase-1) (83). NLRP3 inflammasome complex is responsible for the cleavage of pro-caspase-1 into its active isomeric form caspase-1. Caspase-1 in turn cleaves pro-IL-18 and pro-IL-1β to their active isomeric form IL-18 and IL-1β respectively. The increment in these proinflammatory cytokines ultimately leads to pyroptosis (84).

Inflammasomes are key signaling platforms that detect pathogenic microorganisms, tissue damage, or metabolic imbalances and that activate highly pro-inflammatory cytokines interleukin-1beta and interleukin-18 (83). NLRP-3 inflammasomes are a group of multimeric protein complexes that consist of an inflammation sensor molecule the adaptor protein ASC and caspase-1. Several inflammation sensor molecules can trigger the formation of inflammasomes most of the inflammasomes that have been described to date contain NOD-like receptor sensor molecules namely NLRP-1, NLRP-3, NLRP-6, NLRP-12, and NLRC-4 (84). Inflammasomes have been described that contained Pyrin family members including AIM-2 responsible for ds-DNA sensing. NLRP-1 inflammasome is activated by bacillus anthracis lethal toxin and NLRC-4 inflammasome is activated by gram-negative bacteria was type 3 or 4 secretion system (85). NLRP-3 inflammasome is activated in response to the widest array of stimuli including pathogen-associated molecular patterns such as pore-forming toxins, RNA, M2 protein, Hemozoin, B-Glucans, and Hyphae (86). DAMPs include ATP, Amyloid Beta, Alum, Asbestos, Glucose, Hyaluronic, MSU, and ROS also sensed by NLRP-3

inflammasome (87). Increasing evidence in mouse models supported by human data strongly implicates the involvement of inflammasome in the initiation or progression of diseases with a high impact on public health. Such as metabolic disorders, neurodegenerative diseases, hypertension, inflammatory bowel disease, respiratory diseases, and cancer (87). While the molecular mechanisms linking NLRP3 activation to disease remain poorly understood, researchers are gaining many vital clues. NLRP-3 inflammasome has been reported to consist of three main components: a sensor (NLRP3), an adaptor (ASC/PYCARD), and an effector (caspase-1) each of which is controlled by multiple post-translational modifications (PTMs) (82). The ubiquitin system controls NLRP3 sensors and priming. Phosphorylation controls NLRP3 self-association and interacting networks for the ASC adopter, and ubiquitination control oligomerization. While phosphorylation regulates ASC localization and assembly. Less is known about how PTMs control the caspase-1 effector, although ubiquitination and phosphorylation are reported to play important roles (88). Targeting enzymes that write, read and erase these various PTMs could be one way to treat diseases associated with NLRP3 inflammasome. Further studies are needed to understand the mechanisms of PTM regulation and how to manipulate it to target the origins of various inflammatory diseases.

6.0 Inflammasomes role in health and disease

The inflammasome is increasingly recognized as critical orchestrator of immunity. Inflammasomes are at the center of a variety of pathways in innate immune cells, including cytokine production, cytoskeletal remodeling, and inflammatory cell death. Inflammasome formation is initiated when a PAMP or DAMP is recognized and triggers signaling often via Nod-like receptor protein, such as NLRP3 or NLRC4 (89). This results in nucleation and oligomerization of the adaptor protein Asc at the site of the NLR and recruitment of procaspase-1 to the CARD of Asc. Dimers of procaspase-1 are then cleaved to active caspase-1 through autoproteolysis, which then catalyzes the final processing of pro-IL-1b and IL-18 into their mature, secreted forms (90). Activation of caspase-1 is also accompanied by an inflammatory form of apoptosis, termed pyroptosis. Noncanonical caspase-11 inflammasomes, as well as pathways dependent on caspase-8 or neutrophil proteases, have also been described. Inflammasome-dependent secretion of IL-1b and IL-18 is critical for the immune control of many microbes and may play an important role in vaccine adjuvant-induced responses (91). However, dysregulation or inappropriate activation of inflammasomes can also produce severe autoinflammation and contribute to neurodegenerative, metabolic disorders, autoimmune disorders, Alzheimer's disease, Parkinson's disease, Obesity, Type 2 diabetes, Atherosclerosis, Multiple sclerosis, and many other pathologic processes **(Figure 3)** (91).

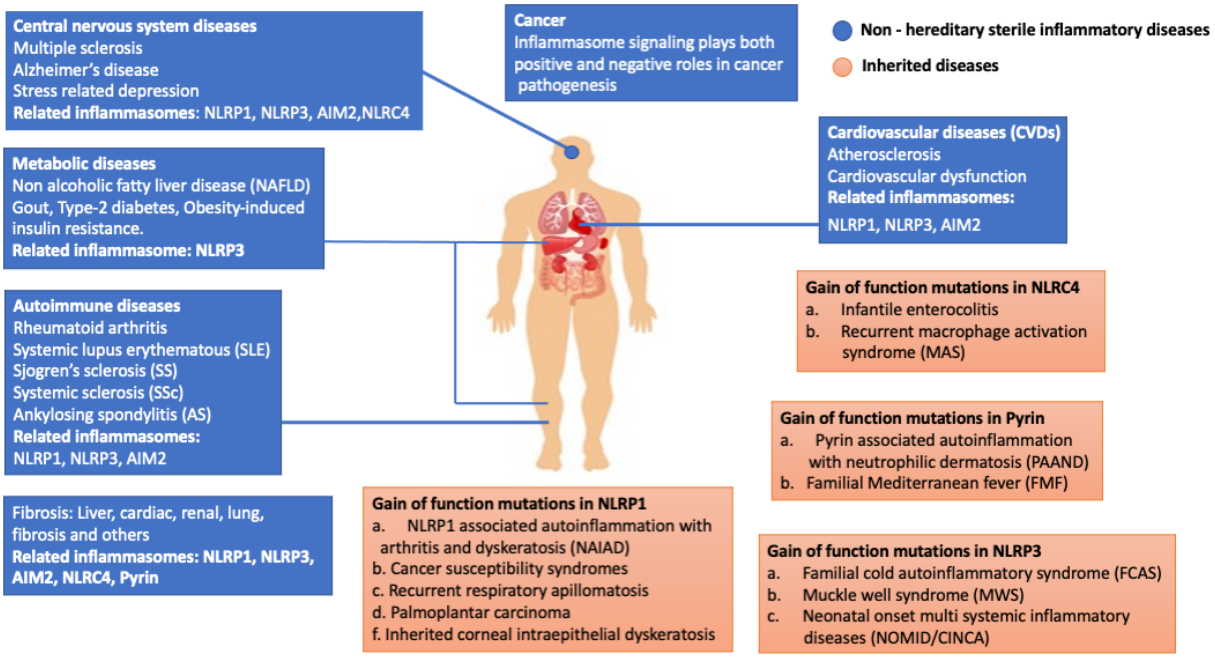

Figure 3. Inflammasomes related human diseases (91). Canonical inflammasomes are involved in multiple inherited diseases and non-hereditary sterile inflammatory diseases . The highly relevant inflammasomes are responsible for sterile inflammatory diseases.

To some extent, the roles of IL-1b and IL-18 overlap. Prominent effects of IL-1b include recruitment of neutrophils to sites of infection, promotion of endothelial cell adhesion, and stimulation of adaptive Th17 responses. An important role of IL-18 is to induce NK and T cells to produce IFN-g, which activates macrophages (92). IL-1b, in particular, tends to cause host tissue damage, whereas IL-18 tends to have a less detrimental effect, although still helping to control infection (93). This can be critical for the clearance of intracellular pathogens and for the efficient activation of adaptive immune responses. Consequently, inflammasome-activated caspase-1 and subsequent levels of IL-1b and IL-18 secretion are key events in many infectious and non-infectious diseases.

7.0 Role of Inflammasomes in inflammation and therapeutics

Inflammasomes always enhance the rate of inflammation through different scientific ways and provide support for long-term persistence. Inflammation is a non-specific defense mechanism of the innate immunity against harmful stimuli (ex: foreign partials include DAMPs, PAMPs & HAMPS), pathogens, dead cells, or irritants) and is scientifically handled by host neutrophils, dendritic cells, and monocytes (94). A low level of tissue injury can lead to the presence of pathogens on the site of tissue injury, while a high level of tissue injury can lead to chronic or systemic inflammatory disorders. Inflammation symptoms are pain, heat, redness

(erythema), swelling (edema), and loss of function, these all symptoms are present at the site of infection (95)(96). Erythema is responsible for increased vascular diameter and blood flow. Due to erythema, both redness and heat increase at the site of infection. The blood vessels become permeable to the passing fluid and proteins leading to swelling. During inflammation, endothelial cells are producing cell adhesion proteins which are responsible for the attachment and extraversion of white blood cells including neutrophils, lymphocytes and monocytes (97) . Inflammatory response is generated by a class of signalling molecules also known as immune regulators, which are responsible for regulation of immune cells. Activated immune cells like macrophages (activated form of monocytes) secrete chemo attractants known as chemokines (98). Chemokines call for immune cells at the site of inflammation. Neutrophils are the first to arrive at the site of inflammation followed by monocytes and dendritic cells (99). Dendritic cells make contact with the antigen coming from the invading pathogens and take them to nearest lymph nodes. Two reported key mediators of inflammatory response are histamine (released by a class of injured cells during inflammation) and kinins (present in blood plasma in inactive form) both are responsible for vasodilation and leads to the permeability of capillaries (100). Kinins are reported nerve stimulators and responsible for pain during inflammation. Inflammasome is essential for health and overcoming inflammatory diseases and also play a crucial role in the maturation and release of proinflammatory cytokines (101). In inflammation damaged cellular contents (malignant or damaged cells and infectious agents) removal requires exposure to the inflammasome. Inflammasome is responsible for initiation and regulation of inflammation as the maturation of pro-inflammatory cytokines (which increase the rate of inflammation) completely depend on the proteolytic activity of caspases and caspases are an important part of the inflammasome complex (102)(103). Caspase proteolytic activity is regulated and controlled by other members of inflammasome assembly. In the case of inflammation, inflammatory response is regulated by pattern recognition receptors especially present on cell surfaces of immune cells (like macrophages). One of the most reported cell surface receptors is TLR-4 responsible for the detection of extracellular lipopolysaccharide (LPS). LPS is a highly immunogenic component of Gram-negative bacterial cell wall (104). NFkβ is a nuclear transcriptional factor that plays a critical role in the initiation of inflammatory signalling pathways. NFkβ is activated by the MyD88 pathway after activation of PRR by ligand molecule and the nuclear transcription factor initiates transcription of proinflammatory cytokines (105). Proinflammatory cytokines are released by activated macrophages and participate in the up-regulation of inflammatory reactions. Pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α are responsible for pain in inflammation (106). The

activated inflammatory signalling pathways participate in the up-regulated expression of pro-inflammatory cytokines and few certain inflammatory genes are also important for the induction of cyclooxygenase-2 (COX-2) (107). The other proinflammatory genes play a very critical role in the synthesis and regulation of inflammatory mediators like nitric oxide (NO) and prostaglandin E2 (PGE2). Prostaglandins originated from eicosanoids (a family of very potent short-range signalling messengers). Eicosanoids also include prostanoids and leukotrienes, all eicosanoids are of common origin and made by 20 carbons of polyunsaturated fatty acids eicosanoic acid, particularly arachidonic acid (108). Prostaglandins are first reported in human semen but in the case of mammalian cells, almost all mammalian cells produce prostaglandins except red blood cells (109). Prostaglandins originate from C20 fatty acid prostanoic acid (which contains a cyclopentane ring in our structure). Arachidonic acid is a reported most prevalent precursor of prostaglandins in humans. In the synthesis of prostaglandins, arachidonic acid is the substrate of the cyclooxygenase (COX) enzyme or prostaglandin H2 synthase. In the case of mammals, two other isozymes are reported COX, COX-1, and COX-2 (110). COX-1 is participating in the synthesis of prostaglandins, which is responsible for the regulation of gastric mucin, and COX-2 is responsible for prostaglandins which mediate inflammation and pain. The other important diverse reported functions of prostaglandins are stimulated uterine contraction, lowering blood pressure, vasodilation, mediation of inflammatory response, regulation of neurotransmission, inhibition of gastric secretion, sensitization to pain, and stimulation of smooth muscle contraction (111). Aspirin (acetylsalicylate) is a reported agent or drug for the inhibition of prostaglandins synthesis. Aspirin has anti-inflammatory, analgesic (pain relieving), and antipyretic (fever reducing) effects. Aspirin is also responsible for active site irreversible inhibition of COX enzymes by acetylating a Serine residue. Inhibition of COX is stopping the synthesis of prostaglandins that in turn reduces the inflammatory response (112).

Conclusion

Inflammasome pathways interact with bacterial secretion systems in many different fashions, both with inhibitory and activating functions. Responses are initiated by pore/translocon formation, instructed by secreted effector proteins or by components, such as flagellin or Lipopolysaccride, channeled via the needle. Each pathogenic microorganism has its own way of interacting with to host primary line of defense and can bunch of inhibitory proteins suppressing activation, but the host may have modified mechanisms to detect these virulence key factors. But, it is often a fight between activating and blocking forces with regard to the net effect on the first line of defense, and there is likely a delicate balance that will decide if

the pathogenic microorganism may cause disease. Inflammasomes study in relation to bacterial virulence pathways are very fascinating field; more progress can likely increase the perceived complexity of these mechanisms but may also help in the design of new therapeutics for autoimmune disorders and inflammatory diseases. Characterizing the molecular mechanisms underlying inflammasome organization and cellular compartments would also be a source of fascination and critical to understanding this magnificent molecular machinery. Is there a key component that integrates all signals ? How do the inflammasome make contact with metabolism and nutrients and form special cellular compartments ? Evidently, inhibitors targeting inflammasome signaling components including cGAS-STING signaling, provide some attractive potential in understanding the fundamental rationale of this immune system and treating numerous inflammasome associated diseases.

Author Contributions

Concept and design of study finalized by AK, BJO, and drafting done by PK and A Kothari. Manuscript editing and manuscript review are prepared by MP.

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Conflict of Interest

The authors declare no conflict of interest.

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