Inflammasome Activation and Regulation During *Helicobacter pylori* Pathogenesis

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Abstract

Helicobacter pylori is a leading cause of gastric cancer worldwide, its type four secretary toxin CagA is cited to be primarily responsible for it. Other virulence factors such as urease, VacA, HopQ, BabA and SabA are responsible for bacterial survival in acidic environment, adherence and cellular damage but its molecular mechanism is not completely understood. A number of pathogens including bacteria, fungi and virus are involved in the regulation of cellular machinery of inflammasome. Inflammasomes are multimeric protein complexes formed after external stimuli such as PAMPs/DAMPs or salt crystals and activates cellular caspases causes inflammation via pro-inflammatory cytokines. Virulence factors associated with microbial pathogens causes' cellular damage through damaging mitochondria, rupturing lysosome, producing endoplasmic stress and dysregulation of cellular ions balance. These cellular dysfunctioning leads to oxidative stress, cathepsin B production, nuclear and mitochondrial DNA damage which activates inflammasome machinery, pro-inflammatory cytokine release and cellular death known as pyroptosis. The mechanism of inflammasome induction by *H. pylori* is not studied extensively and very few virulence factors such as UreB, CagA, FlaA and VacA and their role in inflammasomes is established. This review elaborates the mechanism of inflammasomes regulation and elucidates the pathways through which *H. pylori* regulates inflammasome activation**.**

Key Words*:* *Helicobacter pylori*, Inflammasome, IL-1β, Virulence factors, and Caspases

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Introduction

The Gram-negative microaerophilic bacterium *Helicobacter pylori* colonize in human gastric mucosa and is a leading cause of human gastric diseases. More than half of the world population including 4.4 billion individuals are infected with *H. pylori* (Hooi et al., 2017). Although there is no clear evidence of *H. pylori* source and route of infection, its transmission is expected to occur through feco-oral route (Salama et al., 2013). *H. pylori* has long association with human stomach and therefore is helpful to study the human migration and evolution (Moodley & Linz 2009). *H. pylori* colonized human gastric mucosa is associated with less severe asymptomatic gastritis, prolongation of which leads to severe disease conditions such as peptic ulcer, gastric adenocarcinoma and mucosa-associate lymphoid tissue (MALT) lymphoma. *H. pylori* is categorized as a type I carcinogen by World Health Organization (Parsonnet et al., 1991). Successful persistence of *H. pylori* in human stomach depends on two initial events, neutralization of acidic pH of gastric juice and its penetration to gastric mucosa. A versatile pool of virulence genes, bacterial shape and strategies to modulate self and host responses promote *H. pylori* colonization in gastric mucosa and epithelium (Suerbaum and Josenhans 2007). It secretes urease enzyme which with the help of Ni^{2+} ions as a cofactor catalyses the production of ammonium ions and consequently neutralize highly acidic pH (1-2) through ammonium hydroxide of the stomach (Mobley et al., 1991; Montecucco & Rappuoli 2001). It then penetrates the gastric mucosa and become motile in a mucous layer with the help of a tuft of polar flagella (Celli et al., 2009). The helical shape of *H. pylori* promotes corkscrew like mechanism which eases the process of penetration into mucous layer (Bonis et al., 2010). In mucous layer, *H. pylori* live as free swimming bacteria while a few adhere to stomach epithelium surface and form micro-colonies (Tang et al., 2011).

The surface exposed blood group antigen-binding adhesin (BabA) and sialyly-Lewis x (sLex)-binding adhesin (SabA) are two important adhesins which help in the adherence of *H. pylori*. Other adhesins which involved in colonization are AlpA, AlpB, HopZ and OipA (Odenbreit, 2005; Yamaoka et al., 2002). Apart from adhesins, *H. pylori* lipopolysaccharides (LPS) also facilitate adhesion by the interactions of Lex and Ley epitopes with dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) receptor (Nilsson et al., 2006), epithelial cell

receptor galectin-3 (Fowler et al., 2006) and with mucous layer trefoil factor protein TFF1 (Reeves et al., 2008).

Inflammasomes

Inflammasomes are high molecular weight multimeric protein complexes formed in the cytoplasm in presence of pathogen associated molecular patterns (PAMPs), danger associate molecular patterns (DAMPs) and inorganic salt crystals. Pathogen recognition receptors (PRRs) expressed on epithelial cells and phagocytes including macrophages and neutrophils sense the inflammasome activating ligands which belongs to nuclear oligomerization domain (NOD) like receptor (NLRs) family rich in leucine and AIM2 (absent in myeloma 2) inflammasome which senses DNA. Cytosolic NLRs have three domains (except NLRP1 inflammasome) which is composed of a variable N-terminal effector region with caspase recruitment domain (CARD), pyrin domain (PYD) which helps in signal transduction, acidic domain, or baculovirus inhibitor repeats (BIRs), a centrally located NOD (also referred as the NACHT cassette) which is critical for ATPase activity and its oligomerization and C-terminal leucine-rich repeats (LRRs) that senses PAMPs (Bauernfeind & Hornung 2013). Second component of inflammasome is adaptor protein apoptosis associated speck like containing a CARD domain (ASC) consisting of two death-fold domains which are a PYD and a caspase recruitment domain (CARD) (Broz & Dixit 2016). Through its PYD, ASC interacts with upstream PYD of tripartite NLR and forms multimers of ASC dimers while CARD brings inactivated procaspases together, autoproteolytic action of which leads to cytosolic complexing of inflammasomes and maturation of pro-caspases such as caspase 1, caspase 4 and caspase 5 (inflammatory caspases). The activated caspases further releases pro-inflammatory cytokines IL-1β and IL-18 (Man et al., 2017) and initiates the maturation of a membrane pore forming toxin, activation of which leads to cell death known as pyroptosis (Shi, et al., 2015; Latz et al., 2013). NLRP1, NLRP3, NLRP4 AIM2 are involved in canonical inflammasome formation. There are various types of inflammasome based on the differences in their sensor and effector domains which are listed in table 1.

Activation and Regulation of Inflammasomes

The activation of inflammasome is regulated both positively and negatively by cellular proteins and number of signals are responsible for the induction of these proteins. Inflammasome

priming i.e. activation of PRRs or cytokine receptors is followed by second stimulus produced by extracellular ATP, endogenous cAMP, PAMPs, pore forming toxins or crystalline materials which leads to efflux of potassium ion a major step during activation of inflammasome. The activation is restricted to NLRP3 and AIM2 or NLRC4 are not activated by K^+ release. Genome-wide CRISPR/Cas9 screen study have found a mammalian NIMA-related kinases (NEK) as an important upstream regulator of NLRP3 against the stimuli of an ionophore nigericin in murine macrophages (Schmid-Burgk et al., 2016). K^+ efflux activates downstream NEK7 which binds to NLRP3 inflammasome and leads its oligomerization through ASC domain (He, et al., 2016). It has also been found that NLRP3 activation and mitosis are independent event and NEK7 restricts inflammasome at interphase of cell cycle with downstream production of mitochondrial ROS by binding to NLRP3 leucine-rich repeat domain through kinase-independent manner (Shi, et al., 2016). Kinase receptor interacting protein kinase 3 (RIPK3) signaling leads to necroptosis through downstream effector pseudokinase mixed lineage kinase domain-like (MLKL) translocation to cellular membrane, gasdermin independent disruption of membrane and loss of K^+ ions which activates NLRP3 inflammasome and releases IL-1β (Gutierrez et al., 2017). MLKL translocate to membrane, oligomerize, reduce intracellular cellular level of K^+ ions and induce necroptosis through its four helical bundle domain (Conos et al., 2017).

Two important protein degradation pathways ubiquitin-proteasome and autophagy-lysosomal regulates the innate immune responses by targeting infllamasome. Plasminogen activator inhibitor type 2 and dopamine D1 receptor (DRD1) regulate autophagy-lysosomal dependent NLRP3 degradation (Chuang et al., 2013; Versteeg et al., 2014), through E3 ubiquitin ligase (Versteeg et al., 2014), Tripartite motif (TRIM) containing superfamily proteins expresses in response to interferons (IFNs) and play important role in regulating innate immune responses (Ozato et al., 2008). TRIM31, a member of TRIM superfamily promote degradation of NLRP3 in resting and activated stages of macrophages by promoting K48 polyubiquitination followed by proteasomal degradation (Song et al., 2016). Protein kinase A inhibits NLRP3 inflammasome through prostaglandin E_2 (PGE_2) signaling via PGE2 receptor E-prostonoid 4 (EP4) (Mortimer et al., 2016). PKA directed NLRP3 phosphorylation at Ser295 leads to attenuation of its ATPase activity. Activated PKA promotes cyclic adenosine monophosphate (cAMP) increase which

endogenously binds to NOD-binding domain (NBD) and leads to its degradation autophagy (Yan et al., 2015; Lee et al., 2012 ; Sokolowska et al., 2015) Figure 1.

Reactive oxygen species (ROS) generated by cellular damage initiated by PAMPs, organelles damage and cell death are also responsible for the activation of inflammasome. The NADPH oxidase (NOX) dependent ROS has inhibitory effect on IL-1β production as seen during the knockdown of $p22^{phox}$ subunit (Dostert et al., 2008 & Dostert et al., 2009). The contradictory studies have also shown NOX1-4 independent activation of NLRP3 (van Bruggen et al., 2010). The level of IL-1β remains unaltered irrespective of ROS downregulation when analyzed in chronic granulomatous disease **(**CGD) patient having mutation at *CYBA* gene which codes for $p22^{phox}$ a subunit of NOX responsible for its activation. S100A8/A9 are DAMPs responsible for inflammation by promoting migration of phagocytes, their degranulation and NADPH oxidase dependent ROS production. Cellular upregulation of S100A8/A9 leads to regulation of NLRP3 by ROS dependent activation of NF-B and IL-1β release (Simard et al., 2013).

Inflammasome and Bacterial Pathogenesis

Inflammasomes are host innate defense mechanisms against invading pathogens. Microbial activation of inflammasomes leads to inflammation with the secretion of pro-inflammatory cytokines IL-1β and IL-18 and HMGB1 as a alarmin in extracellular milieu which checks the pathogen growth inside host. Bacteria modify their effectors to regulate the inflammasomes for own survival and spreading of infection. A number of inflammasomes are activated by bacterial, fungal and viral pathogens including NLRP1, NLRP1b, NLRP3, NAIP/NLRC4, AIM2, NLRP6, NLRP7, NLRP9b and NLRP12 (von Moltke et al., 2013, Zhu, et al., 2017, Vladimer et al., 2013, Tavares et al., 2015, Man et al., 2017 & Mathur et al., 2018). NLRC4 and ASC are required for caspase-1 activation and the production of IL-1β in response to infection, with *Salmonella, Shigella, Pseudomonas,* and *Legionella* whereas NLRPb and NLRP3 inflammasome is activated in response to *B. anthracis* and *Listeria* respectively. The bacterial pathogens which regulate the activity of inflammasomes are enlisted in the table 2.

Activation of Inflammasome by *H. pylori*

Two major virulence factors cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA) of *H. pylori* are responsible for inflammation in gastric epithelium. CagA is a type IV secretory system (T4SS) oncoprotein while VacA is a type V secretory system (T5SS) secreted toxin which induce cytosolic vacuolation, mitochondrial dysfunction, endoplasmic reticulum and endosomal mediated stress which leads to oxidative stress (Salama et al., 2013). The PAMPs from *H. pylori* are recognized by PRRs of host innate immune system and initiate the production of pro-inflammatory cytokines and chemokines with the activation of transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signal transducer and activator of transcription 3 (STAT3) (Castaño-Rodríguez et al., 2014). Activation of these transcription factors leads to expression of inflammatory cytokines IL-1β, IL-6, IL-8, IL-18, IL-23 and TNFα which further creates a state of inflammation. Inflammation is one of the major contributor of tumorigenesis and cancer (Mantovani et al., 2008) and is also responsible for *H. pylori* induced gastric carcinomas. It is not only the pathogen virulence factors which induce gastric inflammation during *H. pylori* infection but host factors are also responsible for prolongation of gastritis. Polymorphism in the gene sequences of pro-inflammatory cytokines IL-1β is another factor which contributes to severe and prolonged gastritis and is also associated with gastric cancer during *H. pylori* infection (Figueiredo et al., 2014). *H. pylori* induced inflammasomes play an important role in the production of pro-inflammatory IL-1β and IL-18 with the activation of pro-caspase 1 into caspase 1 and initiation of gastric inflammation (Franchi et al., 2010) Figure 2.

In gastric epithelium niche, *H. pylori* regulates the activation of inflammasome and pyroptosis for survival. *H. pylori* regulated NLRP3 inflammasome formation is major mediator of IL-1β secretion but NLRP3 independent mechanism for the regulation of immune responses also reported. *H. pylori* flagellin FlaA triggers phosphorylation of NLRC-4 Ser533 without activation of NLRC-4 inflammasome and escape NAIP5 detection through unknown mechanism (Matusiak et al., 2015). The gastric epithelial cells surface associated glycoprotein mucin 1 (MUC1) is highly expressed on cells surfaces and protects epithelium lining against inflammation and infection (Sheng et al., 2013). Mice infected with *H. pylori* showed a MUC1

dependent activation of NLRP3 inflammasome through NF-κB dependent signaling which initiates gastric inflammation (Ng et al., 2015). A T4SS, TLR2 and TLR4 independent activation of NLRP3 is reported in in human neutrophils infected with *H. pylori* strains (Pérez-Figueroa, et al., 2016). Caspase 1 dependent ROS, K^+ efflux and Ca^{2+} signaling induced NLRP3 infllammasome formation was also found in *H. pylori* infected THP1 macrophages (Kameoka, et al., 2016). A decrease in autophagy with an increase in oxidative stress and associated protein NADPH oxidase gp91phox was also noticed during H. pylori gastritis which is mediated through inflammasome/caspase 1 signaling (Yang et al., 2013).

Withaferin A (WA), a withanolide extracted from *Withania somnifera* inhibits NF-κB induced NLRP3 in murine bone marrow derived dendritic cells infected with *H. pylori* (Kim et al., 2015). *H. pylori* urease act as a novel immunomodulator and found to be protect against asthma via NLRP3 activation through TLR2 signaling in dendritic cells (Koch et al., 2015). *H. pylori* infection is also protective against inflammatory bowel disease through NLRP3 induced IL-18 secretion (Engler et al., 2015). It is found that Cag Pathogenicity Island (CagPAI) also stimulates the NRP3 inflammasome (Semper et al., 2014). The role of *H. pylori* induced NLRP3 activation is also studied in innate and adaptive immune responses which leads to the development of Treg and suppression of Th1 in humanized mice (Arnold et al., 2017). Few reports showed the role of inflammasome during *H. pylori* infection at transcriptome level where the expression of NLRP3 inflammasome is upregulated by suppressing miR-22 microRNA (Li et al., 2018). Pachathundikandi and Backert (2018) showed hsa-miR-223-3p controlled NLRP3 upregulation which was independent of CagA, VacA, Cgt, FlaA and CagPAI virulence factors.

Although regulation of inflammasome/caspase 1 signaling pathway is not studied in detail during *H. pylori* infection of gastric epithelium but above preliminary reports showed inflammasome induced pro-inflammatory cytokines Il-1β and IL-18 in H. pylori inflammation. Polymorphism associated with these cytokines are responsible for cancer related activities and modulation of Th1 and Th17 T cells responses (Hitzler et al., 2012). IL18 also responsible for T cells induced immunopathology during *H. pylori* infection (Hitzler et al., 2012). Alogwith adaptive immune cells modification, inflammasome also found to regulate the innate immune responses. Arnold et al., 2017 also studied the non-canonical role of NLRP3 in dendritic cells

development and showed that during *H. pylori* pathogenesis CD11b+ dendritic cell require NLRP3 for their recruitment in lymphoid tissues without the help of ASC adaptor or caspase 1. IL-1β play major role in metastatic diseases and its blockade significantly decreases angiogenesis (Dinarello, 2010). Discovering blockades such as monoclonal antibodies or natural inhibitors for the intermediates Inflammasome/caspase 1 pathway can also play an important role in the inhibition of IL-1β and IL-18 during *H. pylori* infection and ultimately in the prevention of gastric cancer.

Recent Advances and Future Prospects of Inflammasome

Inflammasomes play crucial role against pathogen defense but their dysfunction leads to disease conditions such as cancer, autoimmune and metabolic syndromes and neurodegeneration. NLR is a large protein family having more than 23 NLR proteins out of which the functions of most of these are not well characterized. NLRP3, NAIP5 and NLRC-4 are few inflammasomes which are been studied extensively in various human diseases but their post translational regulatory mechanism and their association with *H. pylori* virulence factors is still poorly characterized. Recently a new family member NLRP 9b has been described which has a role against intestinal rotavirus protection. Expression of NLRP 9b specifically in intestinal epithelial cells restricts rotavirus infection. Through RNA helicase Dhx9 short double-stranded RNA stretches are recognized by NLRP9b which forms inflammasome complexes and stimulate the release of interleukin IL-18 and pore forming gasdermin D (released into plasma membrane upon the cleavage by caspase 1 or caspase11) to initiate pyroptosis (Zhu, et al., 2017). Till date, a direct interaction of PAMPs with inflammasomes molecules and the interacting partners of upstream and downstream signaling during *H. pylori* infection is not known. Its detailed mechanism and careful investigation may give some leads to control and reduce the disease severity. Future studies are also required to address the precise role of genetic variants of NLRs to *H. pylori* susceptibility or resistance.

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Figure Legends

Figure 1: Activation of Inflammasome: The PRRs or cytokine receptors are activated by PAMPs, DAMPs, extracellular ATP and a number of crystalline materials. The stimulus leads to efflux of potassium and calcium ion which leads to ROS generation via mitochondrial dysfunction that activates the inflammasome formation which further activates the caspases, all these events along with activation of NF-κB leads to the production of pro-inflammatory cytokines which ultimately increases the inflammation.

Figure 2: Role of virulence factors of *H pylori* in inflammasome activation: The virulence factors of *H. pylori* such as Ure B, CagA induces inflammation via activating NF-κB transcription factor where as vacuolating cytotoxin A (VacA) induces mitochondrial dysfunction and endoplasmic reticulum mediated stress which leads to reactive oxygen species generation and activation of inflammasome which further cleaves and activates the caspases to produce IL-1β and IL-18 cytokines.

Table 1: Different types of inflammasomes and their ligands

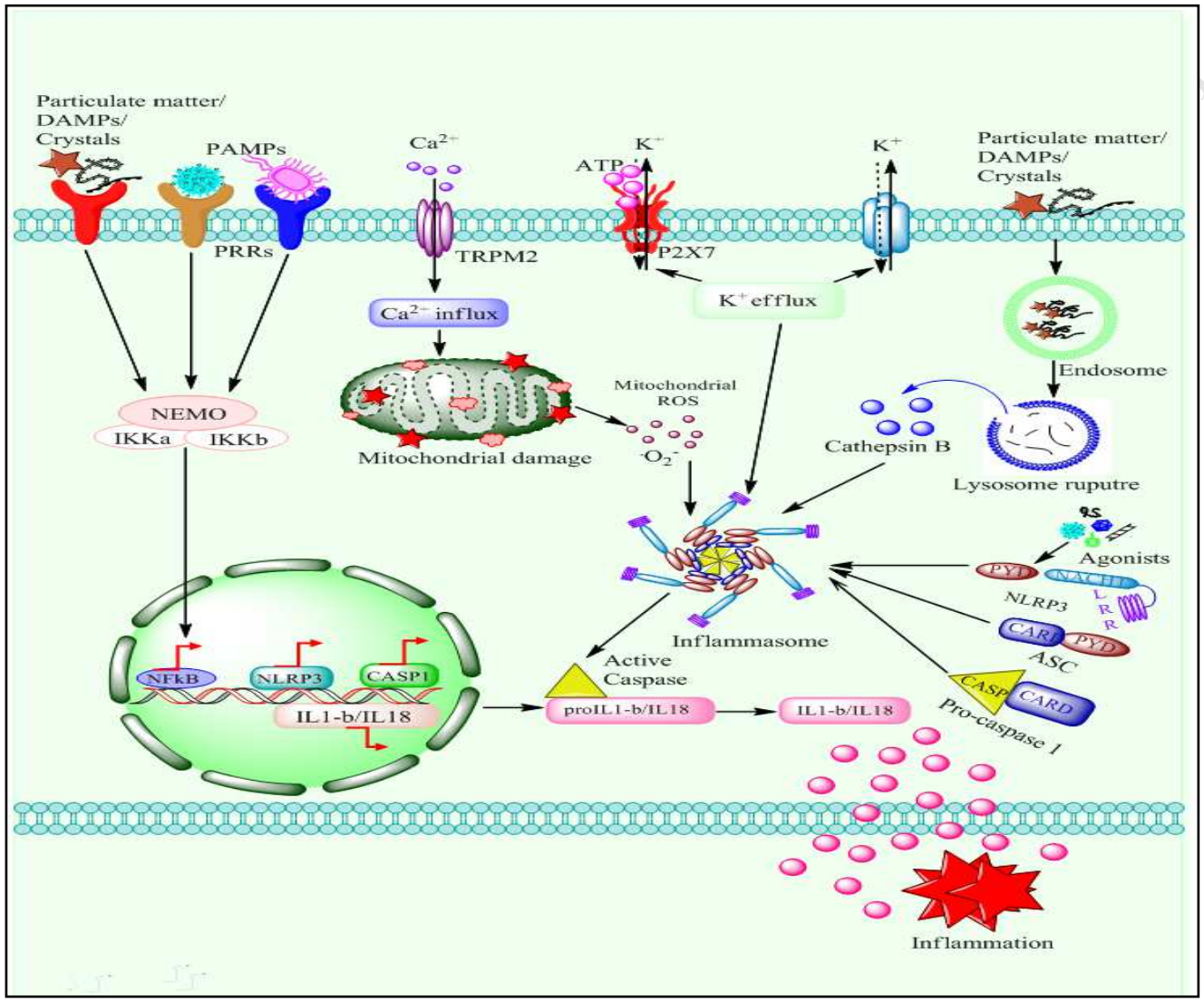
Inflammasome	Activators	Activated caspase	Cytokines released
AIM2	dsDNA	Caspase 1	IL-1β & IL-18
PYRIN	Toxins	Caspase 1	IL-1β & IL-18
NLRC4	Flagellin, T3SS	Caspase 1	IL-1β & IL-18
NLRP1	Bacterial toxins	Caspase 1	IL-1β & IL-18
NLRP3	ROS, ion flux, lysosomal disruption, LPS & OMVs	Caspase 1, 4, 5 & 11	IL-1β & IL-18
NLRP6	Regulators of mucus and antimicrobial peptides	Caspase 1	IL-1β & IL-18
NLRP7	Lipopeptides	Caspase 1	IL-1β & IL-18
NLRP9b	RNA stretches	Caspase 1	IL-1β & IL-18
NLRP10	Fungal components	Caspase 1	IL-1β & IL-18
NLRP12	Negative regulator of colon inflammation	Caspase 1	IL-1β & IL-18

Table 2: Inflammasomes regulated by bacterial virulence factors

Bacteria	Effectors	Inflammasome	References
Acinetobacter baumannii	Unknown	NLRP3	Dikshit et al., 2018 and Kang et al., 2017
Bacillus anthacis	Lethal toxin	NLRP1b	Greaney, Leppla & Moayeri, 2015
Bordetella pertussis	CyaA	NLRP3	Dunne et al., 2010
Burkholderia cenocepacia	T6SS	PYRIN	Gavrilin et al., 2012
Chlamydia pneumoniae	Unknown	NLRP3	Shimada et al., 2012
Clostridium difficile	TcdA and TcdB	NLRP3	Ng et al., 2010
Francisella tularemia	T6SS & DNA	AIM2 & NLRP3	Wallet, Lagrange & Henry, 2016
Helicobacter pylori	CagPAI, UreB, FlaA	NLRP3 & NLRC4	Semper et al., 2014, Ng et al., 2015 & Pérez-Figueroa et al., 2016
Legionella pnemotropophilla	Dot/Icm	NLRP3, NAIP5/NLRC4 & AIM2	Mascarenhas & Zamboni, 2017
Listeria monocytogenes	LLO & DNA	NLRP1b, NLRP3 & AIM2	Kim et. al., 2010 and Neiman-Zenevich et al., 2017
Mycobacterium tuberculosis	Whole Mtb	NLRP3 & AIM2	Wawrocki & Druszczynska, 2017
Porphyromonas gingivalis	NDK	NLRP3	Olsen & Yilmaz, 2016.
Pseudomonas aeruginosa	IpaF, T3SS	NLRC4 & NLRP3	Miao et al., 2008 and Jabir et al., 2015
Salmonella typhimurium	LPS	NLRP3 & NLRC4	Diamond et al., 2017 and Liu et al., 2017
Shigella flexneri	T3SS	NLRP3 & NLRP1b	Berndt 2014 and Neiman-Zenevich et al., 2017
Staphylococcus aureus	α-hemolysin	NLRP3	Muñoz-Planillo et al., 2009
Streptococcus pneumoniae	Pneumolysin	NLRP3 & AIM2	Rabes, Suttorp, & Opitz, 2016
Vibrio cholerea	HlyA and	NLRP3	Toma et al., 2010

	MARTX		
Vibrio parahaemolyticus	VopS	PYRIN	Xu et al., 2014
Yersinia enterocolitica	T3SS	NLRP3	Zwack et al., 2015

Figure 1


Particulate matter/
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Crystals
K^+ efflux
Ca^{2+} influx
Endosome
NEMO
IKKa
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Cathepsin B
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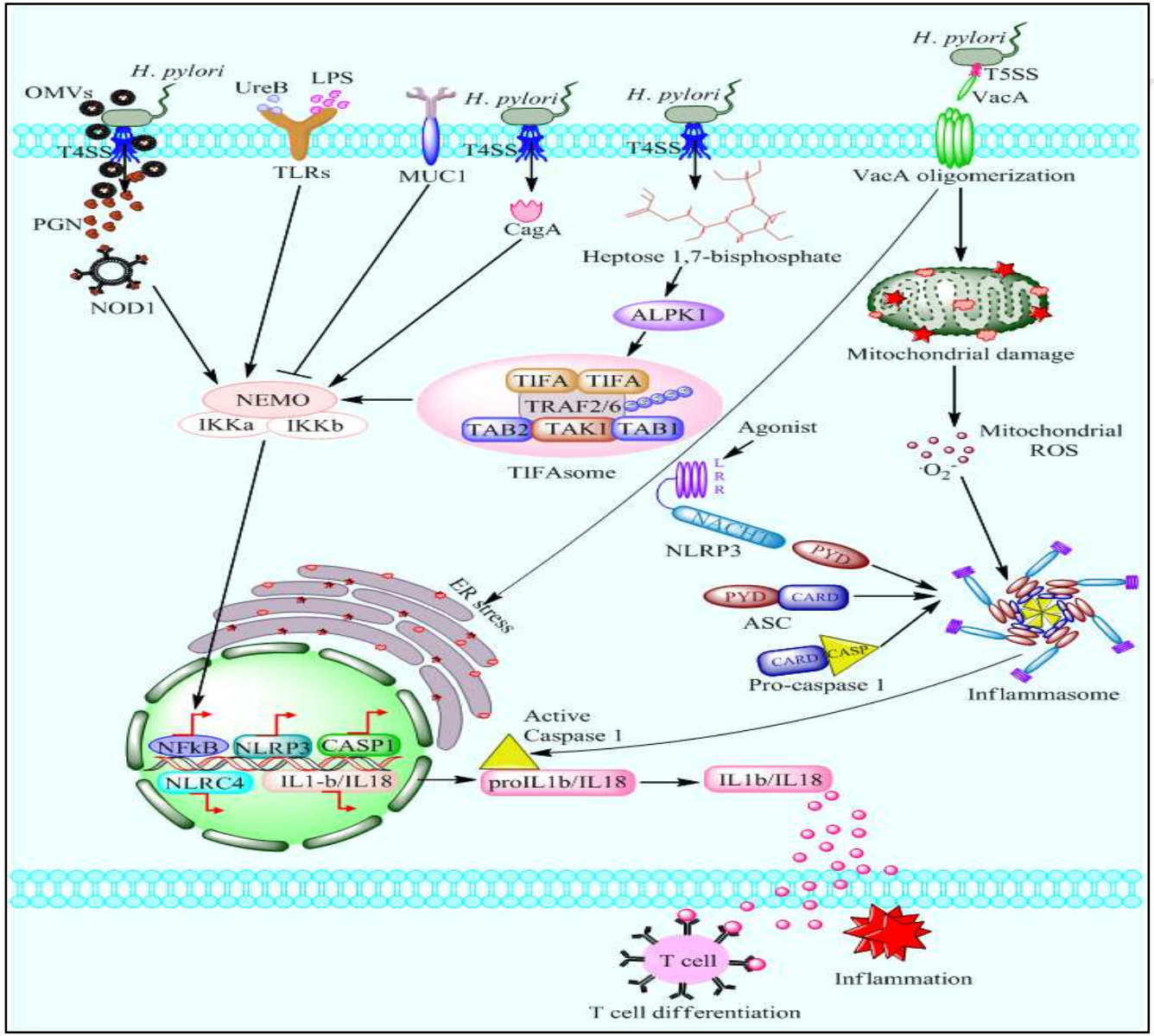
Agonists
PYD
NACHT
NLRP3
LRR
CARD
PYD
ASC
CASP
CARD
Pro-caspase 1
Inflammasome
Active Caspase
NFkB
NLRP3
CASP1
IL1-b/IL18
proIL1-b/IL18
IL1-b/IL18
Inflammation

Figure 2


H. pylori
OMVs
T4SS
PGN
NOD1
H. pylori
UreB
LPS
TLRs
MUC1
H. pylori
T4SS
CagA
H. pylori
T4SS
Heptose 1,7-bisphosphate
ALPK1
H. pylori
T5SS
VacA
VacA oligomerization
Mitochondrial damage
Mitochondrial ROS
O_2^-
NEMO
IKKa
IKKb
TIFA
TIFA
TRAF2/6
TAB2
TAK1
TAB1
TIFAsome
Agonist
LRR
NACHT
NLRP3
PYD
PYD
CARD
ASC
CARD
CASP
Pro-caspase 1
Inflammasome
ER stress
NFkB
NLRP3
CASP1
NLRC4
IL1-b/IL18
Active
Caspase 1
proIL1b/IL18
IL1b/IL18
T cell
T cell differentiation
Inflammation

Highlights

The review “Inflammasome Activation and Regulation during *Helicobacter pylori* Pathogenesis” emphasizes on:

1. The understanding the mechanism of inflammasome activation by various bacterial pathogens and *H. pylori.*
2. The role of virulence factors of *H. pylori* such as UreB, CagA, FlaA and VacA in inflammasomes function.
3. Elaborated mechanism of inflammasomes regulation and elucidates the pathways through which *H. pylori* regulates it.
4. The recent advances and future prospects of inflammasome during *H. pylori* infection.

Introduction

The Gram-negative microaerophilic bacterium *Helicobacter pylori* colonize in human gastric mucosa and is a leading cause of human gastric diseases. More than half of the world population including 4.4 billion individuals are infected with *H. pylori* (Hooi et al., 2017). Although there is no clear evidence of *H. pylori* source and route of infection, its transmission is expected to occur through feco-oral route (Salama et al., 2013). *H. pylori* has long association with human stomach and therefore is helpful to study the human migration and evolution (Moodley & Linz 2009). *H. pylori* colonized human gastric mucosa is associated with less severe asymptomatic gastritis, prolongation of which leads to severe disease conditions such as peptic ulcer, gastric adenocarcinoma and mucosa-associate lymphoid tissue (MALT) lymphoma. *H. pylori* is categorized as a type I carcinogen by World Health Organization (Parsonnet et al., 1991). Successful persistence of *H. pylori* in human stomach depends on two initial events, neutralization of acidic pH of gastric juice and its penetration to gastric mucosa. A versatile pool of virulence genes, bacterial shape and strategies to modulate self and host responses promote *H. pylori* colonization in gastric mucosa and epithelium (Suerbaum and Josenhans 2007). It secretes urease enzyme which with the help of Ni^{2+} ions as a cofactor catalyses the production of ammonium ions and consequently neutralize highly acidic pH (1-2) through ammonium hydroxide of the stomach (Mobley et al., 1991; Montecucco & Rappuoli 2001). It then penetrates the gastric mucosa and become motile in a mucous layer with the help of a tuft of polar flagella (Celli et al., 2009). The helical shape of *H. pylori* promotes corkscrew like mechanism which eases the process of penetration into mucous layer (Bonis et al., 2010). In mucous layer, *H. pylori* live as free swimming bacteria while a few adhere to stomach epithelium surface and form micro-colonies (Tang et al., 2011).

The surface exposed blood group antigen-binding adhesin (BabA) and sialyly-Lewis x (sLex)-binding adhesin (SabA) are two important adhesins which help in the adherence of *H. pylori*. Other adhesins which involved in colonization are AlpA, AlpB, HopZ and OipA (Odenbreit, 2005; Yamaoka et al., 2002). Apart from adhesins, *H. pylori* lipopolysaccharides (LPS) also facilitate adhesion by the interactions of Lex and Ley epitopes with dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) receptor (Nilsson et al., 2006), epithelial cell

receptor galectin-3 (Fowler et al., 2006) and with mucous layer trefoil factor protein TFF1 (Reeves et al., 2008).

Inflammasomes

Inflammasomes are high molecular weight multimeric protein complexes formed in the cytoplasm in presence of pathogen associated molecular patterns (PAMPs), danger associate molecular patterns (DAMPs) and inorganic salt crystals. Pathogen recognition receptors (PRRs) expressed on epithelial cells and phagocytes including macrophages and neutrophils sense the inflammasome activating ligands which belongs to nuclear oligomerization domain (NOD) like receptor (NLRs) family rich in leucine and AIM2 (absent in myeloma 2) inflammasome which senses DNA. Cytosolic NLRs have three domains (except NLRP1 inflammasome) which is composed of a variable N-terminal effector region with caspase recruitment domain (CARD), pyrin domain (PYD) which helps in signal transduction, acidic domain, or baculovirus inhibitor repeats (BIRs), a centrally located NOD (also referred as the NACHT cassette) which is critical for ATPase activity and its oligomerization and C-terminal leucine-rich repeats (LRRs) that senses PAMPs (Bauernfeind & Hornung 2013). Second component of inflammasome is adaptor protein apoptosis associated speck like containing a CARD domain (ASC) consisting of two death-fold domains which are a PYD and a caspase recruitment domain (CARD) (Broz & Dixit 2016). Through its PYD, ASC interacts with upstream PYD of tripartite NLR and forms multimers of ASC dimers while CARD brings inactivated procaspases together, autoproteolytic action of which leads to cytosolic complexing of inflammasomes and maturation of pro-caspases such as caspase 1, caspase 4 and caspase 5 (inflammatory caspases). The activated caspases further releases pro-inflammatory cytokines IL-1β and IL-18 (Man et al., 2017) and initiates the maturation of a membrane pore forming toxin, activation of which leads to cell death known as pyroptosis (Shi, et al., 2015; Latz et al., 2013). NLRP1, NLRP3, NLRP4 AIM2 are involved in canonical inflammasome formation. There are various types of inflammasome based on the differences in their sensor and effector domains which are listed in table 1.

Activation and Regulation of Inflammasomes

The activation of inflammasome is regulated both positively and negatively by cellular proteins and number of signals are responsible for the induction of these proteins. Inflammasome

priming i.e. activation of PRRs or cytokine receptors is followed by second stimulus produced by extracellular ATP, endogenous cAMP, PAMPs, pore forming toxins or crystalline materials which leads to efflux of potassium ion a major step during activation of inflammasome. The activation is restricted to NLRP3 and AIM2 or NLRC4 are not activated by K^+ release. Genome-wide CRISPR/Cas9 screen study have found a mammalian NIMA-related kinases (NEK) as an important upstream regulator of NLRP3 against the stimuli of an ionophore nigericin in murine macrophages (Schmid-Burgk et al., 2016). K^+ efflux activates downstream NEK7 which binds to NLRP3 inflammasome and leads its oligomerization through ASC domain (He, et al., 2016). It has also been found that NLRP3 activation and mitosis are independent event and NEK7 restricts inflammasome at interphase of cell cycle with downstream production of mitochondrial ROS by binding to NLRP3 leucine-rich repeat domain through kinase-independent manner (Shi, et al., 2016). Kinase receptor interacting protein kinase 3 (RIPK3) signaling leads to necroptosis through downstream effector pseudokinase mixed lineage kinase domain-like (MLKL) translocation to cellular membrane, gasdermin independent disruption of membrane and loss of K^+ ions which activates NLRP3 inflammasome and releases IL-1β (Gutierrez et al., 2017). MLKL translocate to membrane, oligomerize, reduce intracellular cellular level of K^+ ions and induce necroptosis through its four helical bundle domain (Conos et al., 2017).

Two important protein degradation pathways ubiquitin-proteasome and autophagy-lysosomal regulates the innate immune responses by targeting infllamasome. Plasminogen activator inhibitor type 2 and dopamine D1 receptor (DRD1) regulate autophagy-lysosomal dependent NLRP3 degradation (Chuang et al., 2013; Versteeg et al., 2014), through E3 ubiquitin ligase (Versteeg et al., 2014), Tripartite motif (TRIM) containing superfamily proteins expresses in response to interferons (IFNs) and play important role in regulating innate immune responses (Ozato et al., 2008). TRIM31, a member of TRIM superfamily promote degradation of NLRP3 in resting and activated stages of macrophages by promoting K48 polyubiquitination followed by proteasomal degradation (Song et al., 2016). Protein kinase A inhibits NLRP3 inflammasome through prostaglandin E_2 (PGE_2) signaling via PGE2 receptor E-prostonoid 4 (EP4) (Mortimer et al., 2016). PKA directed NLRP3 phosphorylation at Ser295 leads to attenuation of its ATPase activity. Activated PKA promotes cyclic adenosine monophosphate (cAMP) increase which

endogenously binds to NOD-binding domain (NBD) and leads to its degradation autophagy (Yan et al., 2015; Lee et al., 2012 ; Sokolowska et al., 2015) Figure 1.

Reactive oxygen species (ROS) generated by cellular damage initiated by PAMPs, organelles damage and cell death are also responsible for the activation of inflammasome. The NADPH oxidase (NOX) dependent ROS has inhibitory effect on IL-1β production as seen during the knockdown of $p22^{phox}$ subunit (Dostert et al., 2008 & Dostert et al., 2009). The contradictory studies have also shown NOX1-4 independent activation of NLRP3 (van Bruggen et al., 2010). The level of IL-1β remains unaltered irrespective of ROS downregulation when analyzed in chronic granulomatous disease **(**CGD) patient having mutation at *CYBA* gene which codes for $p22^{phox}$ a subunit of NOX responsible for its activation. S100A8/A9 are DAMPs responsible for inflammation by promoting migration of phagocytes, their degranulation and NADPH oxidase dependent ROS production. Cellular upregulation of S100A8/A9 leads to regulation of NLRP3 by ROS dependent activation of NF-B and IL-1β release (Simard et al., 2013).

Inflammasome and Bacterial Pathogenesis

Inflammasomes are host innate defense mechanisms against invading pathogens. Microbial activation of inflammasomes leads to inflammation with the secretion of pro-inflammatory cytokines IL-1β and IL-18 and HMGB1 as a alarmin in extracellular milieu which checks the pathogen growth inside host. Bacteria modify their effectors to regulate the inflammasomes for own survival and spreading of infection. A number of inflammasomes are activated by bacterial, fungal and viral pathogens including NLRP1, NLRP1b, NLRP3, NAIP/NLRC4, AIM2, NLRP6, NLRP7, NLRP9b and NLRP12 (von Moltke et al., 2013, Zhu, et al., 2017, Vladimer et al., 2013, Tavares et al., 2015, Man et al., 2017 & Mathur et al., 2018). NLRC4 and ASC are required for caspase-1 activation and the production of IL-1β in response to infection, with *Salmonella, Shigella, Pseudomonas,* and *Legionella* whereas NLRPb and NLRP3 inflammasome is activated in response to *B. anthracis* and *Listeria* respectively. The bacterial pathogens which regulate the activity of inflammasomes are enlisted in the table 2.

Activation of Inflammasome by *H. pylori*

Two major virulence factors cytotoxin associated gene A (CagA) and vacuolating cytotoxin A (VacA) of *H. pylori* are responsible for inflammation in gastric epithelium. CagA is a type IV secretory system (T4SS) oncoprotein while VacA is a type V secretory system (T5SS) secreted toxin which induce cytosolic vacuolation, mitochondrial dysfunction, endoplasmic reticulum and endosomal mediated stress which leads to oxidative stress (Salama et al., 2013). The PAMPs from *H. pylori* are recognized by PRRs of host innate immune system and initiate the production of pro-inflammatory cytokines and chemokines with the activation of transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and signal transducer and activator of transcription 3 (STAT3) (Castaño-Rodríguez et al., 2014). Activation of these transcription factors leads to expression of inflammatory cytokines IL-1β, IL-6, IL-8, IL-18, IL-23 and TNFα which further creates a state of inflammation. Inflammation is one of the major contributor of tumorigenesis and cancer (Mantovani et al., 2008) and is also responsible for *H. pylori* induced gastric carcinomas. It is not only the pathogen virulence factors which induce gastric inflammation during *H. pylori* infection but host factors are also responsible for prolongation of gastritis. Polymorphism in the gene sequences of pro-inflammatory cytokines IL-1β is another factor which contributes to severe and prolonged gastritis and is also associated with gastric cancer during *H. pylori* infection (Figueiredo et al., 2014). *H. pylori* induced inflammasomes play an important role in the production of pro-inflammatory IL-1β and IL-18 with the activation of pro-caspase 1 into caspase 1 and initiation of gastric inflammation (Franchi et al., 2010) Figure 2.

In gastric epithelium niche, *H. pylori* regulates the activation of inflammasome and pyroptosis for survival. *H. pylori* regulated NLRP3 inflammasome formation is major mediator of IL-1β secretion but NLRP3 independent mechanism for the regulation of immune responses also reported. *H. pylori* flagellin FlaA triggers phosphorylation of NLRC-4 Ser533 without activation of NLRC-4 inflammasome and escape NAIP5 detection through unknown mechanism (Matusiak et al., 2015). The gastric epithelial cells surface associated glycoprotein mucin 1 (MUC1) is highly expressed on cells surfaces and protects epithelium lining against inflammation and infection (Sheng et al., 2013). Mice infected with *H. pylori* showed a MUC1

dependent activation of NLRP3 inflammasome through NF-κB dependent signaling which initiates gastric inflammation (Ng et al., 2015). A T4SS, TLR2 and TLR4 independent activation of NLRP3 is reported in in human neutrophils infected with *H. pylori* strains (Pérez-Figueroa, et al., 2016). Caspase 1 dependent ROS, K^+ efflux and Ca^{2+} signaling induced NLRP3 infllammasome formation was also found in *H. pylori* infected THP1 macrophages (Kameoka, et al., 2016). A decrease in autophagy with an increase in oxidative stress and associated protein NADPH oxidase gp91phox was also noticed during H. pylori gastritis which is mediated through inflammasome/caspase 1 signaling (Yang et al., 2013).

Withaferin A (WA), a withanolide extracted from *Withania somnifera* inhibits NF-κB induced NLRP3 in murine bone marrow derived dendritic cells infected with *H. pylori* (Kim et al., 2015). *H. pylori* urease act as a novel immunomodulator and found to be protect against asthma via NLRP3 activation through TLR2 signaling in dendritic cells (Koch et al., 2015). *H. pylori* infection is also protective against inflammatory bowel disease through NLRP3 induced IL-18 secretion (Engler et al., 2015). It is found that Cag Pathogenicity Island (CagPAI) also stimulates the NRP3 inflammasome (Semper et al., 2014). The role of *H. pylori* induced NLRP3 activation is also studied in innate and adaptive immune responses which leads to the development of Treg and suppression of Th1 in humanized mice (Arnold et al., 2017). Few reports showed the role of inflammasome during *H. pylori* infection at transcriptome level where the expression of NLRP3 inflammasome is upregulated by suppressing miR-22 microRNA (Li et al., 2018). Pachathundikandi and Backert (2018) showed hsa-miR-223-3p controlled NLRP3 upregulation which was independent of CagA, VacA, Cgt, FlaA and CagPAI virulence factors.

Although regulation of inflammasome/caspase 1 signaling pathway is not studied in detail during *H. pylori* infection of gastric epithelium but above preliminary reports showed inflammasome induced pro-inflammatory cytokines Il-1β and IL-18 in H. pylori inflammation. Polymorphism associated with these cytokines are responsible for cancer related activities and modulation of Th1 and Th17 T cells responses (Hitzler et al., 2012). IL18 also responsible for T cells induced immunopathology during *H. pylori* infection (Hitzler et al., 2012). Alogwith adaptive immune cells modification, inflammasome also found to regulate the innate immune responses. Arnold et al., 2017 also studied the non-canonical role of NLRP3 in dendritic cells

development and showed that during *H. pylori* pathogenesis CD11b+ dendritic cell require NLRP3 for their recruitment in lymphoid tissues without the help of ASC adaptor or caspase 1. IL-1β play major role in metastatic diseases and its blockade significantly decreases angiogenesis (Dinarello, 2010). Discovering blockades such as monoclonal antibodies or natural inhibitors for the intermediates Inflammasome/caspase 1 pathway can also play an important role in the inhibition of IL-1β and IL-18 during *H. pylori* infection and ultimately in the prevention of gastric cancer.

Recent Advances and Future Prospects of Inflammasome

Inflammasomes play crucial role against pathogen defense but their dysfunction leads to disease conditions such as cancer, autoimmune and metabolic syndromes and neurodegeneration. NLR is a large protein family having more than 23 NLR proteins out of which the functions of most of these are not well characterized. NLRP3, NAIP5 and NLRC-4 are few inflammasomes which are been studied extensively in various human diseases but their post translational regulatory mechanism and their association with *H. pylori* virulence factors is still poorly characterized. Recently a new family member NLRP 9b has been described which has a role against intestinal rotavirus protection. Expression of NLRP 9b specifically in intestinal epithelial cells restricts rotavirus infection. Through RNA helicase Dhx9 short double-stranded RNA stretches are recognized by NLRP9b which forms inflammasome complexes and stimulate the release of interleukin IL-18 and pore forming gasdermin D (released into plasma membrane upon the cleavage by caspase 1 or caspase11) to initiate pyroptosis (Zhu, et al., 2017). Till date, a direct interaction of PAMPs with inflammasomes molecules and the interacting partners of upstream and downstream signaling during *H. pylori* infection is not known. Its detailed mechanism and careful investigation may give some leads to control and reduce the disease severity. Future studies are also required to address the precise role of genetic variants of NLRs to *H. pylori* susceptibility or resistance.

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Figure Legends

Figure 1: Activation of Inflammasome: The PRRs or cytokine receptors are activated by PAMPs, DAMPs, extracellular ATP and a number of crystalline materials. The stimulus leads to efflux of potassium and calcium ion which leads to ROS generation via mitochondrial dysfunction that activates the inflammasome formation which further activates the caspases, all these events along with activation of NF-κB leads to the production of pro-inflammatory cytokines which ultimately increases the inflammation.

Figure 2: Role of virulence factors of *H pylori* in inflammasome activation: The virulence factors of *H. pylori* such as Ure B, CagA induces inflammation via activating NF-κB transcription factor where as vacuolating cytotoxin A (VacA) induces mitochondrial dysfunction and endoplasmic reticulum mediated stress which leads to reactive oxygen species generation and activation of inflammasome which further cleaves and activates the caspases to produce IL-1β and IL-18 cytokines.

Table 1: Different types of inflammasomes and their ligands

Inflammasome	Activators	Activated caspase	Cytokines released
AIM2	dsDNA	Caspase 1	IL-1β & IL-18
PYRIN	Toxins	Caspase 1	IL-1β & IL-18
NLRC4	Flagellin, T3SS	Caspase 1	IL-1β & IL-18
NLRP1	Bacterial toxins	Caspase 1	IL-1β & IL-18
NLRP3	ROS, ion flux, lysosomal disruption, LPS & OMVs	Caspase 1, 4, 5 & 11	IL-1β & IL-18
NLRP6	Regulators of mucus and antimicrobial peptides	Caspase 1	IL-1β & IL-18
NLRP7	Lipopeptides	Caspase 1	IL-1β & IL-18
NLRP9b	RNA stretches	Caspase 1	IL-1β & IL-18
NLRP10	Fungal components	Caspase 1	IL-1β & IL-18
NLRP12	Negative regulator of colon inflammation	Caspase 1	IL-1β & IL-18

Table 2: Inflammasomes regulated by bacterial virulence factors

Bacteria	Effectors	Inflammasome	References
Acinetobacter baumannii	Unknown	NLRP3	Dikshit et al., 2018 and Kang et al., 2017
Bacillus anthacis	Lethal toxin	NLRP1b	Greaney, Leppla & Moayeri, 2015
Bordetella pertussis	CyaA	NLRP3	Dunne et al., 2010
Burkholderia cenocepacia	T6SS	PYRIN	Gavrilin et al., 2012
Chlamydia pneumoniae	Unknown	NLRP3	Shimada et al., 2012
Clostridium difficile	TcdA and TcdB	NLRP3	Ng et al., 2010
Francisella tularemia	T6SS & DNA	AIM2 & NLRP3	Wallet, Lagrange & Henry, 2016
Helicobacter pylori	CagPAI, UreB, FlaA	NLRP3 & NLRC4	Semper et al., 2014, Ng et al., 2015 & Pérez-Figueroa et al., 2016
Legionella pnemotropophilla	Dot/Icm	NLRP3, NAIP5/NLRC4 & AIM2	Mascarenhas & Zamboni, 2017
Listeria monocytogenes	LLO & DNA	NLRP1b, NLRP3 & AIM2	Kim et. al., 2010 and Neiman-Zenevich et al., 2017
Mycobacterium tuberculosis	Whole Mtb	NLRP3 & AIM2	Wawrocki & Druszczynska, 2017
Porphyromonas gingivalis	NDK	NLRP3	Olsen & Yilmaz, 2016.
Pseudomonas aeruginosa	IpaF, T3SS	NLRC4 & NLRP3	Miao et al., 2008 and Jabir et al., 2015
Salmonella typhimurium	LPS	NLRP3 & NLRC4	Diamond et al., 2017 and Liu et al., 2017
Shigella flexneri	T3SS	NLRP3 & NLRP1b	Berndt 2014 and Neiman-Zenevich et al., 2017
Staphylococcus aureus	α-hemolysin	NLRP3	Muñoz-Planillo et al., 2009
Streptococcus pneumoniae	Pneumolysin	NLRP3 & AIM2	Rabes, Suttorp, & Opitz, 2016
Vibrio cholerea	HlyA and	NLRP3	Toma et al., 2010

	MARTX		
Vibrio parahaemolyticus	VopS	PYRIN	Xu et al., 2014
Yersinia enterocolitica	T3SS	NLRP3	Zwack et al., 2015

Figure 1

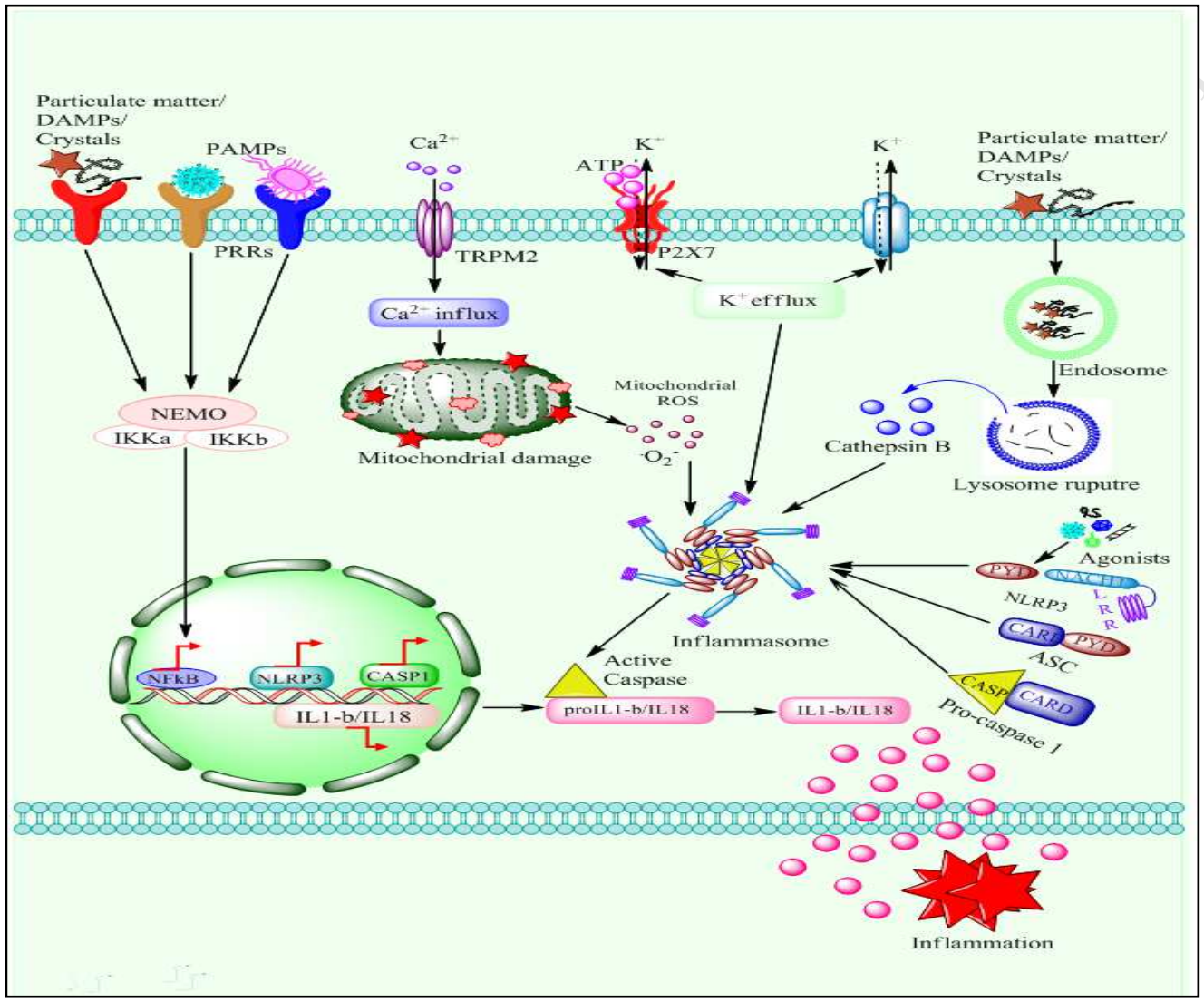
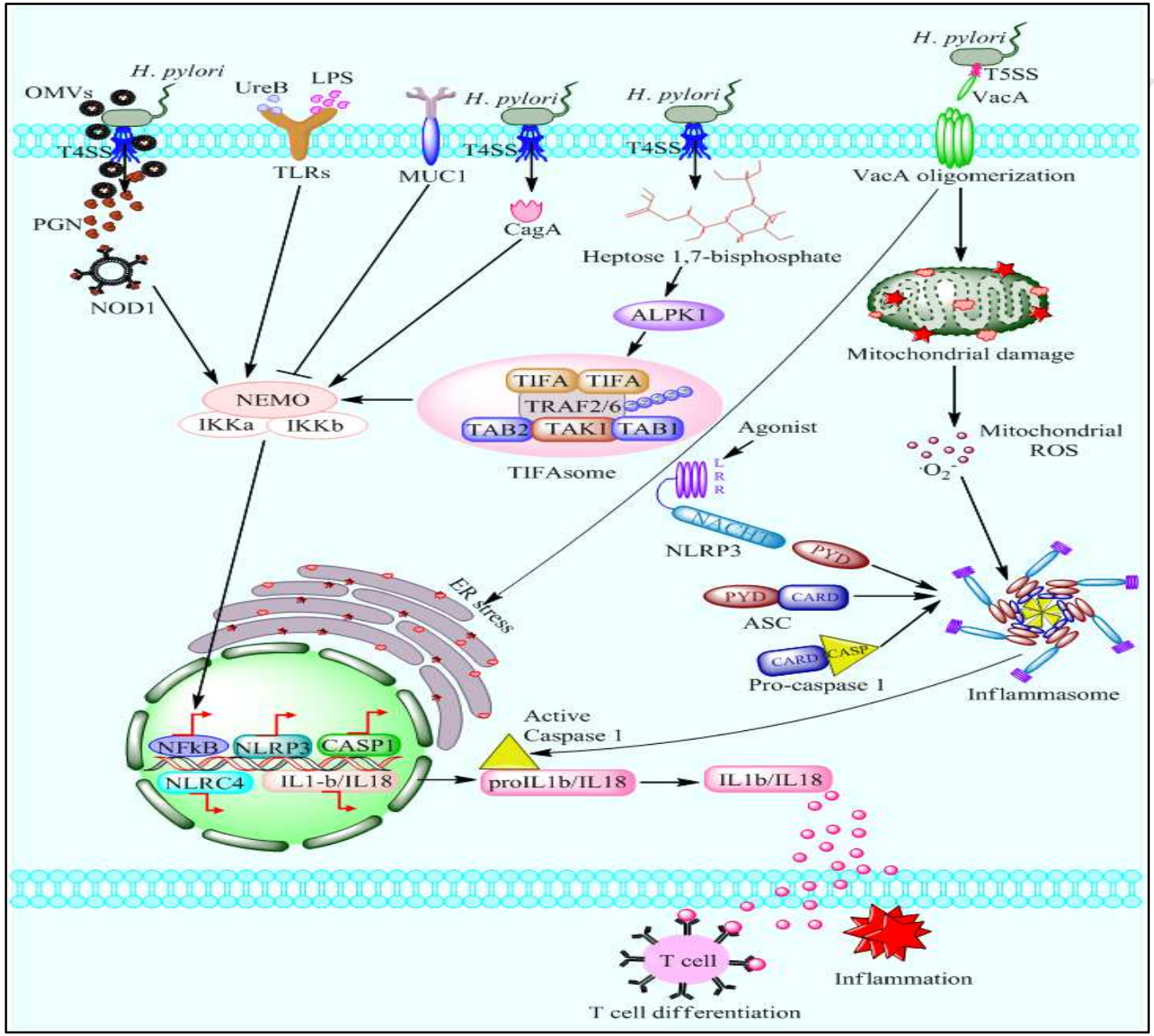

Particulate matter/
DAMPs/
Crystals
PAMPs
PRRs
Ca^{2+}
TRPM2
K^+
ATP
P2X7
K^+
Particulate matter/
DAMPs/
Crystals
K^+ efflux
Ca^{2+} influx
Endosome
NEMO
IKKa
IKKb
Mitochondrial damage
Mitochondrial ROS
$\cdot O_2^-$
Cathepsin B
Lysosome ruptre
Agonists
PYD
NACHT
NLRP3
LRR
CARD
PYD
ASC
CASP
CARD
Pro-caspase 1
Inflammasome
Active Caspase
NFkB
NLRP3
CASP1
IL1-b/IL18
proIL1-b/IL18
IL1-b/IL18
Inflammation

Figure 2


H. pylori
OMVs
T4SS
PGN
NOD1
H. pylori
UreB
LPS
TLRs
MUC1
H. pylori
T4SS
CagA
H. pylori
T4SS
Heptose 1,7-bisphosphate
ALPK1
H. pylori
T5SS
VacA
VacA oligomerization
Mitochondrial damage
Mitochondrial ROS
O_2^-
NEMO
IKKa
IKKb
TIFA
TIFA
TRAF2/6
TAB2
TAK1
TAB1
TIFAsome
Agonist
LRR
NACHT
NLRP3
PYD
PYD
CARD
ASC
CARD
CASP
Pro-caspase 1
Inflammasome
ER stress
NFkB
NLRP3
CASP1
NLRC4
IL1-b/IL18
Active
Caspase 1
proIL1b/IL18
IL1b/IL18
T cell
T cell differentiation
Inflammation