Inflammasomes: A molecular mediators and their functional role in inflammation and cancer

Renuka Verma [1]*, Trilochan Satapathy[1], Prasanna Kumar Panda[2], Jhakeshwar Prasad[1], Ashish Kumar Netam[1], Ruchi Bhattacharya[1], Manisha Sahu[1],

1. Department of Pharmacology, Columbia Institute of Pharmacy, Tekari, Near Vidhansabha, Raipur -493111Dist-Raipur (C.G.) India.

2. University department of Pharmaceutical Sciences, Utkal University, Bhubaneswar-751 004, Odisha, India

Abstract:

An Inflammasome is a multimolecular complex, composed of a NOD-like protein (NLR), the adaptor apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC), and caspase-1 associated with various stages of tumor development. It also has significant impacts on tumor immunity and immunotherapy. Inflammasome are protein complexes that are formed within a cell and has important innate immune pathway critical for the production of active IL-1β and interleukin 18, as well as the induction of pyroptosis. Research output revealed that, inflammasomes play a vital role in infectious and autoimmune diseases and their role in tumor progression remains elusive. Recent studies demonstrated that, inflammasomes promote tumor progression in skin and breast cancer. These results indicated that inflammasomes can promote and suppress tumor development depending on different types of tumors, specific inflammasomes involved, and downstream effector molecules. There are numerous studies on the involvement of toll-like receptors (TLRs) or interferon (IFN) pathways in tumor development. The complicated role of inflammasomes paved the way for new opportunities and challenges to manipulate Inflammasome pathways in the treatment of cancer.

Keywords: Inflammasome, Inflammation, Tumor immunity, cancer, signaling pathway

INTRODUCTION

Inflammasome:

Chronic inflammation plays an important role at all stages of tumor development, including initiation, growth, invasion, and metastasis. [1] Nearly a decade ago, the concept of inflammasomes was introduced. Since then, the biochemical characterization of the inflammasomes has led to a richer understanding of innate immune responses in the context of infection and sterile inflammation. [2] The exact composition of an inflammasome depends on the activator which initiates inflammasome assembly, e.g. dsRNA will trigger one inflammasome composition whereas asbestos will assemble a different variant. The inflammasome promotes the maturation of the inflammatory cytokines Interleukin 1β (IL-1β) and Interleukin 18 (IL-18). [3]

- **IL-1β and IL-18 :**

  The common feature of IL-1β and IL-18 precursor forms do not bind their receptors and require proteolytic cleavage by either intracellular caspase-1 or extracellular neutrophilic proteases. The inflammasome is also responsible for activation of inflammatory processes.[2] Because the pro-inflammatory pathway does not need Toll-like receptors (TLRs), inflammasomes can detect cytoplasmic DNA that may be threatening and strengthen their innate response. Inflammasomes have been shown to induce cell pyroptosis.

- **Pyroptosis** :

  Pyroptosis is a highly inflammatory form of programmed cell death that occurs most frequently upon infection with intracellular pathogens and is likely to form part of the antimicrobial response. [4]

TYPES OF INFLAMMASOMES:

A key player in inflammasome assembly is the adaptor protein Apoptosis-associated Speck-like protein containing a CARD (ASC). It is also termed PYCARD due to it consisting of an N-terminal Pyrin (also DAPIN: Domain in apoptosis and INF response) and a C-terminal CARD domain. ASC is a common interaction partner in the inflammasome scaffold and usually indispensible for caspase-1 recruitment to this pro-inflammatory platform. Despite the presence of ASC as a common adaptor, different types of inflammasomes can be distinguished. A large group is made up by NOD-Like Receptor (NLR) inflammasomes. They exhibit a common domain structure usually containing a Leucine Rich Repeat (LRR), typically representing the receptor domain and a Nucleotide Binding (NBD) or NACHT (NAIP, CIITA, HET-E, TP1) domain that facilitates oligomerization upon ligand interaction. The NLR inflammasomes can be further differentiated. The NLRP (also NALP) inflammasomes additionally harbor a Pyrin domain (PYD) for ASC interaction and NLRC (also IPAF) inflammasomes lack PYD but instead contain a CARD domain for direct interaction with caspase-1. Nevertheless it has been suggested that signaling by the IPAF inflammasome is not entirely independent of ASC. [5] Another NLR inflammasome is NAIP5 (also NLRB) that contains Baculoviral Inhibitor of apoptosis proteins Repeat (BIR) domain repeats instead of PYD or CARD and functions in collaboration with IPAF. [6, 7]

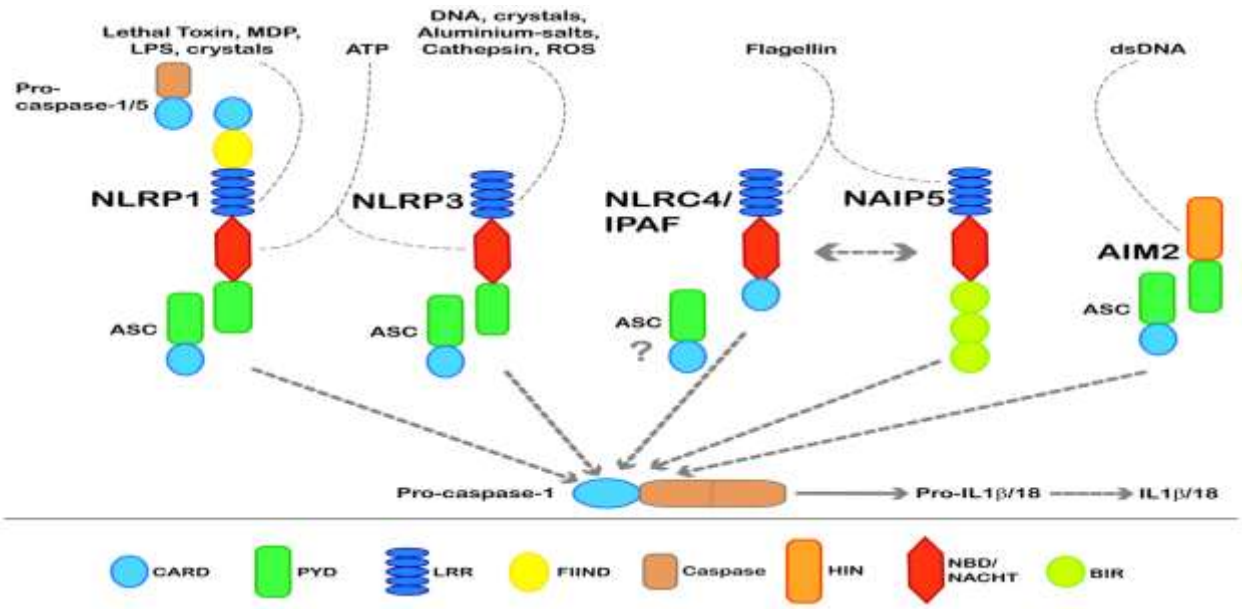

Figure: 1 Overview of the assembly, domain structure and direct or indirect stimuli of different inflammasomes.

INFLAMMASOME SIGNALING PATHWAY:

Human body is exposed to microbes or exogenous stimuli, pattern recognition receptors (PRRs) play a protective role as gatekeepers in the defense system. The PRR family consists of various memberane such as Toll-like receptors (TLRs), nucleotide-binding domain and leucine-rich repeat containing receptors (NLRs). it leads to the release of IL-1β and IL-18. Such proinflammatory cytokines continue to attract a myriad of immune cells and form ROS and RNS which are toxic to DNA and contribute to DNA damage.

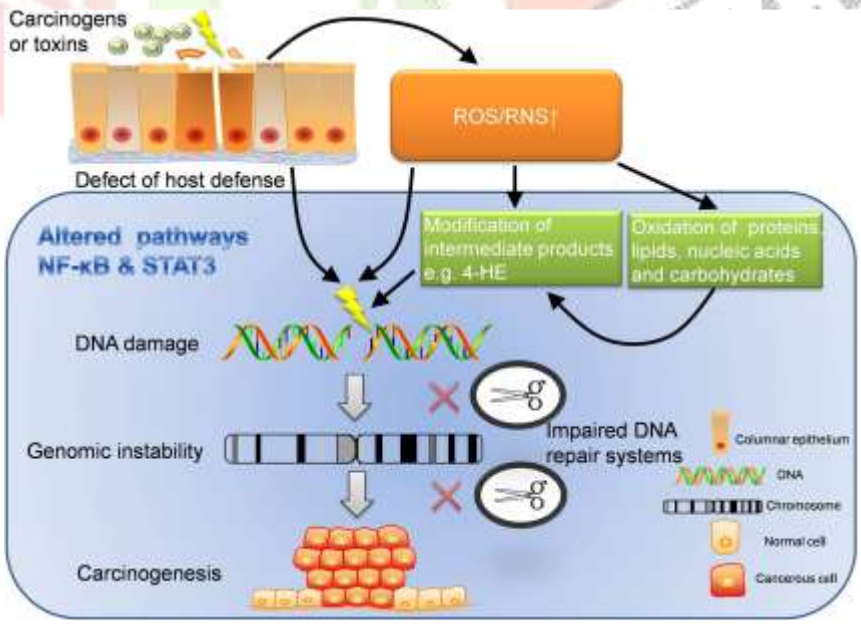

Figure: 2 Activation of carcinogen by ROS/RNS.

When DNA damage occurs where oncogenes or cancer suppressor genes are localized, it will result in the unlock of oncogenes as well as loss-of-function mutations of some cancer suppressor genes. Proteins, lipids, and nucleic acids are also likely to be oxidized directly under the high oxidative stress. Moreover, ROS can induce DNA double-strand breaks or DNA cross-links, which leads to replication mistakes. ROS/ RNS also facilitates carcinogenesis indirectly by the modification of intermediate metabolic products which mostly are also reactive species. [8]

NLRP3-IL-1 activation:

NLRP3-IL-1 activates migration of myeloid-derived suppressor cells (MDSCs) to tumorigenic sites and promotes the gastric cancer. Over expression of interferon (INF) by absent in melanoma 2 (AIM2) and NLRP3 also enhances Janus kinase (JAK)/STAT signaling to promote the colon cancer. Inflammasome is involved in the promotion of prostate cancer through the induction of chronic inflammation. [9]

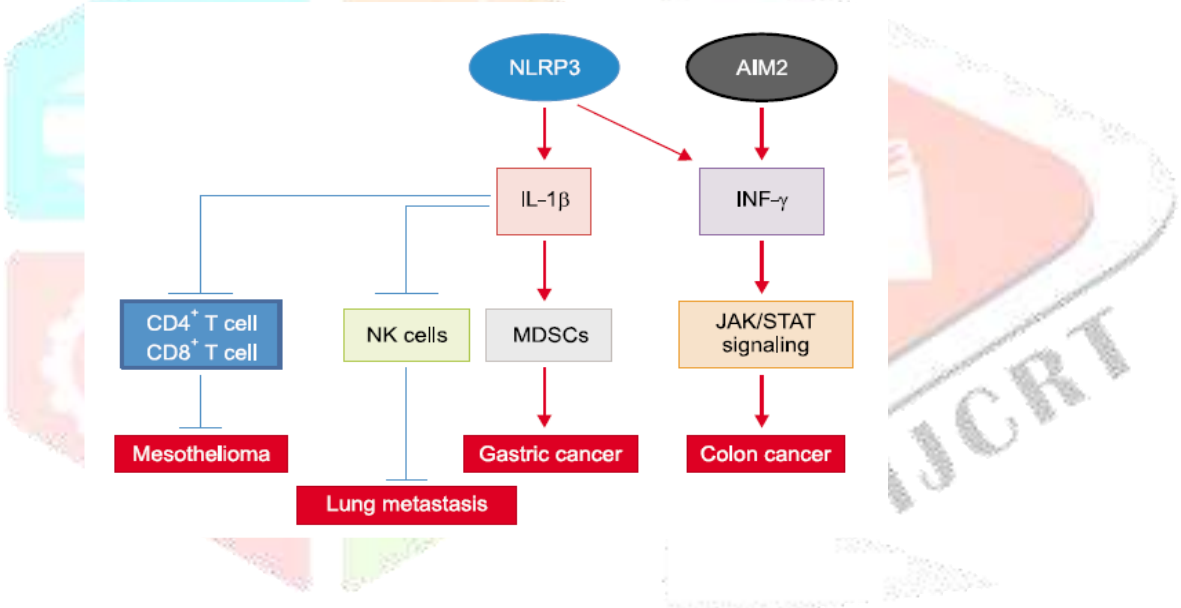

Figure: 3 Activation of NLRP3- IL-1β and interferon (INF).

Inflammasomes in targeting cancer:

Inflammasome dependent anticancer effects are more profound when combined with chemotherapies. When inflammasome damage primary tumor cells, they induce autophagy of tumor cells and lead to the leak of ATP into extracellular space. ATP can bind to P2Y2 receptors on macrophages, resulting in tumor infiltration and it also bind to P2RX7 receptors on DCs to activate NLRP3 inflammasome. Then IL-1β and IL-18 are released and they work together to promote γδT cell-induced secretion of IL-17, which recruits IFN-γ-producing CD8+ αβ T cells. As a result, IFN-γ finally damages therapy-resistant tumor cells. [10]

TOLL LIKE RECEPTORS:

The German word *Toll* is difficult to render precisely in English but approximates to fantastic, mad, or amazing. In a scientific context, N¨ usslein-Volhard and Anderson first used the word Toll to name a gene that they discovered in a genetic screen of *Drosophila*, the phenotype of which they thought to be Toll. [11, 12] Infact, this pioneering work in the early 1980s identified a group of 10 different genes, all of which produced qualitatively similar maternal effect phenotypes, now known as the dorsal group. Null mutations in any of these genes result in the failure to differentiate pattern elements on the dorsoventral axis and lead to early embryonic lethality. During the following 10 years, all the dorsal group genes were cloned, and a compelling picture emerged of how dorsoventral patterning occurred in the *Drosophila* embryo. Shortly after the fertilization of the embryo, a ventrally restricted signal associated with an extracellular membrane structure activates a protease cascade, and the terminal member Easter then processes an inactive precursor of Sp¨atzle, an endogenous protein ligand of Toll, a class I transmembrane receptor. By this mechanism Toll is activated at ventral positions in the embryos, and an intracellular signaling pathway then causes the relocalization of a transcription factor, dorsal, from the cytoplasm to nuclei located at ventral positions in the embryo.

This results in the formation of a ventral-dorsal gradient of the transcription factor, and the information contained in this morphogenetic gradient is then used to direct the subsequent differentiation of the dorsoventral body axis. [13] The sequence of Toll determined in 1988 revealed a tripartite structure with an Nterminal region containing tandem arrays of a short leucine-rich motif, the leucine-rich repeat (LRR), a sequence likely to form a single transmembrane helix and a C-terminal domain of unknown structure and function. [14] An important development in the early 1990s was the recognition that this C-terminal domain was significantly related to that of the vertebrate interleukin-1 receptor (IL-1R). [15,16] IL-1R is activated by a cytokine formerly known as endogenous pyrogen that is now named IL-1. IL-1 is part of the acute phase response to infection characterized by fever and the secretion of defense proteins into the circulation by the liver. This discovery was important as it suggested that this domain, now known as the Toll-interleukin receptor (TIR domain, was involved in signaling processes not only in the restricted context of insect development but also in the generation of initial responses to infection by human immunesystem cells.

Table: 1 TLRs and tumor development and growth.

Tumor-promoting	TLR	References	Anti-tumor	TLR	Reference
Pro-angiogenic	2, 9	58	Anti-angiogenic	7, 9	[17-19]
Proliferation	3, 4	64-67	Apoptosis	3, 4, 7, 9	[20, 21-23, 24]
Chemoresistance	4	81, 82	Chemosensitivity	2, 4, 7	[25-28]

TLRs AS ANTI-TUMOR VACCINES:

The potential of TLR ligands to induce appropriate and effective immune human reactions against a given antigen has been exploited in the vaccine therapy of melanoma.

Table: 2 TLRs in anti-tumor vaccine studies for melanoma.

TLR	Vaccine	Reference
7	Cancer/testis antigen + imiquimod	[29]
Poly	Melanoma antigen + Ribomunyl	[30]
9	Melanoma antigen + CpG7909	[31]
7	Melanoma antigen + Flt3 + imiquimod	[32]

TLRs IN CLINICAL TRIALS AGAINST HUMAN MALIGNANCIES

The promising role of the TLR pathway in the treatment of human malignancies was studied in several clinical trials.

Table: 3 TLRs in clinical trials.

Malignancy	TLR	Reference
Advanced-stage non-small-cell lung cancer	TLR9	[33]
IV stage melanoma	TLR7	[34]
IIIb/c or IV stage melanoma	TLR9	[35]
Incompletely resectable pancreatic carcinoma	TLR2/6	[36]
Recurrent non-Hodgkin lymphoma	TLR9	[37]
Recurrent glioblastoma	TLR9	[38]
Recurrent non-Hodgkin lymphoma	TLR9	[39]
Recurrent non-Hodgkin lymphoma	TLR9	[40]
CLL, skin deposits	TLR7	[41]

Table: 4 Ligands for the Toll-like receptors (TLRs).

TLRs	Ligands	Origin of ligands
TLR1/2	Triacyl lipopeptides (Pam_3CSK_4)	Bacteria, mycobacteria
	Soluble factors	*Neisseria meningitides*
	OspA	*Borrelia burgdorferi*
	Porin PorB	*Neisseria meningitidis*
TLR2	Lipoprotein/lipopeptides	A variety of pathogens
	Diacyl lipopeptides (Pam_2CSK_4 and $MALP2SK_4$)	Synthetic ligands
	Peptidoglycan	Gram-positive bacteria (not accessible in

		gram negative)
	Lipoteichoic acid	Gram-positive bacteria
	Lipoarabinomannan	Mycobacteria
	A phenol-soluble modulin	*Staphylococcus* epidermidis
	Glycoinositolphospholipids	*Trypanosoma cruzi*
	Glycolipids	*Treponema maltophilum*
	Porins	*Neisseria meningitidis*
	Zymosan	Fungi
	Atypical LPS	*Leptospira interrogans*
	Atypical LPS	*Porphyromonas gingivalis*
	Hsp70	Host
	Hyaluronan	Host
	Hemagglutinin	Measles virus
TLR3	Poly (I-C) dsRNA	Virus
TLR4	LPS	Gram-negative bacteria
	Flavolipin	*Flavobacterium meningosepticum*
	ER-112022, E5564, E5531	Synthetic compounds
	Taxol	Plant
	Fusion protein	Respiratory syncytial virus
	Envelope proteins	Mouse mammary tumor virus
	Hsp60	*Chlamydia pneumoniae*
	Hsp60	Host
	Hsp70	Host
	Type III repeat extra domain A of fibronectin	Host
	Oligosaccharides of hyaluronic acid	Host
	Polysaccharide fragments of heparan sulfate	Host
	Fibrinogen	Host
	αA crystallin and HSPB8	Host (recombinant *E. coli*–produced proteins)
TLR5	Flagellin	Bacteria
TLR6/2	Diacyl lipopeptides	Mycoplasma
TLR7	Imidazoquinolines (imiquimod, R-848)	Synthetic compounds
	Bropirimine	Synthetic compounds
	Guanosine analogs	Synthetic compounds
TLR8	R-848	Synthetic compounds
TLR9	Unmethylated CpG DNA	Bacteria, virus, yeast, insects
	Chromatin-IgG complexes	Host

Conservation and divergence in the Toll signaling pathways.

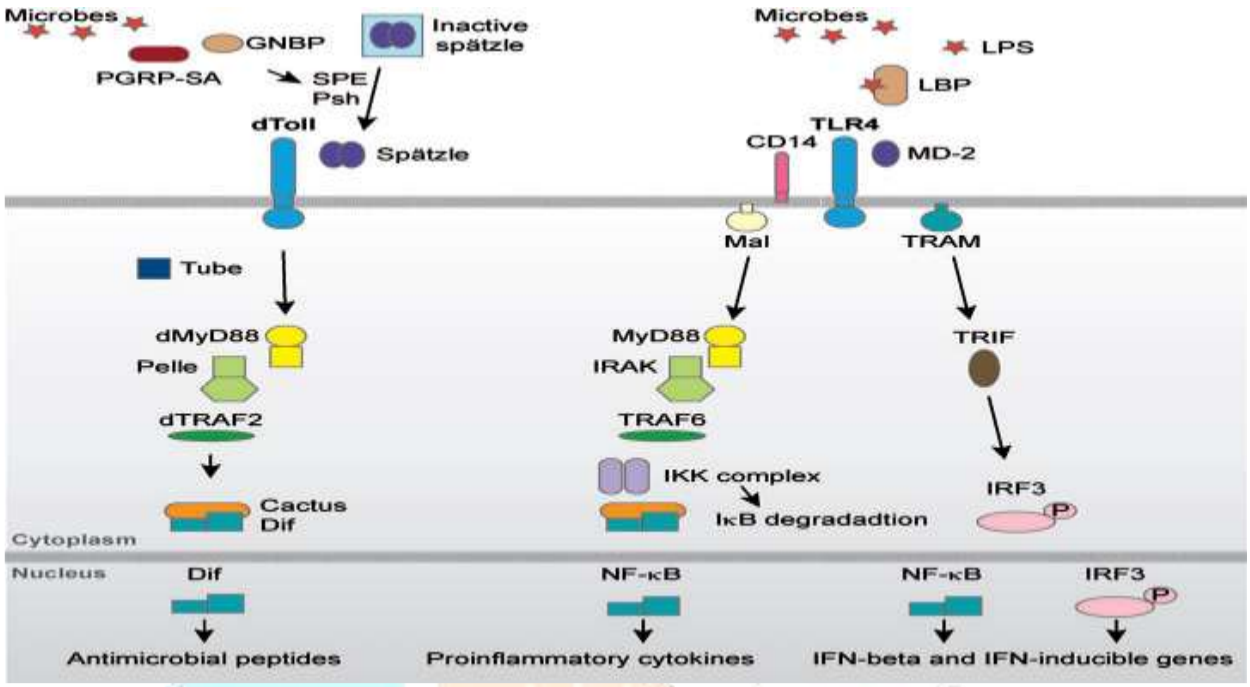

Figure: 4 Conservation and divergence in the Toll signaling pathways. Components of the Drosophila Toll (left) and the human Toll-like receptor (right) pathways are illustrated schematically. Evolutionarily or functionally conserved elements in the two pathways are illustrated in the same color. Abbreviations: CD14, an extrinsic, PI-glycan modified membrane protein; Dif, dorsal-related immunity factor; dMyD88, Drosophila homolog of MyD88; dToll, Drosophila Toll receptor; dTRAF2, Drosophila homolog of TNF receptor-associated factor 2; GNBP1, gram-negative binding protein 1; IFN, interferon; IKK, IκB kinase; LBP, LPS-binding protein; IRAK, interleukin-1 receptor-associated kinase; IRF3, interferon response factor 3; LPS, lipopolysaccharide; MD-2, co-receptor of TLR4; Mal, MyD88 adaptor-like; MyD88, myeloid differentiation primary response protein 88; NF-κB, nuclear factor κ B; Pelle, product of the Drosophila pelle gene a protein kinase and homolog of IRAK; PGRP-SA, a peptidoglycan recognition protein; SPE, Sp¨atzle processing enzyme; P, phosphorylation of IRF3; Psh, Persephone, a Drosophila serine protease; TLR4, Toll-like receptor 4; TRAF6, TNF receptor-associated factor 6; TRAM, TRIF-related adaptor molecule; TRIF, TIR domain–containing adaptor protein inducing interferon-β.

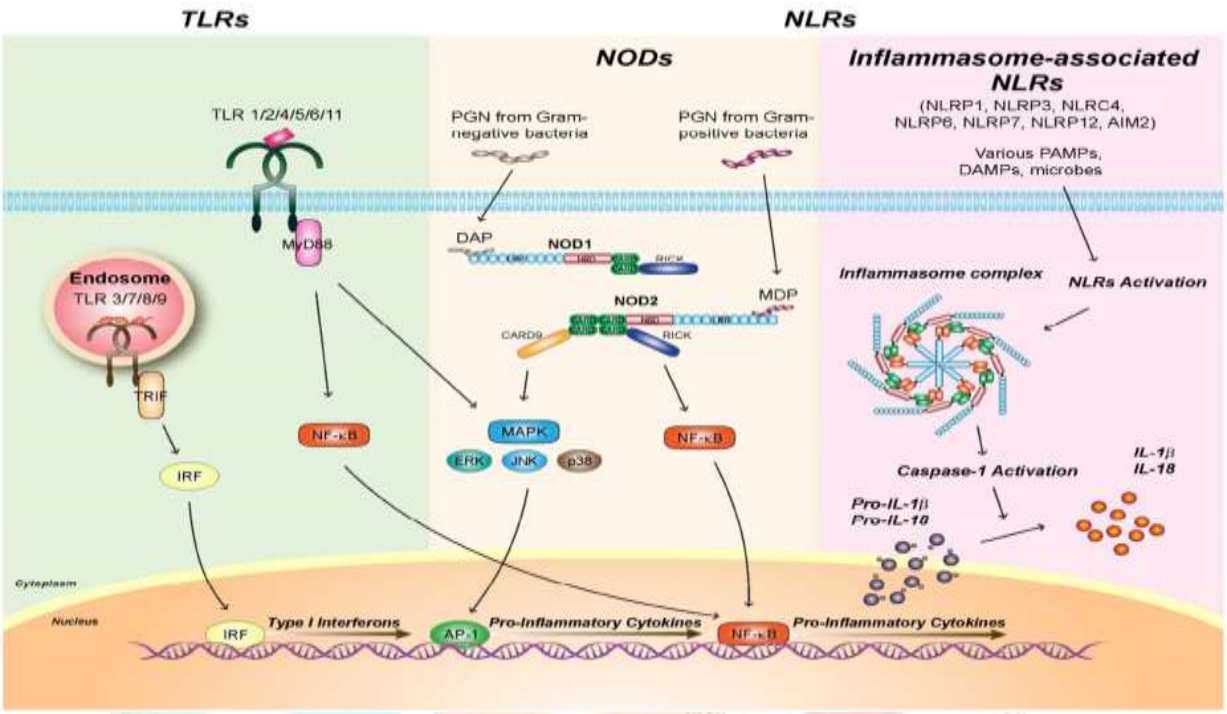

Figure: 5 Signal transduction mechanism of toll like receptor.

PATTERN RECOGNITION RECEPTORS:

Pattern recognition receptors (PRRs) expressed by innate immune cells are essential for detecting invading pathogens and initiating the innate and adaptive immune response. There are multiple families of PRRs including the membrane-associated Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), and the cytosolic NOD like receptors (NLRs), RIG-I-like receptors (RLRs), and AIM2-like receptors (ALRs). PRRs are activated by specific pathogen-associated molecular patterns (PAMPs) present in microbial molecules or by damage-associated molecular patterns (DAMPs) exposed on the surface of, or released by, damaged cells. In most cases, ligand recognition by PRRs triggers intracellular signal transduction cascades that result in the expression of pro-inflammatory cytokines, chemokines, and antiviral molecules. [42] In contrast, activation of some ALRs and NLRs leads to the formation of multiprotein infl ammasome complexes that serve as platforms for the cleavage and activation of Caspase-1. [43] and IL-18, which further amplifi es the pro-inflammatory immune response. Since a single pathogen canβCaspase-1 promotes the maturation and secretion of IL-1 simultaneously activate multiple PRRs, crosstalk between diff erent receptors may also play a role in enhancing or inhibiting the immune response. [44] Therefore, tight regulation of PRR signaling is required in order to eliminate infectious pathogens and at the same time, prevent aberrant or excessive PRR activation, which can lead to the development of inflammatory and autoimmune disorders.

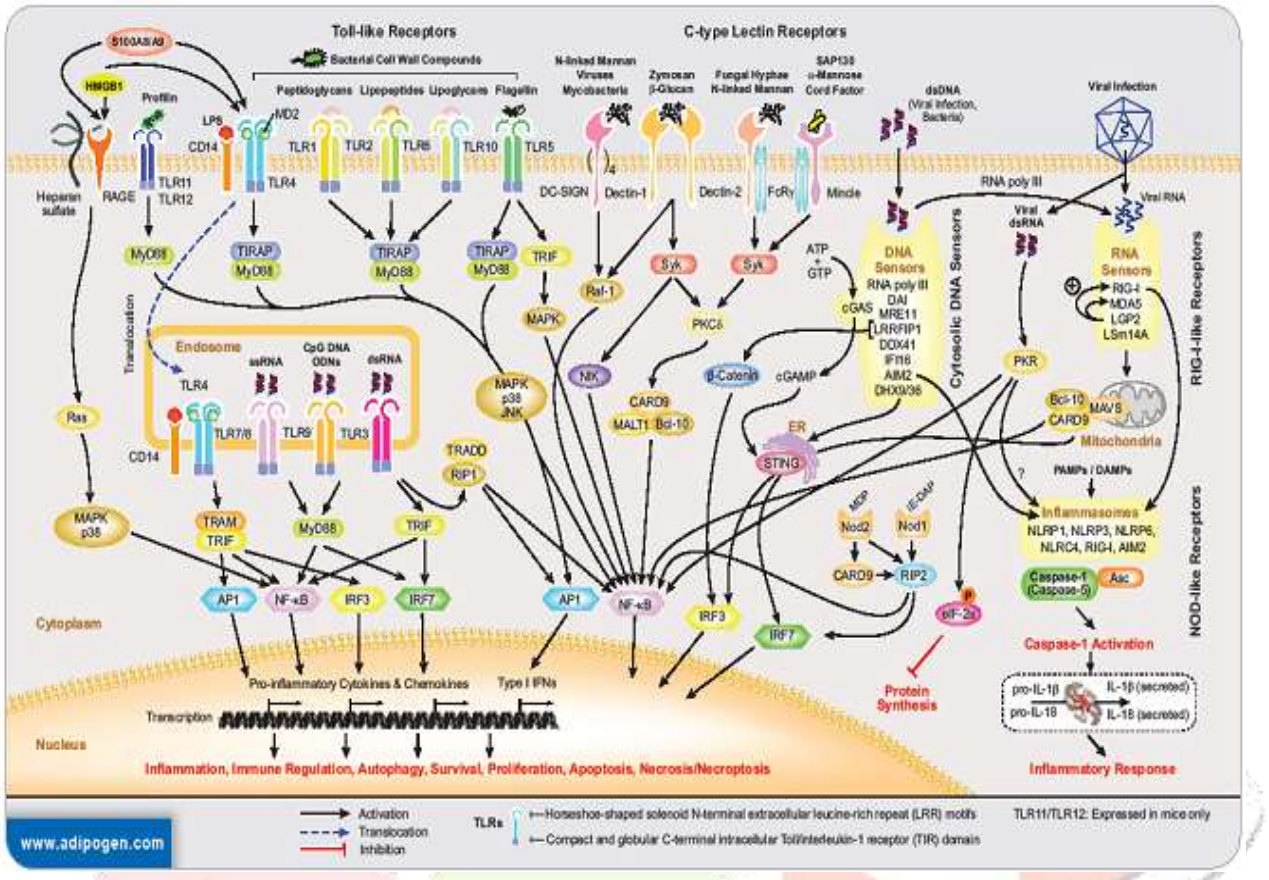

Figure: 6 Pattern Recognition Receptors Signaling Pathways.

NUCLEIC ACID RESPONSIVE PATTERN RECOGNITION RECEPTORS (PRRs):

Viral and pathogen derived RNA is either recognized by Toll-like receptors or by RIG-I-like Receptors or Helicases (RLR or RLH). The latter are a group of cytosolic super family 2 (SF2) helicases comprising RIG-I, Melanoma Differentiation Associated protein 5 (MDA5) and Laboratory of Genetics and Physiology 2 (LGP2). [45] RLRs are ubiquitously expressed and even found in cells primarily involved in adaptive immunity. [46] On the other hand, the presence of foreign DNA in the cytosol has been shown to be sensed by DAI [47] and indirectly by NLRP3 (NOD-Like Receptor family, Pyrin domain containing 3). [48] Recently, the IFN-inducible protein AIM2 has been also implicated in pathogenic DNA sensing in the cytosol. It has been shown to form a multimeric inflammasome complex upon DNA binding and by recruiting ASC (Apoptosis-associated Speck-like protein containing a CARD; also PYCARD) and caspase-1. [49] Moreover, another pathogenic DNA recognition mechanism has been revealed to link to RLR signaling. RNA Polymerase III has been shown to produce DNA derived RNA intermediates that can be sensed by RIG-I in the cytosol inducing type I interferon production. [50, 51] The existence of PRRs and pathways

responsive to exogenous or abnormal DNA has not been known for long and it is assumed that yet more remain to be discovered. Most of the so far described PRRs are cell-type or ligand specific. The group of High Mobility Group Box (HMGB) proteins is more versatile. Originally, they had been known to be nuclear proteins regulating chromatin structure and transcription. Only recently they have been implicated in nucleic acid delivery to PRRs for detection, by acting as more universal receptors. [52]

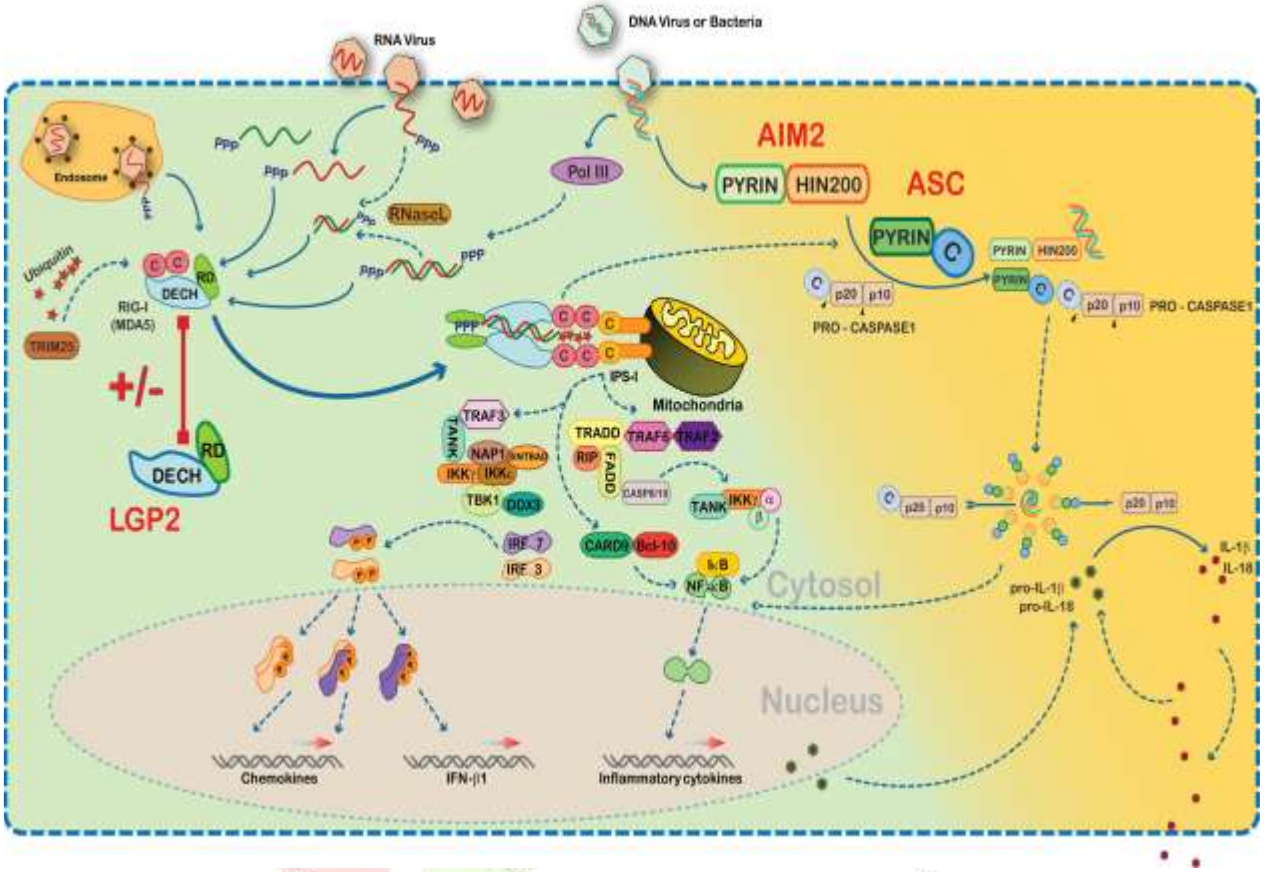

Figure: 7 Schematic overview of some signaling pathways of the innate immune system directed against pathogenic nucleic acids with focus on the AIM2 inflammasome and RLR LGP2 as a regulator of RIG-I and MDA5 signaling (C = CARD).

INTERLEUKIN-1β (IL-1β) AND INTERLEUKIN – 18:

Interleukin-1 β and IL-18 contribute to host defense against infection by augmenting antimicrobial properties of phagocytes and initiating Th1 and Th17 adaptive immune responses. Protein complexes called Inflammasomes activate intracellular caspase-1 auto catalytically, which cleaves the inactive precursors of IL-1b and IL-18 into bioactive cytokines. IL-1 and IL-18 are members of the IL-1 family of ligands, and their receptors are members of the IL-1 receptor family. Although several biological properties overlap for these cytokines, differences exist. IL-18 uniquely induces IFN-from T lymphocytes and natural killer cells but does not cause fever,

whereas fever is a prominent characteristic of IL-1 in humans and animals. In the present study, human epithelial cells were stably transfected with the IL-18 receptor chain and responded to IL-18 with increased production of IL-1, IL-6, and IL-8. Five minutes after exposure to either cytokine, phosphorylation of mitogen activated protein kinase (MAPK) p38 was present; specific inhibition of p38 MAPK reduced IL-18 activity to background levels. Whereas IL-1 induced the expression of the NF-B-reporter gene and was suppressed by competitive inhibition of NF-B binding, IL-18 responses were weak or absent. In contrast to IL-1, IL-18 also did not activate degradation of the NF-B inhibitor. After 4 h, both cytokines induced comparable levels of mRNA for the chemokine IL-8 but, in the same cells, steady-state levels of cyclooxygenase (COX)-2 mRNA were high after IL-1but low or absent after IL-18. After 30 h, IL-18-induced COX-2 appeared in part to be IL-1 dependent. Similarly, low levels of prostaglandin E2 were measured in IL-18- stimulated A549 cells and freshly obtained primary human monocytes and mouse macrophages. We conclude that in epithelial cells, IL-18 signal transduction is primarily via the MAPK p38 pathway rather than NF-B, which may explain the absence of COX-2 and the failure of IL-18 to cause fever.

Inflammasome-independent activation of IL-1β and IL-18:

In experimental animal models of turpentine-induced inflammation, Il1b –/– mice are protected against inflammation but caspase-1-deficient mice are not. [53,54] Furthermore, caspase-1 appears to be redundant in the host defense against certain types of microorganisms, such as Chlamydia trachomatis, although IL-1b is involved in the inflammatory responses induced by these microorganisms. [55,56] These data suggest that caspase-1 and inflammasome activation is important in some, but not all, types of IL-1b-driven inflammation and argue for inflammasome-independent activation of IL-1b in certain infectious processes. Apart from the cysteine protease caspase-1, serine proteinases such as cathepsin G, elastase and, in particular, proteinase 3 (PR3) are able to cleave pro-IL-1b. [57] In addition, matrix metalloproteinases (MMP), such as stromelysin 1, gelatinases A and B, have been shown to process pro-IL-1b into bioactive IL-1b. [58] It was shown that PR3, which is present predominantly in activated neutrophils, is one of the most potent proteases that can process pro-IL-1b into the active IL-1b fragment. In streptococcal cell wall-induced arthritis, caspase-1 deficiency had no effect on IL-1b processing in vivo. [59] However, the concentration of bioactive IL-1b in vivo was highly dependent on PR3. This reflects the importance of the presence of neutrophils to process IL-1b. Two other models of autoimmune arthritis, collagen-induced arthritis (CIA) and antigen-induced arthritis (AIA), which are known to be highly dependent on IL-1, also demonstrated that these inflammatory responses were caspase-1-independent. [60,61] Neutrophils are abundant during the onset of both models, suggesting that PR3 is responsible for IL-1b processing in the acute phase of inflammation, conditions that are characterized by a strong neutrophil infiltrate. However, in chronic stages of autoimmune arthritis, in which inflammatory

monocyte and macrophage infiltration becomes more prominent, caspase-1 has been shown to play a predominant role. These earlier results support a general concept in which neutrophils are the major source for processing IL- 1b via PR3 during acute bacterial and fungal infection, and that caspase-1 and inflammasome activation become more important for the production of mature IL-1b in the later stages of infection. An interesting observation regards the role of the inflammasome in host defense against M. tuberculosis. IL-1 plays an important role for the host defense and the survival of mice infected with M. tuberculosis. [62] Surprisingly, caspase-1-deficient mice are reported to have a normal resistance to M. tuberculosis [63], suggesting activation of IL-1b by alternative mechanisms.

However, ASC-deficient mice are reported to be more susceptible to mycobacterial infection, opening the intriguing possibility of biological functions of inflammasome components that are not related to caspase-1 activation. Indeed, earlier studies on the function of ASC have reported its interaction with nuclear factor kappa B and an influence on gene transcription. [64,65] Whether ASC can function independently of inflammasome activation during other infections has not been studied. In addition to the alternative mechanisms of processing IL-1b, non-caspase proteases have been indicated to cleave pro-IL-18 into bioactive IL-18. PR3 cleaves pro-IL-18 [66], however, it is still a matter of debate whether the cleaved product is bioactive. In addition, mast cell chymase can cleave IL-18. [67] It was suggested recently that granzyme B, which is present mostly in cytotoxic T cells and NK cells and in neutrophils, can cleave pro-IL-18. [68] This raises the intriguing possibility that T cells and neutrophils contribute to the availability of bioactive IL-18 in inflammatory conditions.

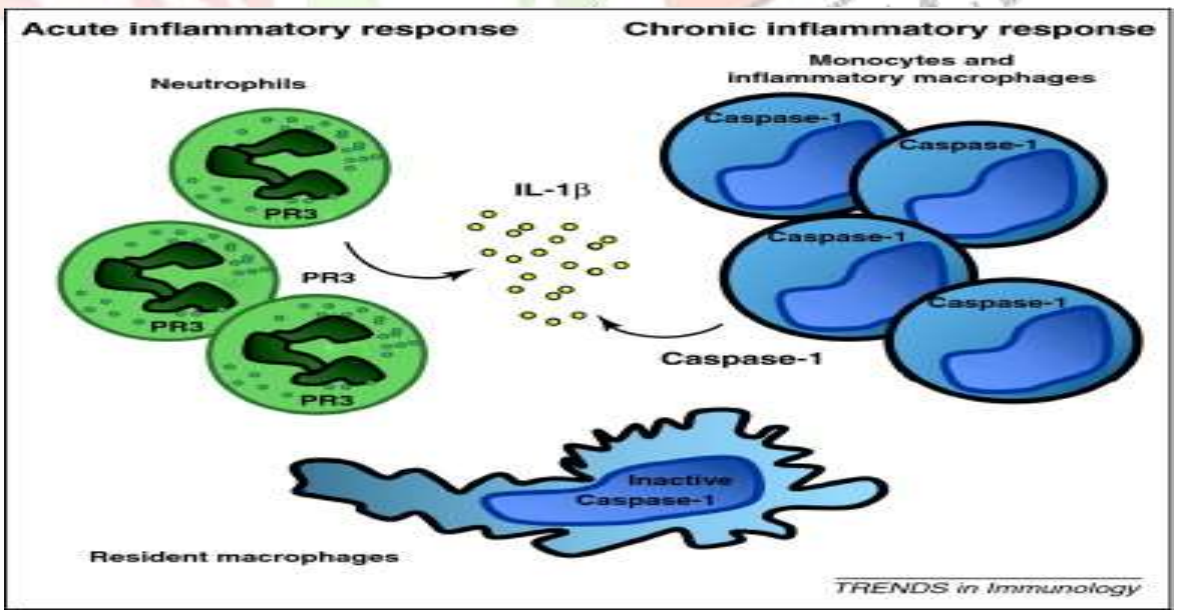

Figure: 8 IL-1β processing in acute and chronic stages of inflammation. Neutrophils are the major source for processing IL-1β via PR3 during acute inflammatory conditions. In chronic stages of inflammation when

monocytes and macrophages play a more dominant role, caspase-1 and inflammasome activation become more important for the production of mature IL-1β.

CONCLUSIONS:

In this review, we have tried to throw a light and provided a brief overview of the functional role of inflammasomes in different forms of inflammation and cancer. Inflammasomes are large multimolecular complexes they possess both protumorigenic and antitumorigenic properties which are largely determined by the types of cells, tissues, and organs involved. In some conditions such as colorectal cancer, activation of inflammasome sensors is largely beneficial owing to the epithelial healing effects of the IL18 signaling pathway, regulation of cellular proliferation, maturation and cell death, and maintenance of a healthy gut microbiota. In the other way, the activation of inflammasome with various insults enhances the secretion of inflammatory cytokines, leading to infiltration of more immune cells and resulting in the generation and maintenance of an inflammatory microenvironment surrounding cancer cells. The important signaling pathways described in this review are nonspecific regulators of inflammasome and some are specific regulators of specific inflammasomes. Despite much more information available from scientific evidence, still the exact molecular mechanisms by which some NLR inflammasomes are activated remain unresolved; furthermore, still it is not understood clearly that, how cells 'decide' to engage death pathways after inflammasome activation. Therefore a detail study and scientific evidence is required to understand this dual and controversial role of inflammasomes at molecular level to decide the target and to improve the therapeutic effectiveness of inflammasomes.

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