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Deubiquitinases (DUBs): A Sub - Family of Master Regulators in Modulating Inflammatory Response through Toll Like Receptor (TLR) Signalling

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Abstract: ***Inflammation is an inductive response stimulated by foreign pathogens or infection which trigger host defence machinery for the recruitment of immune components to the site. However, the mechanisms, context, and significance of inflammation throughout normal immune responses and pathology, on the other hand, is constantly evolving. Toll like receptors (TLR) are receptors on the cell surface and plays pivotal role in innate immune system. It recognizes various microbial components and induce a signalling cascade that drives the expression of inflammatory cytokines genes. In addition, DUBs play key role in modulation TLR mediated inflammatory responses and are known to be regulator of various cellular events. Studies showed that Ubiquitin specific peptidase (USP) to be involved inflammation via TLR mediated pathway. Studies regarded TLRs and USPs was not fully understood. Here we explained structure and function of both TLRs and DUBs. Also, we have highlighted the TLR mediated DUBs inflammation and their possible therapeutic targets.***

Keywords: Inflammation, Toll like receptors, Deubiquitinates, Ubiquitin specific peptidase (USP), Inflammatory response

1. Introduction

Immune system is an immensely versatile biological mechanism that protects animals from invading foreign pathogenic antigens. It actuates wide varieties of cells and generates molecules, especially small peptides, capable of specific recognition and elimination of foreign microbes in a dynamically regulated and complex network system. The pioneering stages of observations from the historical perspective of immunological concept has started around 430 BC when Thucydides has categorically mentioned that "only who has once recovered from plague could be able to physically nurse the fresh plague patients as they wouldn't be infected for the same disease again. " First experimental attempt to develop immunity was in fifteen centuries when Chinese and Turk scientists tried to insert pustules of smallpox infected individual into a healthy individual, a process called "variolation". In 1978, English physician Edward Jenner took this concept significantly farther when he inoculated a young boy with fluids from pustules of smallpox infected individual and later, he intentionally infected the young boy with smallpox virus. In agreement to his anticipation, the boy didn't develop the disease. The series of above observations and experimentations are the pioneering steps towards understanding molecular immunology what has been developed in present day research.

Functionally immune system is categorized into two broad components.1) Innate immunity - the first line defence barrier for microbes exists even before the first infection. Its response timing is rapid but non - specific whereas, 2) Acquired immunity develops after exposure to a foreign pathogen. It shows target specificity and subsequent exposure to same antigen triggers memory response as a result of which efficiency to clear the infection increases vigorously thereafter. Innate immunity comprises of physical barriers like skin, epithelial membranes, blood proteins of complement system and different cell types such as phagocytes (macrophages, neutrophils), dendritic cells and natural killer (NK) cells whereas unique components of acquired immunity are lymphocytes (B - cells, T - cells) and its secreted cytokines or chemical mediators. Apart from inducing an immune response it is vital for immune system's ability to distinguish self from non - self. Despite having significant differences in mechanism, innate and adaptive immunity are component of a highly integral system where numerous cells and molecules work in a cordial manner.

Inflammation ordinarily regarded as due to infection of microbes but many at times it is possible inflammation is triggered by host immune response to the pathogen [1]. Infection is a pathological aspect which is well appreciated in various host - pathogen interactions but physiological aspect is very less understood. Dissecting the pathophysiological mechanism in inflammation triggered by pathogens could take us a way forward in exploring the disease related to inflammation. Innate and adaptive immune response to pathogens triggers inflammation in host cells. The range of inflammation varies with regard to pathogen/injury and imbalances in tissue homeostasis leading to acute and chronic inflammation respectively. Chronic inflammation is wide spread largely in CVD, type II diabetes, cancer etc. Host defence mechanism is quite natural in case of any infection/ injury but in many cases most of the complications arouse due to chronic inflammation that perhaps contributed by dysregulation in tissue homeostasis/inflammation machinery.

Basically, inflammation is triggered by infection/ injury involves recruitment of blood components such as macrophages and mast cells to the site of infection. The physiological role of host immune cell - pathogen interaction is well explored. One of the focal points of understanding is microbial recognition by resident macrophages and mast cells results in modulating the production inflammatory

cytokines, chemokines, adapters and mediators of proteolytic cascade. The inflammatory pathway generally consists of inducers, sensors, mediators and effectors. Macrophages play an important role in production of pro - inflammatory cytokine and chemokines which result in inflammation. Macrophages are the result of bone marrow haematopoietic stem cells (HSC) and are distributed throughout the immune system with diversified functions. It is present in all vertebrates' tissues from mid - gestation throughout life, constituting a widely dispersed organ system. Macrophages are activated by several membrane embedded surface receptors which facilitates downstream signalling cascade, also by crosstalk of signalling pathways. Here the main focus is to review TLR mediated macrophage inflammation promoted by deubiquitinase.

Majority of the cellular protein undergoes Post Translational Modification (PTM) by ubiquitin enzymes [2] and phosphorylation, acetylation, methylation, sumoylation and glycosylation to modulate their activities. This versatile nature of ubiquitin due to its lysine residues (K6, K11, K27, K29, K33, K48, K63) and Methionine at the N - terminal serves as linking site for another Ub leading to formation of polyUb chains [3]. Lysine linked cleavage is specific in few class of DUBs, such as MINDY shows selective cleavage K - 48 linked Poly - Ub [4] ZUFSP1/ZUP1 cleaves selectively K - 63 chains [5] where other DUBs cleaves all types of Poly - Ub [6]. Approximately 100 DUBs reported and classified into five distinct families [7, 8], other two families MINDY family [9] and ZUFSP family [5]. The members of DUBs in human are divided into seven families: Ubiquitin specific proteases (USPs), Ovarian tumor proteases (OTUs), Ubiquitin C - terminal hydrolases (UCHs), Machado - Josephin Domain proteases (MJDs) [7], Motif interacting with ubiquitin containing novel DUB family (MINDY) [9], Zinc finger containing ubiquitin peptidase 1 (ZUP1) [5] and Jab1/Mov34/Mpr1 Pad1 N - terminal+ (JAMM) [10]. These further divided two categories: Six are cysteine (thiol) proteases (ZUFSP, MINDY, UCH, USP, OTU and Josephin) and one is metalloprotease (JAMM) binds to Zinc. Proteins are ubiquitinated by ubiquitin machinery which consists three enzymes: Ubiquitin conjugating enzymes (E2s), ubiquitin activating enzymes (E1s) and Ubiquitin ligases (E3s) [11] together regulate function and stability of the proteins.

The physiological and pathological roles of DUBs explored widely. Dysregulation in DUBs activity results into various disease state conditions in Humans. DUBs have potential role in diseases such as: Neurological, Cancer progression and biomarker, autoimmune disorder, etc. Recently, DUBs like viral papain like proteases (PLPs) alter the pathways involved in severing the infection in SARS - CoV - 2 reported. Other, members of the virus family also encode PLPs such as SARS - CoV PLP, MERS - CoV PLP. Here, we mostly focus on the role of DUBs in triggering inflammation acting as key mediators in immune signalling pathways. DUBs in conjugation other molecules modulates inflammation by affecting the assembly and activation of inflammasome. Several DUBs reported to modulate the inflammasome such as: USP7 and USP47, STAMBP, UAF1, A20, etc regulates NLRP3 inflammasome activation.

2. Methodology

This study's research technique involves a complete review of available literature. We searched databases PubMed, ScienceDirect, and Google Scholar for literature published in the last decade on the involvement of Deubiquitinases (DUBs) in Toll Like Receptor (TLR) signaling. Articles that particularly examined the mechanisms of DUBs and their significance in immunological signalling pathways were included in the selection criteria. The information gleaned from these articles was then analysed and synthesized to provide a comprehensive understanding of the subject.

Toll Like Receptors (TLRs) Structure and function

Toll like receptors are the toll gates to which the extracellular molecules binds and leading to cytosolic signalling cascade for the production of inflammatory cytokines. TLR resembles to type 1 transmembrane family member where extracellular (N - terminal) domain consists of leucine rich repeats, transmembrane domains span the membrane and intracellular domain. Its occurrence was reported over the animal kingdom such as Drosophila, purple sea urchin, mice and human with a number of 9, 222, 12 and 10 respectively which serve as Pattern recognition receptors (PPRs). In particular humans encode 10 TLRs which gets activated upon binding to microbial components. Toll protein discovered in dorsoventral development of Drosophila melanogaster also has role in immune response in it [12]. Structural information reveals that the extracellular domain participates in ligand binding and is facilitated by ectodomains. The ectodomains present as homodimers in TLR 3, 4, 5, 7, 8, 9 and heterodimers in TLR 1, 2, 6 [13]. Binding specificity of TLRs is greatly diversified and that depends on the different microbial components. Microbial components (PAMPS/MAMPS) detected by TLRs such as two molecules of Lipoploysaccaride (LPS) - TLR4 [14], [15], flagellin – TLR5 [16, 17], di or tri acylated lipoprotein - TLR2/1, TLR2/6 [18], unmethylated two molecules CpG - containing DNA – TLR9 [19], Bacterial ribosomal RNA – TLR13 [20], ssRNA – TLR7, 8 [21], dsRNA - TLR3 [22], Prolifin protein – TLR11 [23]. This collection of TLRs mentioned above are not limited to humans rather all organisms. Many Human TLR ligand remain to explore such as TLR10 is an inactive pseudogene which is result of retrovirus insertion [24]. Structural determination of TLR is important to outlet the role of TLR in provoking immune response. Out of all methods X - ray diffraction and NMR spectroscopy are the gold standard methods for protein structure determination which are laborious and time consuming. Computational approaches in determining the structure starts form homology modelling but at some instances it also fails to predict the structure of TLRs encoded by 3000 genes whereas LRR template assembly methods proved to have efficient structure prediction of protein [25]. It has been also reported TLR 1 - 5 shares direct homology between human and fly which are positioned on chromosome 4 (TLR 1, 2, 3), 9 (TLR4) and 1 (TLR5) [26]. Evolutionary studies indicate the emergence of various TLRs and their ligand resulted due to natural selection over the primates [27].

TLR 1 and 2

Toll like receptor 2 (TLR2) mediated inflammation triggered by lipoproteins covalently connected through two ester bond and one amide exception to gram - negative [28] and only two ester bonds in gram positive bacteria [29, 30]. TLR2 is specific to lipoprotein but besides lipoprotein, lipoteichoic acids, NAM and NAG, phenol soluble modulins, lipomannans and zymosans also activates TLR2 [31]. Structural anatomy of TLR2 and its ligand reveals to consisting of hydrophobic residues from the Leucine Rich Repeats (LLR 9 - 12) modules [30]. TLR2 and TLR6 share a similar structure and sequence conservation and belong to same family. LRR regions of TLRs are conserved motifs consist parallel Beta strands and infrequently leucine and arginine are replaced by other hydrophobic residues and serine, threonine, cysteine respectively [32]. The biochemical event that occurs in binding of TLR2 and its ligand is not well understood. It has been shown that ester bound lipids are inserted between LLR11 and LLR12 loops and extended to internal hydrophobic pockets. Activation of TLR2 triggered by TLR2 - lipid interaction where TLR2 ligands such as lipoprotein consists two lipids chains which binds to TLR2 and activates inflammatory response. Any irregularity in the lipid chains lead to inefficacious TLR activation. Its activation is further governed by TLR 2 and 6 with which dimerize for its activation [30]. TLR1 associated core TLR6, TLR2 and TLR10 aids TLR1 for the initiation of signalling cascade of TLR1 by heterodimerization on ligand binding [33], [34], [35]. The initiation of Signalling cascade by TLR1 - ligand complex induces signalling adaptors such as TIRAP and Myeloid differentiation primary response gene 88 (MyD88) for production of inflammatory cytokines through expression of NF - κB [36] [37].

TLR3

TLR3 recognises double stranded RNA (dsRNA), a molecular signature of most viruses and triggers inflammatory response. The ectodomains of TLR3 comprises solenoid like structure and appears as horseshoe shape consisting 23 leucine rich repeats (LRR). This structure terminated at both the N terminal (LRR - NT) and C terminal (LRR CT). TLR3 receptor activation is facilitated upon binding of dsRNA results in dimerization of TLR3 ECD. The interaction can be divided in two events: interaction at C terminus LRR19 to LRR21 and assisted but few conserved residues such as Asn517, Asn515, His539, Asn541 and Arg544 whereas the second interaction is at LRR - NT to LRR3 assisted by Glu110, His108, Arg64, Ser86, Phe84, His60 and His39 conserved residues. Glu110, Ser86, Phe84 and Arg64 also contributed in ligand binding to the receptor [38].

TLR4

Indeed, inflammation plays an important role throughout various diseases, one such pathway that induces inflammation to bacterial component response is NF - B signalling pathway. For activation, TLR4 plays a vital role by establishing the interaction with Lipoploysaccaride (LPS). Among mammal TLRs, TLR4 is first identified [39]. TLR4 - LPS complex initiates downstream signalling by recruitment of various adaptors. Strong Receptor ligand specific interaction has been suggested due to low concentrations [40] as well as high concentration [41] of ligand molecules. TLR4 is stimulated by a specific ligand called lipoploysaccaride (LPS) which is embedded in bacterial cell wall. LPS encompasses hydrophilic repeating O antigen, core oligosaccharide and Lipid A is embedded in the glucosamine units of peptidoglycan of bacteria. Lipid A represents as main stimulant for TLR4 signalling. Ectodomain axillary molecules acts upstream of TLR4 such as LPS binding protein (LBP) makes strong contact with LPS and CD14. Further, CD14 helps in extracting a single molecule of LPS and transfer it to MD2 protein [40]. MD - 2 consist two antiparallel B - sheets with a molecular weight ~18kDa, 160 amino acids and devoid of disulphide bonds. Similarly, the acute phase protein LBP contains 481 amino acids and has significant propensity binding to Gram - negative bacteria [42]. The ectodomain TLR4 - MD - 2 heterodimers forms a functional receptor for LPS in macrophages. In cells like B cells RP105 with MD - 1 co receptors upon binding to LPS stimulates TLR4 activation and promotes inflammatory response against LPS [42]. In this heterodimer hydrophobic pocket of MD - 2 interacts with five acyl chains of hexacylated lipid A and the 6th one interacts with another TLR4. Lack of at least five acyl chains leads to weak interaction and finally weakened inflammatory response [14]. The sequential ectodomain events TLR4 downstream signalling includes Myddosome and Triffosome as adaptor seeded by TIRAP/MAL and TRAM respectively. It makes TLR signalling cascade unique among all TLRs. MyD88 is a member of IRAK1 family of serine threonine kinase that occupy core of the myddosome. In vitro study shows the interaction of MyD88 and IRAK1, IRAK2 represented by six to eight molecules of MyD88 interact with each IRAK1 and IRAK4. In vivo stoichiometrices remains unclear [43]. Oligomerization of MyD88 appears due to interaction between C - terminal TIR domain of MyD88 and TIR domains of TLRs and TIRAP/MAL. Further, MyD88 consist N - terminal death domain and TIR proteins in its core. The stochiometry of IRAK1 and IRAK2 in Myddosome differ in mice and human [44]. The next cascade is initiated by through driving autophosphorylation by activating latent kinase activity of IRAKs within Myddosome and subsequent recruitment of the E3 ubiquitin ligases TRAF6 [45], [46], [47]. Two transcriptional responses stimulated by TRAF6 are: activation of TAK1 which stimulates IKκ (IKK) mediated NF - κB and mitogen activated protein kinase (MAPK) mediated AP - 1 transcriptional response [48], [49]. An IKK – related kinase TBK1 and its substrate kinase AKT stimulates induction of glycolysis activation by phosporylation of hexokinase within minutes of TLR activation. TRAF6 activities such as myddosome activation, transcriptional response, glycolysis remains mysterious but E3 ubiquitin ligases pellino - 1 and - 2 function resembles TRAF6 function [50], [51]. In case of triffosome it functions as a SMOC in the TLR pathway [52] upon LPS detection by dimerized TLR4 on membrane of endosome. TRAF6 interacts with TRIF and TRAF3 dependent activation of the kinase TBK1 which induce IFN expression. It also induces glycolysis in similar way within myddosome. The highlighted point here is due to identification of a 39 amino acid Plxis Motif present within TRIF results in TBK1 mediated IFN response. This motif interacts with IFN inducing transcription factor IRF3 open phosphorylation by TBK1. Further triffosome dependant respond is restricted to

TLR4 where TBK1 - TRIF3 enzyme substrate complex leads to activation of IRF3 and expression of IFN.

TLR5

TLR5 Induces inflammation in epithelial cells by crosstalk with Notch1 signalling pathway [53]. It interacts with flagellin of the Gram Negative uropathogenic bacteria which activates TLR5 signalling cascade [42]. Initiation of TLR5 signalling activates the adaptors which results in consequence of production inflammatory cytokines [54]. TLR5 signalling proceeds further upon ligand binding adaptors such as MyD88, TRAF6, NF - κB [55] for the production of pro - inflammatory and Pro - labour cytokines and chemokines [56]. Inflammation is triggered by TLR5 and its deficiency ceases the production of long inflammatory cytokines. Upon ligand binding TLR5 increases the Urokinase Plasminogen Activator (uPA) expression in dental pulp cells via NF - κBand MAPK signalling pathways [57]. The lightened and receptor binding interior 4 have a pivotal role in initiating the signalling pathway. The domains of flagellin Core consists of three domains D1, D2, D3. Looking into the anatomy of the D1 domain comprises 3 Alpha helix and a beta hair pin that is predominantly conserve and has important role in assembling fliC subunits and their polymerisation into a helical filament. The ectodomain events of TLR5 system signalling cascade involves the interaction of D1 domain of flagellin from LRRNT to LRR9. The dimerization of TLR5 leads to stabilization of this homodimer structure upon ligand binding.

TLR 6

TLR6is embedded in cell membrane and functioning as heterodimer with TLR2 - TLR6 and TLR4 - TLR6 [58]. Structural anatomy reveals hydrophobic pockets consists two huge phenyalanine amino acid residues. Ligand and signalling cascade of TLR6 shares with its corresponding heteromonomers i. e. TLR2 and TLR4. In case of TLR4, TLR4 - TLR6 - CD16 ectodomain complex is formed which is the result of heterodimerization [59]. TLR2 - TLR6 heterodimer formation has some unique changes. Here, instead of triacylated lipopeptides (three lipid chains) TLR2 - TLR6 complex recognises diacylated lipopeptides activates TIRAP adaptor and induction NF - κB expression by MyD88 - mediated pathway results in the production of inflammatory cytokines [54].

TLR 7

TLR7comprises two ligand binding pockets which bind to bacterial and viral (GU rich) single stranded (ss) RNA. At high concentration of ligand TLR7 dimerises. The binding sites assist each other for the ligand binding and TLR7 homodimerization. A structural feature reveals that first binding site is promoted by second binding site [42]. The propensity of binding to Guanine of ssRNA to first binding site assists by second binding site. Taken together TLR7 activates the MyD88 - mediated signalling pathway leading to production of Type - I interferon (IFN) production by activation of IRF5 and IRF7 [54, 60]

TLR 8

TLR8 forms homodimers on binding to ligand appears as horseshoe like external ectodomain comprises the highest LRRs domains among identified TLR. TLR8 comprises two binding sites (LRR 11 - 14 and LRR 16 - 18). Specific ligand binds to TLR8 are bacterial and viral (GU rich) ssRNA, free uridines, mostly it recognises guanosine rich single - stranded RNA (ssRNA). Between LRR 14 and 15, TLR8 consist a z loop comprising ~40 amino acids. Z - Loop is structurally characterized by an Alpha helix which is stabilized by disulfide bonds. Thus, Z - loop plays an important role in identification of single stranded RNA. TLR8 induced the expression of IRF7 and IRF5 through MyD88 - dependent pathway leading to the production of Type - 1 Interferons and cytokines respectively [42].

TLR 9

TLR9 appears as horseshoe like domain comprises of 25 LRRs. Like TLR 7 and 8 it also consist a Z - loop present between the LRR 14 and 15. It detects pathogenic CpG DNA within endoysosomal structures. TLR9 mounts acquired and innate immune response by its signalling cascade. It has implicated in systemic lupus erythromatosus (SLE) pathogenesis. TLR9 role is spread across various cells such as pDC, CDC and B - cells. After ligand stimulation TLR9 signalling cascade results in production of cytokines such as TNF - alpha IL - 6 and 12 through induction of transcription factors. Nuclear factor kappa b and activated protein (AP1) the host immune responses such antimicrobial response, cytotoxicity and antigen presentation, etc are the key results of TLR9 stimulation [61].

TLR 10

TLR consists of leucine - rich repeats (LRRs), a transmembrane domain and a cytoplasmic Toll/interleukin - 1 receptor homology (TIR) domain**.** TLR10 considered as orphan receptor due to its unknown ligand. It appears as heterodimer TLR1 and TLR2 and homodimer TLR10. Studies show that homology of TLR10 with TLR1and TLR6 which is well established. The heterodimers TLR1/2 - TLR10 capable of binding to bacterial lipoproteins [62], [63] which results in recruitment of MyD88. But no activation of transcription detected in TLR10 mediated signalling. It is unique among TLR family. Interestingly, TLR10 is a negative regulator among the TLR family this was revealed in a study where TLR can act as suppressor, inhibitor and reducer of responses that is mostly mediated by the TLR 2/1. It also acts as antagonistic to TLR responses inhibiting the production of IL - 6 and if beta mediated TLRs pathway. Surprisingly TLR10 - mediated pathway of the cytokine production through process was report it also inhibits myddosome and triffosome mediated pathway [64].

Table 1: Toll like Receptors (TLRs) ligands and Adaptors

TLR	Ligands	Adaptors
TLR 1	Triacyl lipoproteins	BCAP, MAL (TIRAP), MyD88, SCIMP
TLR 2	Atypical lipopolysaccharide (LPS), glycoinositolphospholipids, glycolipids, lipoproteins, lipoteichoic acid, zymosans, mannan, peptidoglycan, sporozoite, Lipoarabinomannan, porins	BCAP, MAL (TIRAP), MyD88, SCIMP
TLR 3	Viral double - stranded (ds) RNA	SARM, SCIMP, TRIF (TICAM1)
TLR 4	Lipoploysaccaride, type 1, and P fimbriae	BCAP, MAL (TIRAP), MyD88, SARM, SCIMP, TRAM (TICAM2), TRIF (TICAM1)
TLR 5	Flagellin	MyD88, TRIF (TICAM1)
TLR 6	Diacyl lipoproteins, lipoteichoic acid, zymosans	BCAP, MAL (TIRAP), MyD88, SCIMP
TLR 7	Bacterial and viral (GUrich) single - stranded (ss) RNA	MyD88
TLR 8	Bacterial and viral (GUrich) single - stranded (ss) RNA, free uridines	MyD88
TLR 9	Viral and bacterial unmethylated cytosine phosphate guanine - dideoxy nucleotide (CpG) DNA, DNA: RNA hybrids	MAL (TIRAP), MyD88, SCIMP
TLR 10	Diacyl lipoprotein, triacyl lipoprotein, viral glycoprotein, doublestranded (ds) RNA	MyD88

Ubiquitination and Deubiquitination

Several mechanisms are known to regulate TLR signalling pathway. Post translational modifications (PTM) of proteins such as phosporylation, methylation, acetylation, Ubiquitination etc. modulate their function and in turn regulate diverse signaling cascade. Like other posttranslational modification of proteins, Ubiquitination has a key role in the regulation of expression and activity of different proteins. Ubiquitination occurs in a series of steps each mediated by different enzyme (E1 activating enzyme, E2 conjugating enzyme and E3 ligases).

1) Ubiquitin is activated in a two - step manner. First, ubiquitin - adenylate complex is formed and subsequently the ubiquitin group is transferred to the cysteine residue located in the active site of E1 - activating enzyme.
2) The ubiquitin group from E1 is transferred to E2 - conjugating enzyme by transesterification reaction.
3) Finally, E3 ligase catalyzes the formation of an isopeptide bond between the lysine residue of the target protein and the C - terminal glycine of ubiquitin.

The following are two very common examples of ubiquitination in TLR/IL1R signaling pathway.

1) TRAF6, an E3 ligase, promotes Lys - 63 (K - 63) linked ubiquitination of TAK1 leading to its auto - activation. Activated TAK1 phosphorylates IKK complex thereby activating downstream NF - κB [65].
2) The E3 ligaseTRAF7catalyzes Lys - 29 (K - 29) linked polyubiquitination of IκB Kinase (IKKγ) and p65/RelA protein, tagging them for degradation. This results in the repression of NF - κB pathway, Hence a negative regulator of NF - κB pathway. [66].

Deubiquitinationor removal of ubiquitin from the target proteins by a group of proteases known as deubiquitinating enzymes (DUBs) plays a crucial role in the regulation of several biological processes including the regulation of immune responses. For example,

1) **A20**, a zinc finger protein, negatively regulates NF - □ B signaling. A20 inhibit E3 ligase activity of TRAF6, TRAF2 by antagonizing interaction with E2 ubiquitin conjugating enzymes Ubc13 and Ubc5c, leading to ubiquitination - dependent degradation of TRAF6 and TRAF2 [67].
2) ***Deubiquitinating enzyme A*** (DUBA) is a negative regulator of innate immune responses. DUBA cleaves the lysine - 63 linked polyubiquitin chain of TRAF3 (E3 ubiquitin ligase) resulting its dissociation from downstream signaling leading to dampened production of IFN - b [68].
3) Tumor suppressor ***cylindromatosis*** (CYLD) is induced by TLR2/4signaling, deubiquitinatesE3 ligases, TRAF6 and TRAF7 and thereby negatively regulates TLR - induced immune responses [69].

Apart from the above few common Structure and functions of Deubiquitinase (DUB) family of enzymes we have discussed about the recent vast developments regarding the role of DUBs in inflammatory responses.

Role of Deubiquitinases in inflammation

NF - κB is the chief player in modulating the immune response through transcription of several genes that regulate its inflammation. Ubiquitin specific proteases regulate transcriptional activity of NF - κB after the translocation of NF - κB into the nucleus. USP7 activity negatively regulates the expression of target genes thus representing USP7 is critical for the target gene expression through NF - kB signalling [70]. Interaction between NAMO and HSCARG down regulates polyubiquitination of NAMO by deubiquitinase USP7. Thus, USP7 negatively regulate TNF - alpha stimulated NF - κB activity. Nlrp3 in inflammasomes activation is censorious component in the innate immune response that triggers caspase - 1 activation and production of proinflammatory cytokines IL - 1beta/ IL - 18 during microbial response and injury. Deubiquitinases USP7 negatively regulated NLRP3 inflammasome activation that is assessed by treatment with P22077 inhibitor of USP7. The critical role of USP7 is further demonstrated by inducible crisper/cas9 knockout for USP7 which confirms its role in NLRP3 inflammasomes activity [71]. USP7 also modulates TLR signalling by the aid of HSBC immediate early protein ICPO for its translocation from nucleus to cytoplasm. Here USP7 deubiquitinase TRAF6 and IKK gamma results in blocking of TLR mediated NF - □ B and JNK signalling cascade activation. Thus, USP7 is a negative regulator of TLR mediated inflammation response [72]. Lysophospatidic acid receptor 1 (LPA1) belongs to the G protein coupled receptor Superfamily which plays an

important role in regulating inflammation. Study shows upon knockdown or inhibition of USP11 down regulates the stability of LPA1 and resulting in attenuated LPA1 mediated inflammatory [73].

Among all ubiquitin specific proteases, the function of USP12 is unveil poorly. Although less reported it has been explored in regulating LPS - induced macrophage responses through catalytic activity of USP12, which inhibits phosphorylation of IKBα to enhance the LPS - induced macrophage response through phosphorylation of ERK1/2 and p38 results in production of proinflammatory cytokines.

Infectious disease (viral and bacterial) response from the body is tightly regulated. While in few cases the aberrant activation of inflammatory response to the pathogen which suppresses the control of immune system. Interferons (IFN) are the vital player in provoking inflammatory response that defence against microbial response. Further, it establishes and modulates transducer and activator of JNK signalling and STAT transcription respectively via inducing IFN - stimulated genes (ISGs). IFN I, II and III are the critical components of host defence system that fortify from viral infection and these are believed to modulate by deubiquitinase. STAT modulates IFN production it is positively regulated by USP13. This suggests that the expression level of STAT and USP13 is significantly associated [74].

In spite of several deubiquitinases USP14 plays an inhibitory role in proteosomal degradation remained to explore. USP14 has established its role in regulating NF - κB, similarly it also regulates I - kβ. USP14 interacts with phosphorylated ReIA and negatively regulate I - kβ level without altering IKK - α expression [75] in lung inflammation. Thus, USP14 degrades I - kβ which is regulated by overexpression of it and promote upon LPS induction resulting in production of through NF - κB signalling without altering phosphorylation of JNK 1/2 [75]. Upon detection of LPS TLR4 mediated signalling triggers NF - κB activation for producing inflammatory cytokines. USP15 deubiquitinating activity is down regulated by Hrd1 which results in overactivation of NF - κB and enhances the production of inflammatory cytokines [76].

Deubiquitinases collaborates with adaptors of signal transduction and do regulation in Cancer microenvironment. Tumor microenvironment specific leukocyte i. e. macrophages are critical for tumor growth and suppression of tumour [77]. These macrophages are called tumor associated macrophages. Receptor binding signal transduction and responses are the vital steps where the Receptor such as TLR, TNFR and IL - 1 induces immune response through activating NF - κB and resulting in expression of inflammatory genes in Cancer cells [78].

NF - κB is the critical transcription factor act as a molecular switch in regulating various cellular events such as inflammation immune response cell proliferation and cell death [79]. TAK1 - TAB2/TAB3 complex is an adaptor Complex that facilitates NF - κB activation and transcription of inflammation genes. USP19 has shown opposite Association with the TAK1 - TAB2/TAB3 complex to induce NF - κB. Here TNF - α and IL - 1β induce NF - κB activation but USP19 negatively regulate the both inducers. Further, hydrolysis of K27 and K63 linked polyubiquitination chains of TAK1 results in dissociation of TAK1 - TAB2/TAB3 complex along with inhibition of phosphorylation of TAK1 Complex and disruption of NF - □ B activation pathway. It has been shown that mice deficient with USP19 are more susceptible to septicaemia induced by overexpression of TNF - α and IL - 1β (31127032). Similarly, TLR3/4 mediated signalling cascade negatively regulated by USP19 where USB 19 hydrolyzes TRIF K27 - linked polyubiquitination thereby inhibiting TRIF recruitment TLR3/4 [80].

USP20 is reversely associated with TRAF6 (tumour necrosis factor receptor associated factor 6). Ubiquitination and deubiquitination are the two antagonistic events involved in modulating different pathway that results in inflammation. So, β - arrestin 2, a molecule that is promoted by tlr4 activation and it loses its activity by USP20 deubiquitination. Overall USP20 and TLR4 function antagonistically to each other to provoke inflammation [81]. The roles of USP20 in regulation of NF - κB activation is greatly appreciated and explored. To achieve NF - □ B inhibition USP20 interacts with a set of intermediate of TNFR1 (Tumour necrosis factor receptor 1) dependent NF - κB activation. USP20 majorly interacts with Cia1, Ubiquitin E3 ligase TRAF2, two components of LUBAC, HOIL - 1interacting protein (HOIP), RIPK1, IKBα and β - arrestin 2. Of these, results suggest USP20 act on RIPK1in downstream of TNFR1 activation in smooth muscle cells. Taking together USP20 impairs TNF and IL - 1R evoked inflammatory response by inhibiting NF - κB activation [82].

An unexplored deubiquitinase is USP22 involved in a wide range of cellular processes. One among is inflammation where it has been greatly recognised for its manifested role. Inflammation in podocytes is triggered by high level of D - glucose which further induces apoptosis. Knockdown results revealed that USP22 negatively regulate high D - glucose induce apoptosis in podocytes [83]. This involvement of ubiquitin specific proteases is in a myriad of cellular events such as virus induced inflammation through Type - I IFN signalling. Interaction with TRAF2, TRAF2 and RIG - 1 with USP25 results in deubiquitination of these intermediates. USP25 enzyme activity relies on its cysteine (Cys - 178) and histidine (His - 607) residue are the catalytic domains thereby deubiquitinating Lysine - 48 and Lysine - 68 linked polyubiquitin in vitro. Taken together USP25 is a negative regulator in virus induced Type - I IFN signalling. Further, USP25 is also a negative regulator by hydrolysing TRAF5 and TRAF6 linked lysine - 63 polyubiquitin chain with the aid of adaptor act1 of IL - 1 evoked signalling and inflammation [84].

A crucial setup event occurs in a well tightly maintained manner to avoid any aberrant event which can be harmful to the host in developing chronic inflammation. One such is Type - I IFN signalling pathway where USP38 is a negative regulator of it. Insights into molecular mechanism reveal that USP38 deubiquitinates K - 33 linked polyubiquitin of TBK1 but adds K - 48 linked ubiquitin chains to TBK1 after

viral infection. This editing machinery of USP38 is mediated by DTX4 and TRIP. In the absence of USP38 the events are just reverse of in the presence. Taken together the critically USP38 maintain its dynamic nature for ubiquitin editing of TBK1 triggered by viral infection [85].

TLR2 and TLR4 are well known Toll like Receptors among 10 human TLRs and both has been studied extensively. TLR2 activates upon binding of lipoproteins of gram negative and gram - positive bacteria. Its activation is critical for induction of various proximal and distal signalling transducers such as TRAF6 and TRAF7, etc. These are crucial for the activation of transcription and thereby enhancing the inflammatory cytokine level. Tumor suppressor Cylindromatosis (CYLD) a deubiquitinases directly regulates various signalling cascade, such as MAPK, NF - Kb. The function of CYLD in various maladies is mediated by its catalytic domain which deubiquitinates the polyubiquitin chain of intermediate adaptors [86]. This negative regulation of CYLD is extensively implicated in regulation of TLR2 dependent activation of both NF - κB and p38 pathway. It is reported that CYLD is a negative regulator of NF - κB activation which is TLR2 mediated but the goal is accomplished by deubiquitinating TRAF6 and TRAF7. Thus, TLR2 regulation is vital for production of inflammatory cytokines cooperative interaction with TRAF6 and TRAF7 which further regulated by CYLD [87]. CYLD regulates TGF - b by acting on the polyubiquitin chain of Akt, which is a signal transducer. Further, CYLD decreases samd3 stability via Akt - Gsk3B - CHIP pathway. Samd3 instability is achieved by deubiquitinating K63 - polyubiquitin of Akt by CYLD. Taken together CYLD is a negative regulator of TGF - B by inhibiting Akt.

A20 is a deubiquitinating enzyme that is shown in various inflammatory scenarios such as it prevents LPS - induced shock in mice. It deubiquitinates K - 63 polyubiquitin molecule of TRAF6 which implies the regulating link between deubiquitinating activity and the regulation of TLR signals. CYLD, A20 and OTULIN regulate mechanism overlaps to each other and these three deubiquitinasesare the last resort is to regulate NF - κB signalling cascade [88]. In brief, A20 protects mice from endotoxic by terminating TLR - mediated activity of NF - κB transcription factor thereby halting the expression of pro - inflammatory cytokines in macrophage. It removes ubiquitin from TRAF6, which is a proximal signalling transducer in TLR mediated signalling cascade. Further, A20 negatively regulates polyubiquitination of TRAF6 and TRAF7 along with the interaction of TAX1BP1. This interaction also disintegrates the interaction of E2 and E3 enzyme in the TNFR and TLR4/IL - 1R pathways [67].

3. Discussion

The array of pathways that control various cellular activities, including immunological responses, is widened by post translational modifications (PTMs). The most extensively researched PTMs in the control of immunological responses are phosphorylation processes regulated by kinases and phosphatases. The study of additional PTMs, such as ubiquitination and its reverse mechanism, deubiquitination, in the regulation of inflammatory responses has recently attracted a lot of attention. The innate immune system is heavily associated with a number of deubiquitinases that function to reverse the ubiquitination process by cleaving monoubiquitin groups or ubiquitin chains. Based on mechanism of catalysis deubiquitinating enzymes are divided into several sub - families such as USP (Ubiquitin Specific Peptidase), UCH, OTU, MJD, MINDY, ZUFSP and JAMM. [5, 9, 89]. All the sub - family members play a crucial role in various cellular processes like cell cycle regulation, DNA repair, Kinase activation, Protein degradation, transcription factor activation etc. However, being the largest group among all, the USP (Ubiquitin Specific Peptidase) sub - family consists of few important players what we have discussed in this study. USP 13, whose expression modulates the antiviral activity of IFN - α against dengue virus serotype 2 (DEN - 2). Thus, USP13 positively regulates type I and type II IFN signalling by deubiquitinating and stabilizing STAT1 protein. Moreover, USP13 is the first DUB identified to modulate STAT1 and play a role in the antiviral activity of IFN against DEN - 2 replication [74]. I - κB polyubiquitination was reduced in USP14 overexpressed condition, suggesting that USP14 regulates I - κB degradation by removing its ubiquitin chain, thus promoting the deubiquitinated I - κB degradation within the proteasome. Further it was found that USP14 was associated with RelA, a binding partner of I - κB, suggesting that RelA is the linker between USP14 and I - κB [75]. USP15 is also reported to play crucial role in regulating bacterial endotoxin stimulated TLR4 signalling cascade [76]. Similarly, USP12, USP20, USP22, USP25, USP38 regulates ubiquitin mediated inflammatory response via various TLR pathways as well as other mechanism independent of TLR pathways.

4. Conclusion

Our review of literature highlights the critical role of Deubiquitinases (DUBs) in controlling the inflammatory response via Toll Like Receptor (TLR) signaling. The work demonstrates DUBs' potential as prospective therapeutic targets in a variety of inflammatory disorders. However, further research is required to completely comprehend the complicated mechanics of DUBs and to create effective therapy techniques that target these enzymes.

References

[1] Medzhitov R: **Origin and physiological roles of inflammation**. *Nature* 2008, **454** (7203): 428 - 435.
[2] Kulathu Y, Komander D: **Atypical ubiquitylation - the unexplored world of polyubiquitin beyond Lys48 and Lys63 linkages**. *Nat Rev Mol Cell Biol* 2012, **13** (8): 508 - 523.
[3] Yau R, Rape M: **The increasing complexity of the ubiquitin code**. *Nat Cell Biol* 2016, **18** (6): 579 - 586.
[4] Faesen AC, Luna - Vargas MP, Geurink PP, Clerici M, Merkx R, van Dijk WJ, Hameed DS, El Oualid F, Ovaa H, Sixma TK: **The differential modulation of USP activity by internal regulatory domains, interactors and eight ubiquitin chain types**. *Chem Biol* 2011, **18** (12): 1550 - 1561.
[5] Kwasna D, Abdul Rehman SA, Natarajan J, Matthews S, Madden R, De Cesare V, Weidlich S, Virdee S,

Ahel I, Gibbs - Seymour I *et al*: **Discovery and Characterization of ZUFSP/ZUP1, a Distinct Deubiquitinase Class Important for Genome Stability**. *Mol Cell* 2018, **70** (1): 150 - 164. e156.

[6] Mevissen TE, Hospenthal MK, Geurink PP, Elliott PR, Akutsu M, Arnaudo N, Ekkebus R, Kulathu Y, Wauer T, El Oualid F *et al*: **OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis**. *Cell* 2013, **154** (1): 169 - 184.

[7] Clague MJ, Barsukov I, Coulson JM, Liu H, Rigden DJ, Urbé S: **Deubiquitylases from genes to organism**. *Physiol Rev* 2013, **93** (3): 1289 - 1315.

[8] Ronau JA, Beckmann JF, Hochstrasser M: **Substrate specificity of the ubiquitin and Ubl proteases**. *Cell Res* 2016, **26** (4): 441 - 456.

[9] Abdul Rehman SA, Kristariyanto YA, Choi SY, Nkosi PJ, Weidlich S, Labib K, Hofmann K, Kulathu Y: **MINDY - 1 Is a Member of an Evolutionarily Conserved and Structurally Distinct New Family of Deubiquitinating Enzymes**. *Mol Cell* 2016, **63** (1): 146 - 155.

[10] Reyes - Turcu FE, Ventii KH, Wilkinson KD: **Regulation and cellular roles of ubiquitin - specific deubiquitinating enzymes**. *Annu Rev Biochem* 2009, **78**: 363 - 397.

[11] Leestemaker Y, Ovaa H: **Tools to investigate the ubiquitin proteasome system**. *Drug Discov Today Technol* 2017, **26**: 25 - 31.

[12] Gay NJ, Keith FJ: **Drosophila Toll and IL - 1 receptor**. *Nature* 1991, **351** (6325): 355 - 356.

[13] Takeda K: [**Toll - like receptor]**. *Nihon Rinsho Meneki Gakkai Kaishi* 2005, **28** (5): 309 - 317.

[14] Park BS, Song DH, Kim HM, Choi BS, Lee H, Lee JO: **The structural basis of lipopolysaccharide recognition by the TLR4 - MD - 2 complex**. *Nature* 2009, **458** (7242): 1191 - 1195.

[15] Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C *et al*: **Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene**. *Science* 1998, **282** (5396): 2085 - 2088.

[16] Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL: **Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression**. *J Immunol* 2001, **167** (4): 1882 - 1885.

[17] Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A: **The innate immune response to bacterial flagellin is mediated by Toll - like receptor 5**. *Nature* 2001, **410** (6832): 1099 - 1103.

[18] Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik SG, Lee H, Lee JO: **Crystal structure of the TLR1 - TLR2 heterodimer induced by binding of a tri - acylated lipopeptide**. *Cell* 2007, **130** (6): 1071 - 1082.

[19] Collins B, Wilson IA: **Crystal structure of the C - terminal domain of mouse TLR9**. *Proteins* 2014, **82** (10): 2874 - 2878.

[20] Hidmark A, von Saint Paul A, Dalpke AH: **Cutting edge: TLR13 is a receptor for bacterial RNA**. *J Immunol* 2012, **189** (6): 2717 - 2721.

[21] Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C: **Innate antiviral responses by means of TLR7 - mediated recognition of single - stranded RNA**. *Science* 2004, **303** (5663): 1529 - 1531.

[22] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA: **Recognition of double - stranded RNA and activation of NF - kappaB by Toll - like receptor 3**. *Nature* 2001, **413** (6857): 732 - 738.

[23] Yarovinsky F, Zhang D, Andersen JF, Bannenberg GL, Serhan CN, Hayden MS, Hieny S, Sutterwala FS, Flavell RA, Ghosh S *et al*: **TLR11 activation of dendritic cells by a protozoan profilin - like protein**. *Science* 2005, **308** (5728): 1626 - 1629.

[24] Narayanan KB, Park HH: **Toll/interleukin - 1 receptor (TIR) domain - mediated cellular signaling pathways**. *Apoptosis* 2015, **20** (2): 196 - 209.

[25] Gong J, Wei T: **Structure modeling of Toll - like receptors**. *Methods Mol Biol* 2014, **1169**: 45 - 53.

[26] Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF: **A family of human receptors structurally related to Drosophila Toll**. *Proc Natl Acad Sci U S A* 1998, **95** (2): 588 - 593.

[27] Nakajima T, Ohtani H, Satta Y, Uno Y, Akari H, Ishida T, Kimura A: **Natural selection in the TLR - related genes in the course of primate evolution**. *Immunogenetics* 2008, **60** (12): 727 - 735.

[28] Hantke K, Braun V: **Covalent binding of lipid to protein. Diglyceride and amide - linked fatty acid at the N - terminal end of the murein - lipoprotein of the Escherichia coli outer membrane**. *Eur J Biochem* 1973, **34** (2): 284 - 296.

[29] Mühlradt PF, Kiess M, Meyer H, Süssmuth R, Jung G: **Isolation, structure elucidation, and synthesis of a macrophage stimulatory lipopeptide from Mycoplasma fermentans acting at picomolar concentration**. *J Exp Med* 1997, **185** (11): 1951 - 1958.

[30] Kang JY, Nan X, Jin MS, Youn SJ, Ryu YH, Mah S, Han SH, Lee H, Paik SG, Lee JO: **Recognition of lipopeptide patterns by Toll - like receptor 2 - Toll - like receptor 6 heterodimer**. *Immunity* 2009, **31** (6): 873 - 884.

[31] Zähringer U, Lindner B, Inamura S, Heine H, Alexander C: **TLR2 - promiscuous or specific? A critical re - evaluation of a receptor expressing apparent broad specificity**. *Immunobiology* 2008, **213** (3 - 4): 205 - 224.

[32] Kim HM, Park BS, Kim JI, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ *et al*: **Crystal structure of the TLR4 - MD - 2 complex with bound endotoxin antagonist Eritoran**. *Cell* 2007, **130** (5): 906 - 917.

[33] Behzadi E, Behzadi P: **The role of toll - like receptors (TLRs) in urinary tract infections (UTIs).** *Cent European J Urol* 2016, **69** (4): 404 - 410.

[34] Behzadi P, Behzadi E, Pawlak - Adamska EA: **Urinary tract infections (UTIs) or genital tract infections (GTIs) ? It's the diagnostics that count**. *GMS Hyg Infect Control* 2019, **14**: Doc14.

[35] Matsushima N, Miyashita H, Mikami T, Kuroki Y: **A nested leucine rich repeat (LRR) domain: the precursor of LRRs is a ten or eleven residue motif**. *BMC Microbiol* 2010, **10**: 235.

[36] Qiu Y, Ding Y, Zou L, Tan Z, Liu T, Fu X, Xu W: **Divergent roles of amino acid residues inside and outside the BB loop affect human Toll - like receptor (TLR) 2/2, TLR2/1 and TLR2/6 responsiveness**. *PLoS One* 2013, **8** (4): e61508.

[37] Behzadi P, Behzadi E, Ranjbar R: **IL - 12 Family Cytokines: General Characteristics, Pathogenic Microorganisms, Receptors, and Signalling Pathways**. *Acta Microbiol Immunol Hung* 2016, **63** (1): 1 - 25.

[38] Liu L, Botos I, Wang Y, Leonard JN, Shiloach J, Segal DM, Davies DR: **Structural basis of toll - like receptor 3 signaling with double - stranded RNA**. *Science* 2008, **320** (5874): 379 - 381.

[39] Szatmary Z: **Molecular biology of toll - like receptors**. *Gen Physiol Biophys* 2012, **31** (4): 357 - 366.

[40] Gioannini TL, Teghanemt A, Zhang D, Coussens NP, Dockstader W, Ramaswamy S, Weiss JP: **Isolation of an endotoxin - MD - 2 complex that produces Toll - like receptor 4 - dependent cell activation at picomolar concentrations**. *Proc Natl Acad Sci U S A* 2004, **101** (12): 4186 - 4191.

[41] Fitzgerald KA, Kagan JC: **Toll - like Receptors and the Control of Immunity**. *Cell* 2020, **180** (6): 1044 - 1066.

[42] Behzadi P, García - Perdomo HA, Karpiński TM: **Toll - Like Receptors: General Molecular and Structural Biology**. *J Immunol Res* 2021, **2021**: 9914854.

[43] Bonham KS, Orzalli MH, Hayashi K, Wolf AI, Glanemann C, Weninger W, Iwasaki A, Knipe DM, Kagan JC: **A promiscuous lipid - binding protein diversifies the subcellular sites of toll - like receptor signal transduction**. *Cell* 2014, **156** (4): 705 - 716.

[44] Sun J, Li N, Oh KS, Dutta B, Vayttaden SJ, Lin B, Ebert TS, De Nardo D, Davis J, Bagirzadeh R *et al*: **Comprehensive RNAi - based screening of human and mouse TLR pathways identifies species - specific preferences in signaling protein use**. *Sci Signal* 2016, **9** (409): ra3.

[45] Cao Z, Xiong J, Takeuchi M, Kurama T, Goeddel DV: **TRAF6 is a signal transducer for interleukin - 1**. *Nature* 1996, **383** (6599): 443 - 446.

[46] Ferrao R, Zhou H, Shan Y, Liu Q, Li Q, Shaw DE, Li X, Wu H: **IRAK4 dimerization and trans - autophosphorylation are induced by Myddosome assembly**. *Mol Cell* 2014, **55** (6): 891 - 903.

[47] Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S *et al*: **TRAF6 deficiency results in osteopetrosis and defective interleukin - 1, CD40, and LPS signaling**. *Genes Dev* 1999, **13** (8): 1015 - 1024.

[48] Emmerich CH, Ordureau A, Strickson S, Arthur JS, Pedrioli PG, Komander D, Cohen P: **Activation of the canonical IKK complex by K63/M1 - linked hybrid ubiquitin chains**. *Proc Natl Acad Sci U S A* 2013, **110** (38): 15247 - 15252.

[49] Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ: **TAK1 is a ubiquitin - dependent kinase of MKK and IKK**. *Nature* 2001, **412** (6844): 346 - 351.

[50] Moynagh PN: **The Pellino family: IRAK E3 ligases with emerging roles in innate immune signalling**. *Trends Immunol* 2009, **30** (1): 33 - 42.

[51] Schauvliege R, Janssens S, Beyaert R: **Pellino proteins: novel players in TLR and IL - 1R signalling**. *J Cell Mol Med* 2007, **11** (3): 453 - 461.

[52] Akira S, Hoshino K: **Myeloid differentiation factor 88 - dependent and - independent pathways in toll - like receptor signaling**. *J Infect Dis* 2003, **187 Suppl 2**: S356 - 363.

[53] Aziz M, Ishihara S, Ansary MU, Sonoyama H, Tada Y, Oka A, Kusunoki R, Tamagawa Y, Fukuba N, Mishima Y *et al*: **Crosstalk between TLR5 and Notch1 signaling in epithelial cells during intestinal inflammation**. *Int J Mol Med* 2013, **32** (5): 1051 - 1062.

[54] Kawai T, Akira S: **The role of pattern - recognition receptors in innate immunity: update on Toll - like receptors**. *Nat Immunol* 2010, **11** (5): 373 - 384.

[55] Lim R, Barker G, Lappas M: **The TLR2 ligand FSL - 1 and the TLR5 ligand Flagellin mediate pro - inflammatory and pro - labour response via MyD88/TRAF6/NF - κB - dependent signalling**. *Am J Reprod Immunol* 2014, **71** (5): 401 - 417.

[56] Lim R, Barker G, Lappas M: **TLR2, TLR3 and TLR5 regulation of pro - inflammatory and pro - labour mediators in human primary myometrial cells**. *J Reprod Immunol* 2017, **122**: 28 - 36.

[57] Hwang HS, Kim JW, Oh SH, Song JH, Yang JW, Zang Y, Kim YH, Lee SE, Hwang YC, Koh JT: **TLR5 activation induces expression of the pro - inflammatory mediator Urokinase Plasminogen Activator via NF - κB and MAPK signalling pathways in human dental pulp cells**. *Int Endod J* 2019, **52** (10): 1479 - 1488.

[58] Dannemann M, Andrés AM, Kelso J: **Introgression of Neandertal - and Denisovan - like Haplotypes Contributes to Adaptive Variation in Human Toll - like Receptors**. *Am J Hum Genet* 2016, **98** (1): 22 - 33.

[59] Iwami KI, Matsuguchi T, Masuda A, Kikuchi T, Musikacharoen T, Yoshikai Y: **Cutting edge: naturally occurring soluble form of mouse Toll - like receptor 4 inhibits lipopolysaccharide signaling**. *J Immunol* 2000, **165** (12): 6682 - 6686.

[60] Arleevskaya MI, Larionova RV, Brooks WH, Bettacchioli E, Renaudineau Y: **Toll - Like Receptors, Infections, and Rheumatoid Arthritis**. *Clin Rev Allergy Immunol* 2020, **58** (2): 172 - 181.

[61] Kumagai Y, Takeuchi O, Akira S: **TLR9 as a key receptor for the recognition of DNA**. *Adv Drug Deliv Rev* 2008, **60** (7): 795 - 804.

[62] Mikami T, Miyashita H, Takatsuka S, Kuroki Y, Matsushima N: **Molecular evolution of vertebrate Toll - like receptors: evolutionary rate difference between their leucine - rich repeats and their TIR domains**. *Gene* 2012, **503** (2): 235 - 243.

[63] Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A: **The evolution of vertebrate Toll - like receptors**. *Proc Natl Acad Sci U S A* 2005, **102** (27): 9577 - 9582.

[64] Jiang S, Li X, Hess NJ, Guan Y, Tapping RI: **TLR10 Is a Negative Regulator of Both MyD88 -**

Dependent and - Independent TLR Signaling. *J Immunol* 2016, **196** (9): 3834 - 3841.

[65] Adhikari A, Xu M, Chen ZJ: **Ubiquitin - mediated activation of TAK1 and IKK**. *Oncogene* 2007, **26** (22): 3214 - 3226.

[66] Wullaert A, Heyninck K, Janssens S, Beyaert R: **Ubiquitin: tool and target for intracellular NF - kappaB inhibitors**. *Trends Immunol* 2006, **27** (11): 533 - 540.

[67] Shembade N, Ma A, Harhaj EW: **Inhibition of NF - kappaB signaling by A20 through disruption of ubiquitin enzyme complexes**. *Science* 2010, **327** (5969): 1135 - 1139.

[68] Kayagaki N, Phung Q, Chan S, Chaudhari R, Quan C, O'Rourke KM, Eby M, Pietras E, Cheng G, Bazan JF *et al*: **DUBA: a deubiquitinase that regulates type I interferon production**. *Science* 2007, **318** (5856): 1628 - 1632.

[69] Yoshida H, Jono H, Kai H, Li JD: **The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll - like receptor 2 signaling via negative cross - talk with TRAF6 AND TRAF7**. *The Journal of biological chemistry* 2005, **280** (49): 41111 - 41121.

[70] Colleran A, Collins PE, O'Carroll C, Ahmed A, Mao X, McManus B, Kiely PA, Burstein E, Carmody RJ: **Deubiquitination of NF - κB by Ubiquitin - Specific Protease - 7 promotes transcription**. *Proc Natl Acad Sci U S A* 2013, **110** (2): 618 - 623.

[71] Palazón - Riquelme P, Worboys JD, Green J, Valera A, Martín - Sánchez F, Pellegrini C, Brough D, López - Castejón G: **USP7 and USP47 deubiquitinases regulate NLRP3 inflammasome activation**. *EMBO Rep* 2018, **19** (10).

[72] Daubeuf S, Singh D, Tan Y, Liu H, Federoff HJ, Bowers WJ, Tolba K: **HSV ICP0 recruits USP7 to modulate TLR - mediated innate response**. *Blood* 2009, **113** (14): 3264 - 3275.

[73] Zhao J, Wei J, Dong S, Bowser RK, Zhang L, Jacko AM, Zhao Y: **Destabilization of Lysophosphatidic Acid Receptor 1 Reduces Cytokine Release and Protects Against Lung Injury**. *EBioMedicine* 2016, **10**: 195 - 203.

[74] Yeh HM, Yu CY, Yang HC, Ko SH, Liao CL, Lin YL: **Ubiquitin - specific protease 13 regulates IFN signaling by stabilizing STAT1**. *J Immunol* 2013, **191** (6): 3328 - 3336.

[75] Mialki RK, Zhao J, Wei J, Mallampalli DF, Zhao Y: **Overexpression of USP14 protease reduces I - κB protein levels and increases cytokine release in lung epithelial cells**. *J Biol Chem* 2013, **288** (22): 15437 - 15441.

[76] Lu Y, Qiu Y, Chen P, Chang H, Guo L, Zhang F, Ma L, Zhang C, Zheng X, Xiao J *et al*: **ER - localized Hrd1 ubiquitinates and inactivates Usp15 to promote TLR4 - induced inflammation during bacterial infection**. *Nat Microbiol* 2019, **4** (12): 2331 - 2346.

[77] Sica A, Allavena P, Mantovani A: **Cancer related inflammation: the macrophage connection**. *Cancer Lett* 2008, **267** (2): 204 - 215.

[78] Hoesel B, Schmid JA: **The complexity of NF - κB signaling in inflammation and cancer**. *Mol Cancer* 2013, **12**: 86.

[79] Vallabhapurapu S, Karin M: **Regulation and function of NF - kappaB transcription factors in the immune system**. *Annu Rev Immunol* 2009, **27**: 693 - 733.

[80] Wu X, Lei C, Xia T, Zhong X, Yang Q, Shu HB: **Regulation of TRIF - mediated innate immune response by K27 - linked polyubiquitination and deubiquitination**. *Nat Commun* 2019, **10** (1): 4115.

[81] Jean - Charles PY, Zhang L, Wu JH, Han SO, Brian L, Freedman NJ, Shenoy SK: **Ubiquitin - specific Protease 20 Regulates the Reciprocal Functions of β - Arrestin2 in Toll - like Receptor 4 - promoted Nuclear Factor κB (NFκB) Activation**. *J Biol Chem* 2016, **291** (14): 7450 - 7464.

[82] Jean - Charles PY, Wu JH, Zhang L, Kaur S, Nepliouev I, Stiber JA, Brian L, Qi R, Wertman V, Shenoy SK *et al*: **USP20 (Ubiquitin - Specific Protease 20) Inhibits TNF (Tumor Necrosis Factor) - Triggered Smooth Muscle Cell Inflammation and Attenuates Atherosclerosis**. *Arterioscler Thromb Vasc Biol* 2018, **38** (10): 2295 - 2305.

[83] Shi JX, Wang QJ, Li H, Huang Q: **Silencing of USP22 suppresses high glucose - induced apoptosis, ROS production and inflammation in podocytes**. *Mol Biosyst* 2016, **12** (5): 1445 - 1456.

[84] Zhong B, Liu X, Wang X, Chang SH, Liu X, Wang A, Reynolds JM, Dong C: **Negative regulation of IL - 17 - mediated signaling and inflammation by the ubiquitin - specific protease USP25**. *Nat Immunol* 2012, **13** (11): 1110 - 1117.

[85] Lin M, Zhao Z, Yang Z, Meng Q, Tan P, Xie W, Qin Y, Wang RF, Cui J: **USP38 Inhibits Type I Interferon Signaling by Editing TBK1 Ubiquitination through NLRP4 Signalosome**. *Mol Cell* 2016, **64** (2): 267 - 281.

[86] Mathis BJ, Lai Y, Qu C, Janicki JS, Cui T: **CYLD - mediated signaling and diseases**. *Curr Drug Targets* 2015, **16** (4): 284 - 294.

[87] Yoshida H, Jono H, Kai H, Li JD: **The tumor suppressor cylindromatosis (CYLD) acts as a negative regulator for toll - like receptor 2 signaling via negative cross - talk with TRAF6 AND TRAF7**. *J Biol Chem* 2005, **280** (49): 41111 - 41121.

[88] Lork M, Verhelst K, Beyaert R: **CYLD, A20 and OTULIN deubiquitinases in NF - κB signaling and cell death: so similar, yet so different**. *Cell Death Differ* 2017, **24** (7): 1172 - 1183.

[89] Amerik AY, Hochstrasser M: **Mechanism and function of deubiquitinating enzymes**. *Biochim Biophys Acta* 2004, **1695** (1 - 3): 189 - 207.