



Universiteit  
Leiden  
The Netherlands

## The Function of Toll-like receptor 2 in Infection and Inflammation

Hu, W.

### Citation

Hu, W. (2021, December 16). *The Function of Toll-like receptor 2 in Infection and Inflammation*. Retrieved from <https://hdl.handle.net/1887/3247321>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3247321>

**Note:** To cite this publication please use the final published version (if applicable).

## **Chapter 1**

### **General introduction:**

#### **The role of TLR2: from cell biology to therapeutic target**



## 1. Innate immunity and Toll-like receptors

### 1.1 Innate immunity

The host cells, rely on membrane-localized and germline-encoded pattern recognition receptors (PRRs) to initiate protective innate immune responses [2, 3]. PRRs recognize invading microbial pathogens through pathogen-associated molecular patterns (PAMPs) of the pathogens in combination with recognition of danger-associated molecular patterns (DAMPs) produced by infected or damaged tissues [2, 3, 15]. PRRs are comprised of four well-characterized groups, including Toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG)-I-like receptors (RLRs), the nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and C-type lectin receptors (CLRs) [16]. TLRs and CLRs belong to transmembrane proteins, while RLRs and NLRs are cytoplasmic proteins [16]. TLRs are the most important and widely studied PRRs (See Fig 1).

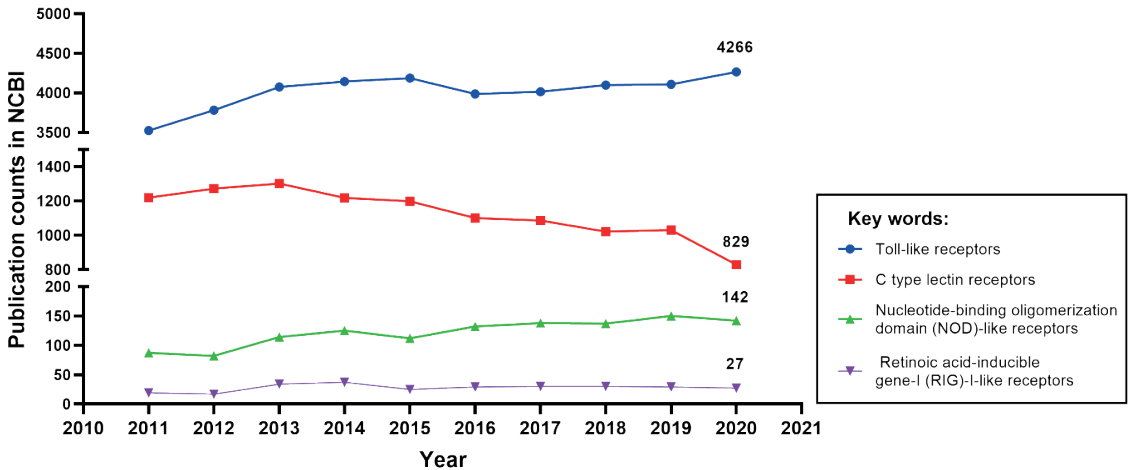


Figure 1 Publication counts for four PRRs in the NCBI data base.

### 1.2 The structure of TLRs

The function of TLRs has been studied extensively in the last decades (Fig 1). Their capacity as a key control factor of innate immune responses makes them attractive therapeutic targets. TLRs are homologs of the *toll* gene that was first discovered to be involved in embryonic development in *Drosophila* [5, 6]. The investigation of TLRs became very intense after their function in defense against microbial infection in *Drosophila* and vertebrates was demonstrated [17]. TLRs are made up of an ectodomain, also known as the periplasmic extracellular domain,

1 a cytoplasmic signaling domain, and a single transmembrane domain [3, 18]. The ectodomain of TLRs contains leucine-rich repeats (LRRs) and selectively recognizes PAMPs and DAMPs [3]. We summary the different PAMPs and DAMPs are recognized by the specific TLRs in Fig 2. The cytoplasmic signaling domain of TLRs comprises an evolutionary conserved Toll/IL-1 receptor (TIR) homology domain that is responsible for signal transduction [3]. Different species have different numbers of genes that encode TLRs. In the human genome, 10 TLRs are encoded, whereas the mouse and zebrafish genomes encode at least 12 and 17 TLRs, respectively [19-21]. TLRs can be divided into two subgroups based on their cellular location. TLRs are expressed either on the cell surface or in intracellular compartments. In humans, TLR1, 2, 4, 5, 6 and 10 are expressed on the cell surface, while TLR3, 7, 8 and 9 are localized in intracellular membranes [3, 22]. In mice, the cellular distribution of the conserved TLRs is assumed to be the same as the distribution in humans, while TLR12 is expressed on the cell surface and TLR13 is probably expressed within endosomes [22]. Interestingly, TLR11 can be expressed on both cell surface and intracellular compartments [23].

### 1.3 TLR2, an important member of the TLR family

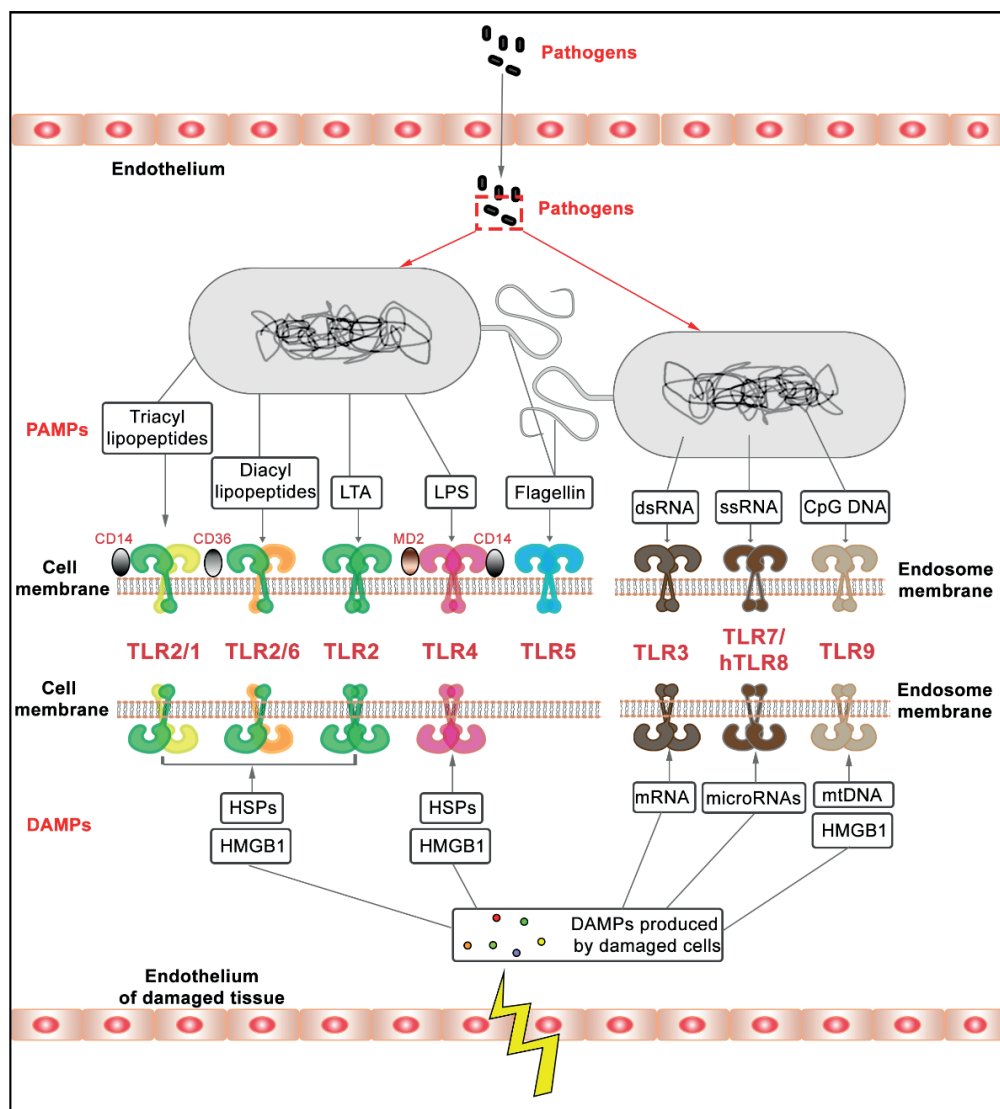
After the identification of TLR2 in 1998, much progress has been made in our understanding of its function [9, 24]. Its functions, in the recognition of a large number of ligands, including PAMPs and DAMPs, are complicating the studies of the underlying recognition mechanisms (Fig 2). In addition, the ubiquitous distribution of TLR2 on various types of cells, e.g., immune, endothelial, and epithelial cells, also determines its wide range of functions [9]. Considering the broad functions of TLR2, the drive for the development of TLR2 related therapeutic targeted vaccine or treatment has accelerated in the last decades [7, 25, 26]. However, some studies on TLR2 are still controversial [7]. It is because of the complex functions of TLR2 that its role in immune regulation is not black or white [8]. For example, TLR2 plays a dual role in infection processes. TLR2 has been shown to play a protective role during infection by triggering a strong pro-inflammatory response which is considered beneficial for bacterial clearance [27, 28]. However, excessive inflammation caused by TLR2 can lead to tissue damage and even affect healing of damaged tissues [11]. The mechanisms of TLR2 signaling and its regulation are discussed in detail below.

## 2. Regulation of TLR2 signaling

### 2.1 TLR2 signaling pathway

The binding of the LRR domain in TLRs and its ligands stimulates the recruitment of adaptor proteins to interact with the intracellular TIR domain in TLRs to trigger the downstream cascades. Myeloid differentiation factor (Myd88) is a well-known adaptor protein that interacts with almost all TLRs except TLR3 [3]. TIR domain-containing adaptor protein (Tirap), which is also called Myd88 adaptor-like (Mal), is required in the TLR2/6 signaling via Myd88 while it is not necessary in the TLR2/1 signaling [29]. In addition to Myd88 and Tirap, other adaptor proteins in mammalian cells include TIR domain-containing adaptor protein inducing interferon- $\beta$  (TRIF), TIR-containing adaptor molecule (TICAM), TRIF-related adaptor molecule (TRAM), and sterile  $\alpha$ - and armadillo motif-containing protein (SARM) [30]. The recruitment of distinct adaptor proteins can trigger different downstream signaling pathways. Recent reviews have discussed in detail the known differences between downstream signaling pathways of the mammalian TLR receptors [3, 31, 32] and therefore we only briefly describe TLR2 signaling here and summarize it in Fig 3.

After the interaction of TLR2 and its associated adaptor proteins, the IRAK complex is activated to recruit TRAF6. Activated TRAF6 triggers the activity of a complex of TAK1/TABs to stimulate both the activation of the MAPKs and the IKK complex (IKK1, 2, and IKK- $\gamma$ , also named NEMO). The MAPKs family includes, but is not limited to JNKs and p38. The IKK complex promotes the nuclear translocation of NF- $\kappa$ B. In the end, the production of pro-inflammatory cytokines is induced by the AP-1 and NF- $\kappa$ B, which in turn controls inflammation and modulates cell survival and proliferation [9, 33].



**Figure 2 TLRs and its ligands.** TLRs can recognize PAMPs from invading microbial pathogens and DAMPs from infected or damaged tissue. TLR2, its heterodimers and TLR4 recognize pathogens through their cell wall surface components. TLR2 conjugates with TLR1 or with TLR6 to sense triacyl or diacyl lipopeptides, and lipoteichoic acid (LTA) on the cell wall of gram-positive bacteria, and mycobacteria [9, 34-36]. The process of the recognition of triacyl or diacyl lipopeptides by heterodimers needs the participation of accessory molecules. For example, CD14 and CD36 are well characterized as ligand delivery molecules to enhance TLR2 responses to ligands especially with a lower concentration of ligands, although the participation of these molecules is not essential [9, 36]. TLR4 can sense Gram-negative bacteria through the lipopolysaccharide (LPS) located on their outer membrane [35]. During this process, the formation of a complex of TLR4 with MD2 and CD14 is essential for recognizing LPS [35, 37]. TLR5 functions in the recognition of flagellin from bacterial surfaces. There is still relatively little knowledge about the function of TLRs in the recognition of DAMPs compared with its function in their cognition of PAMPs. TLR3, 7, and 9 have been reported to play a role to sense these nucleic acids released from damaged cells [38, 39]. It has been demonstrated that TLR2 and TLR4 can be activated by the intracellular proteins or extracellular matrix components released from damaged cells [38, 39]. It is controversial that DAMPs directly interact with extracellular TLRs, during this DAMPs recognition process [38]. It has been shown that recognition can be indirect for instance by the involvement of high-mobility group box 1 protein (HMGB1), which is a widely studied endogenous danger signal that induces inflammatory response through its interaction with DAMPs recognized by TLR2, TLR4 and TLR9 [40, 41].

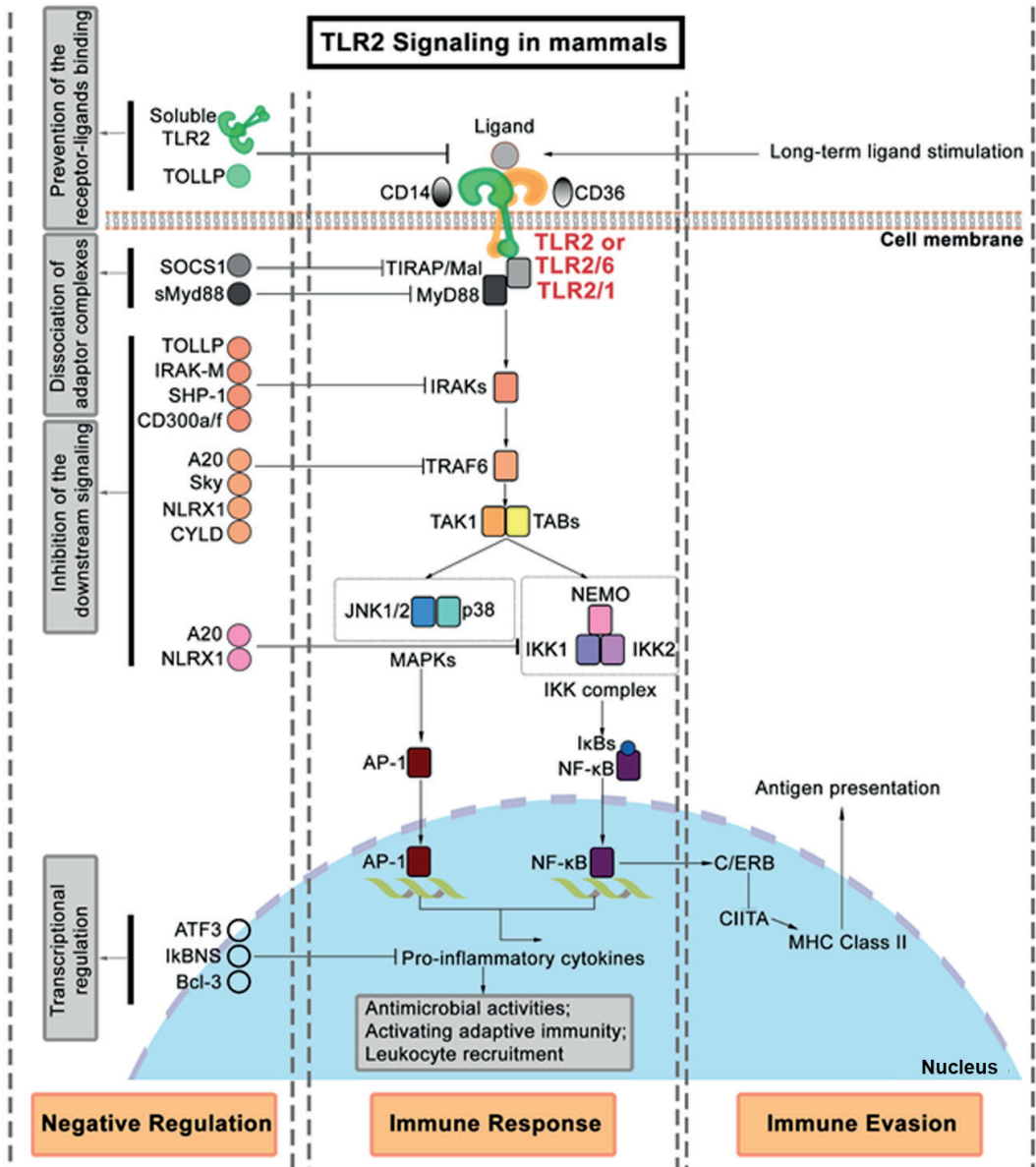
## 2.2 Negative regulation in TLR2 signaling

Accumulating evidence has been reported that the activation of TLR2 signaling is a benefit for the host defense against invading pathogens [42-44]. Excessive activation of TLR signaling, can lead to over-expression of pro-inflammatory cytokines, which have been implicated in chronic inflammatory diseases, autoimmune diseases, and even aggravation of infection diseases [45-47]. For example, a deficiency of TLR2 in diabetic mice can accelerate wound healing, which indicates that excessive activation of TLR2 signaling might be harmful for wound healing [48]. Thus, it appears that TLR2 signaling needs to be tightly regulated by negative regulation mechanisms that are still poorly understood. Recent reviews have summarized many different mechanisms of negative regulation and their molecular components [49-52]. Negative regulators are including the following categories of molecules: ubiquitin ligases, deubiquitinases, transcriptional regulators, and microRNA, which induce different negative regulations [52]. The mechanisms inhibiting TLR2 signaling are based on (1) prevention of receptor-ligand binding; (2) dissociation of adaptor complexes; (3) inhibition of TLR2 downstream kinase signaling; (4) negative transcriptional regulation by TLR2 and other factors as described below [50, 51].

Soluble TLR2, which is a smaller isoform of the TLR2 protein that has been reported to be secreted by human monocytes, has been characterized to compete with TLR2 located on cell membranes to bind with the ligands to inhibit TLR2 signaling [53, 54]. As a negative regulator that leads to dissociation of adaptor complexes it has been shown that a short form of Myd88 (sMyd88) is unable to bind with IRAK4 and thereby its expression can inhibit NF- $\kappa$ B activation [55]. Another described mechanism for inhibition adaptor signaling is the induction of TIRAP degradation by the suppressor of cytokine signaling 1 (SOCS1) [56]. Moreover, TRIF can be degraded by a disintegrin and metalloprotease 15 (ADAM15) in a TRIF-dependent pathway [57]. In terms of TLR2 downstream kinase signaling inhibition, Toll-interacting protein (TOLLIP) inhibits the TLR2 signaling by targeting with IRAK1 to suppress its phosphorylation or directly interacting with TLR2 [58, 59]. Thus, TOLLIP is widely utilized as an inhibitor to inhibit TLR2 signaling [59]. IRAK-M is another IRAK inhibitor, which belongs to the IRAK kinase family but cannot induce NF- $\kappa$ B activation [60]. In addition, tyrosine phosphatase SHP-1, CD300a, and CD300f are also reported as IRAKs inhibitors. SHP-1 inhibits IRAK1 activation by interaction with a kinase tyrosine-based inhibitory motif (KTIM) [61], and CD300a/f can associate with SHP-1 to inhibit signaling [62]. In addition to the inhibitors



1 targeting IRAKs, proteins bind with TRAF6, namely A20 (also called TNF- $\alpha$  induced protein 3, TNFAIP3), a non-receptor tyrosine kinase MyD88, NLR family member X1 (NLRX1), and the cylindromatosis protein (CYLD) have also been shown to be negative regulators of TLR2 signaling [63]. Furthermore, A20 and NLRX1 can also block the activation of the IKK complex [64]. The last category is the negative regulatory of transcription. The transcription of some pro-inflammatory genes, like IL-6, are negatively regulated by transcription factor 3 (ATF3) [65], A TLR-inducible I $\kappa$ B protein (IkBNS) [66], and B-cell CLL/lymphoma 3 (Bcl-3) [67]. It is not yet known how these negative regulators are controlled. It has been hypothesized that Myd88 might be involved in a feedback loop that could be under control of TLR2 or other Toll like receptors [68]. Therefore, it is very likely that TLR2 is an important control factor of negative regulation of transcription of genes involved in inflammation.



**Figure 3** A brief overview of the TLR2 signaling pathway in mammals [9, 33]. To be noted, the shown TLR2 signaling components are not exclusive for this TLR receptor, and the phosphorylation and ubiquitination processes are not mentioned in this figure. TLR2 or its heterodimers are ubiquitously located on the cell membranes. The TLR2 signaling activation through TLR2/1 requires the participant of accessory molecule CD14, while TLR2/6 requires CD36. The TLR2 signaling pathway is activated after TLR2 ligand-recognition (PAMPs or DAMPs). Subsequently, the adaptor proteins, Myd88 and Tirap/Mal, are recruited. After a series of cascades involving NF-κB and MAPKs, various transcription factors are activated to induce pro-inflammatory cytokines.

### 3. TLR2 function in mycobacterial infection studies

#### 3.1 Tuberculosis and non-tuberculosis disease

Tuberculosis (TB) is a communicable disease, which is caused by infection with *Mycobacterium tuberculosis* (Mtb) [69]. At present, TB remains one of the top 10 diseases which give rise to death and currently its death toll is higher than that caused by other major infectious disease such as malaria, AIDS or COVID-19 [69]. It has been reported that in recent years, incidence and death of TB are falling, but it is not reaching any of the global TB eradication goals set by the WHO [70]. Currently, in the COVID-19 pandemic situation the numbers of deaths due to tuberculosis are rapidly rising due to a lack of diagnostic and treatment capacity [69]. Nontuberculous mycobacteria (NTM) diseases are defined as caused by mycobacterial pathogens other than Mtb and *Mycobacterium leprae* [71]. Besides TB, NTM infection diseases have recently attracted wide attention because the disease prevalence of NTM infection diseases is increasing sharply since 2000 [72]. It is hard to combat TB or NTM infection incidence due to the rapid increase in multi-drug resistant mycobacterial strains [69, 73]. Therefore, there is an urgent need to discover novel preventive or therapeutic strategies for TB and NTM infection diseases. Currently, host-directed therapies (HDT) are one of the most promising strategies to combat NTM infectious diseases by making the NTM antibiotic treatment regimens more effective [73, 74]. In mycobacterial infection, TLR2 is a key receptor to recognize mycobacteria, modulate immune cells recruitment, modulate phagocytosis, and trigger pro-inflammatory responses to eliminate the invading mycobacteria [9]. Thus, it is helpful to discover new host directed therapeutic targets by better understanding the mechanism of TLR2 modulating the host-mycobacterial interactions.

#### 3.2 TLR2 recognizes mycobacterial components

As we described in this introduction, TLR2 plays a crucial role in recognizing bacteria such as Mtb through their cell wall components [75]. TLR2 lipoprotein ligands of the cell surface of Mtb include 19-kDa lipoprotein (Rv3763, LpqH), 24-kDa lipoproteins (Rv1270c, LprA and Rv1411c, LprG), and 38-kDa glycolipoprotein (PhoS1). Other categories of TLR2 ligands include lipoarabinomannan (LAM), lipomannans (LM), phosphatidylinositol dimannoside (PIMs) and trehalose dimycolate (TDM). and mycobacterial heat shock protein 70 (HSP70). The TLR2 ligands from Mtb are briefly summarized and described in Table 1 [76]. As we described in this introduction these ligands activate macrophages by activating NF- $\kappa$ B through TLR2 (Fig 3). However, prolonged TLR2 signaling triggered by these ligands might help Mtb

to evade immune surveillance. For example, long-term exposure of macrophages to LpqH, LprG, LprA, PhoS1, LM, and PIM leads to IL-10, IL-4, and TGF- $\beta$  expression, which in turn inhibits the activation of macrophages [76]. Furthermore, it has been demonstrated that prolonged TLR2 signaling activated by LpqH and LprG inhibits the expression of MHC class II molecules and exogenous antigen processing for presentation to CD4<sup>+</sup> T cell, which can be a basis for Mtb immune evasion (Fig 3) [77-80].

### 3.3 TLR2 is associated with susceptibility to various mycobacteria

The structural integrity of TLR2 is crucial for defense against invading pathogens. Single nucleotide polymorphisms (SNPs) in human TLR2 have been reported to associate with increased susceptibility to infectious diseases [81]. For example, one of the TLR2 polymorphisms, Arg753Gln has been demonstrated to lead to higher susceptibility to TB [82]. Moreover, the TLR2 polymorphism R753Q impairs the activation of TLR2 signaling upon *M. smegmatis* infection [83]. These studies indicate that TLR2 plays a protective role in mycobacterial infection diseases, although a small number of studies found no effect of TLR2 polymorphism [8, 84, 85]. Thus, an animal model for TLR2 polymorphisms is needed to investigate the functions of the polymorphisms in tuberculosis.

Mice is a widely used animal model to study the function of TLR2 in resistance to mycobacterial infection. The evidence for the role of TLR2 in defense against NTM infection is still limited, and no correlation has been found between TLR2 polymorphism and human susceptibility to NTM infection until now [86]. Interestingly, TLR2<sup>-/-</sup> mutant mice were more susceptible to *M. avium* infection [87]. It has been demonstrated that TLR2 deficient mice, but not TLR4 deficient mice, were more susceptible to a high dose Mtb infection than wild type mice [88-90]. However, the results of the studies of the role of TLR2 in low-dose Mtb infection are controversial. As a result, it is not clear at which infectious stages TLR2 functions in defense against Mtb infection: it is undecided whether it functions at an acute infectious stage or chronic infectious stage. Some researchers hypothesize that TLR2 plays a protective role during Mtb chronic infection while it does not affect Mtb acute infection [89, 91, 92]. In contrast, Tjärnlund et al. demonstrated that TLR2 has a function in Mtb acute infection (at 3 weeks post infection, wpi) but not in Mtb chronic infection (at 8 wpi) [27]. Interestingly, other studies found no significant differences between TLR2 defective mice or wild type mice upon low-dose Mtb infection, in both acute or chronic infection [88, 93, 94]. The reasons for these different results may be because (1) different Mtb strains were used. Most researchers used the Mtb H37Rv

strain, while some studies utilized the Mtb Kurono strain or the Mtb Erdman strain. (2) They application of different infection methods. Aerosol challenging is the most extensively used method, but some studies also use intranasal (i.n.), intravenous (i.v.), or intratracheal infection methods (i.t.). (3) Differences in the definition of acute or chronic infection. For example, how long is an infection considered a chronic infection? In some studies, 8 weeks are considered as a chronic infection, while other studies 21 weeks is taken as a threshold. In summary the lack of standardization in mice studies has given rise to many uncertainties as to the function of TLR2 in defense against tuberculosis.

### 3.4 Macrophage-mycobacterium interactions mediated by TLR2

Macrophages are not only the primer cells to recognize the invasion of mycobacteria, but are also the main cellular components of granulomas [95]. TLR2 plays an essential role in mediating the interaction of macrophages and mycobacteria. At the early infection stage, TLR2 enhances the entrance of Mtb bacteria into macrophages by binding PE\_PGR33, a mycobacterial protein from the Mtb [96]. The binding of TLR2 and PE\_PGR33 can activate macrophages by inducing the expression of TNF- $\alpha$  and some other pro-inflammatory cytokines (Table 1), while it can also trigger the PI3K pathway that can impair the macrophage antimicrobial responses [96]. In addition to promoting inflammatory responses, TLR2 also plays a role in promoting apoptosis of macrophages [97], which is an important defense mechanism of the host against intracellular pathogens. For example, Sánchez et al. has reported that the apoptosis triggered by Mtb infection is depending on the TLR2 signaling pathway [98]. In addition, it has been demonstrated that the apoptosis induced by ESAT-6 is a TLR2-dependent event [99]. ESAT-6, an abundantly secreted protein of Mtb is an important virulence factor. Furthermore, TLR2-dependent microRNA-155 (miR-155) expression is required to elicit macrophage apoptosis by *Mycobacterium bovis* BCG [100]. The antimicrobial activity of macrophages is an essential function of the host to combat invading mycobacteria and is mediated by TLR2 [101]. In human macrophages, the stimulation of TLR2 by mycobacteria results in the upregulation of Cyp27B1 and VDR, which have a function in the induction of transcription of antimicrobial factors, like the antimicrobial peptide cathelicidin [102, 103]. To be noted, mouse macrophages and human macrophages utilize different mechanisms to kill intracellular Mtb through TLR2 activation [28]. TLR2 activation leading to killing of intracellular bacilli is an iNOS-dependent process in mouse macrophages, whereas human macrophages do not depend on it [27]. Thus, other animal models are needed to confirm the

TLR2-mediated mechanisms of triggering macrophage antimicrobial activity. There is only one *in vitro* study describing how mycobacteria can directly control macrophage migration by rearranging the cytoskeleton via activation of TLR2 [104]. Konowich et al. have demonstrated by using various chimeric mice that TLR2 signaling in hematopoietic cells plays a role in controlling bacterial burden and granuloma integrity, while TLR2 signaling in non-hematopoietic cells may play a role in promoting granulomatous inflammation and bacterial dissemination [92]. Furthermore, Carlos et al. found that TLR2<sup>-/-</sup> mice displayed increased bacterial burden, diminished myeloid cell recruitment, and defective granuloma formation [90]. Interestingly, the adoptive transfer of TLR2 positive mast cells into these TLR2<sup>-/-</sup> mice reversed the phenotype [90]. In conclusion, TLR2 participates in mediating macrophage-mycobacteria interactions in many ways during phagocytosis, apoptosis, antimicrobial activity, cell recruitment, and granuloma formation. But, the mechanisms underlying these function of TLR2 are still not clear and need to be further studied.

### 3.5 Neutrophil-mycobacterium interactions mediated by TLR2

In addition to macrophages, neutrophils are innate immune cells that have an important function in defense against mycobacterial infection. A large number of neutrophils can be detected in TB lesions and in the sputum of TB patients, which indicates that neutrophils play a crucial role during Mtb infection [105, 106]. There is consensus that neutrophils are activated upon mycobacterial infection via TLR2-mediated recognition of LAM on the surface of bacteria (Table 1) [107]. However, the reports on the function of TLR2 in regulation of the recruitment of neutrophils during mycobacterial infection are contradictory. In TLR2<sup>-/-</sup> mutant mice, the bacterial burden after Mtb infection was increased compared to the wild type, and this was accompanied by increased neutrophil influx in the lungs and tissue damage [108]. Conversely, after alveolar epithelial cells were infected by *Mycobacterium bovis* BCG *in vitro*, the recruitment of neutrophils was significantly reduced by blocking TLR2 [109]. Moreover, injection of non-mannose-capped lipoarabinomannan (AraLAM), which is a TLR-ligand from *Mycobacterium smegmatis*, led to a stronger reduction of neutrophils influx in the pulmonary compartment in TLR2<sup>-/-</sup> mice than compared to WT mice [110]. These results demonstrate that it is important to study, the function of TLR2 in neutrophils migration in further detail.

### 3.6 Therapeutic targeting of TLR2 signaling in mycobacterial infection disease

TLR2, as one of the most important representatives of PRRs, can recognize many mycobacterial components which are known as PAMPs. Some of these TLR2 ligands constitute the main

protein component of TB vaccines or adjuvants [96]. For example, the ESAT-6 and PPE18 proteins (Rv1196) are important components of the M72/AS01 and H56/IC31 vaccine candidates [96]. In addition, the mycobacterial MPT38 and PE\_PGRS33 proteins have been reported to be TLR2-targeted secreted proteins that are promising pulmonary TB vaccines [96, 111]. At present, *Mycobacterium bovis* *Bacille Calmette and Guérin* (BCG) remains the only available vaccine for TB, but it is only validated for prevention of TB in children [112, 113]. Furthermore, there is no effective vaccine for infection disease caused by NTM strains.

TLR2 plays a dual role to trigger both pro-inflammatory and anti-inflammatory responses after infection. Thus, modulation of TLR2 signaling has become a popular approach for the design of host-directed therapeutics against infectious diseases. A recent review has described in detail how TLR2 could be used as a therapeutic target to cure bacterial infections [114]. TLR2 ligands from mycobacteria constitute a large group of natural TLR2- agonists and TLR2- antagonists which we summarize in Table 1. These TLR2 agonists or antagonists not only can be used to study the function of TLR2 in infectious diseases, but also provide new possibilities as therapeutic that target TLR2 signaling to treat hyper-inflammation and auto-inflammatory diseases. For example, recombinant PPE18 protein (rPPE18), which is a TLR2 ligand derived from Mtb, has been demonstrated to be a promising novel therapeutic to control sepsis [115]. Because rPPE18 significantly decreases the secretion of serum pro-inflammatory cytokines and reduces organ damage in mice infected with high doses of *E. coli* bacteria [115].

## 4. Zebrafish as a model to study the innate immune system

### 4.1 General advantages of the zebrafish larval model

In the last decades, extensive disease models have been established for using zebrafish larvae to study hematology [116, 117], oncology [118] and other pathogenic processes [119]. Zebrafish models contributed to uncovering pathogenic mechanisms and to the discovery and efficacy screening of innovative drugs [120, 121]. As an animal model, zebrafish possesses various advantages. The zebrafish larvae already have a functional innate immune system within 5 days post-fertilization, when the adaptive immune system is still not functional, providing a great advantage for studying the mechanisms of acute inflammation[122]. Moreover, its optical transparency and small size are the most significant advantages of the zebrafish embryos and larvae, because it provides an ideal *in vivo* system to directly observe cell- cell or cell- microbe interactions [122]. This is very difficult to achieve in other vertebrate

models. In addition, the large number of zebrafish offspring makes it possible for omics studies of large groups of larvae. In this thesis, we make use of these advantages of zebrafish larvae to investigate the role of the innate immune system in inflammation and defense against mycobacteria.

## 4.2 Confocal real-time imaging as a tool for investigating cell function in zebrafish larvae

Cell migration is an important physiological parameter for many pathological processes, including inflammatory responses, immune defense and metastasis of malignant tumor cells. Single-cell tracking using confocal real-time imaging is one of the most popular methods to analyze cell migration. Transgenic zebrafish larvae with fluorescently labeled cells are highly suited for non-invasive real-time imaging because of their transparency at early developmental stages. To visualize and quantify the trajectories of cell migration, a large number of algorithms and software programs have been developed in the last decades. In this thesis we also have developed new automated methods for cell tracking (chapter 3). Notwithstanding these efforts, for much biological research it is needed to track the cell movements manually. In the discussion chapter of this thesis, we will discuss future research directions that could make such time-consuming steps of cell migration research less of a bottleneck.

### Overview of this thesis

TLR2 plays a pivotal role in triggering the innate immune responses in inflammation and infection. This makes TLR2 an attractive therapeutic target for developing cures to many diseases. However, its dual role in inflammation and infection makes it very difficult to use the available results from basic research for the development of clinical trials. In addition, it is still not clear and controversial what is the function of TLR2 in regulating phagocytic cell migration. In this thesis, we used the advantage of the transparent zebrafish larval model to observe the dynamics of cell migration *in vivo* under various conditions. We not only aimed to acquire new insights into the function of Tlr2 in regulating cell migration in inflammation and infection, but also aimed to extend our understanding of the role it thereby plays in host defense against pathogens.

The role of TLR2 in host defense against Mtb is still controversial in reports based on rodent *in vivo* studies. In addition, its function in host innate immunity during infection is still not clear.



Moreover, there is still a lack of research of the systemic transcriptome regulation of TLR downstream signaling in a whole animal model after infection. Therefore, **in Chapter 2**, we study the function of *tlr2* in defense against *Mycobacterium marinum* (Mm) infection in zebrafish by measuring infection phenotypes and corresponding transcriptome responses. We show that infection of a *tlr2* mutant in zebrafish larvae leads to a higher Mm bacterial burden, accompanied with a lower number of granulomas and increased extracellular bacterial growth, compared to wild type siblings. These results suggest that Tlr2 plays a protective role in defense against mycobacteria at early infection stages. To obtain explanations and genetic markers for further studies of the effect of the *tlr2* mutation on infection we performed deep RNA sequencing to study the whole transcriptome profile in our mycobacterial infection model at the systems level. From this RNAseq analysis, we found that the role of Tlr2 in controlling mycobacterial infection can be explained by several mechanisms, like reduction of mycobacterial dissemination by dampening of CXCR3-CXCL11 signaling, and modulation of anti-mycobacterial activity by regulating vitamin D signaling.

In a previous study, Torraca et al. found the CXCR3-CXCL11 axis signaling executes an important function in regulating macrophage recruitment in zebrafish larvae [123]. In chapter 2, we found that the expression of *cxcl11aa* and *cxcl11ac* is significantly decreased in the *tlr2* mutant infected with *M. marinum*. Considering that the expression of many chemokines is controlled by Tlr2, we hypothesized that Tlr2 is a key factor in the control of chemokine expression in order to regulate cell recruitment in innate immunity. To test this hypothesis, **in Chapter 3**, we first investigated the function of Tlr2 and Myd88 in leukocytes migration behavior upon tissue damage by using a zebrafish tail wounding model. In this chapter, live fluorescent imaging was performed to study the effect of the *tlr2* mutation and *myd88* mutation on leukocyte migration upon tail wounding. We observed reduced numbers of recruited neutrophils and macrophages at the wounding area in both *tlr2* mutants and *myd88* mutants, compared to their wild type sibling controls. Extensive mathematical analyses have been performed of the cell migration trajectories in the zebrafish larvae upon wounding. Through these analyses, we demonstrated that both *tlr2* and *myd88* control the migration direction of neutrophils upon wounding. Furthermore, in both the *tlr2* and the *myd88* mutants, macrophages migrated more slowly toward the wound edge.

The migration of leukocytes is important during the infection process for bacterial clearance, containment, dissemination, and granuloma formation at the early mycobacterial infectious

stage [124-127]. However, the role of Tlr2 in modulating leukocyte migration in infection is still not clear. In chapter 3, we show that *tlr2* is involved in regulating leukocyte migration in response to inflammatory signaling. Thus, we hypothesize that *tlr2* could also be involved in the regulation of migratory behavior of macrophages and neutrophils to the sites of mycobacterial infection. To test this hypothesis, in **Chapter 4**, we studied the function of *tlr2* during the infection with two different NTM mycobacterial species with special attention to the responsive cell migration behavior. In this chapter, *M. marinum* Mma20 strain and *M. avium* MAC 101 strain were used to study the function of *tlr2* in infection. *M. marinum* infected zebrafish larvae is a recognized model for tuberculosis infection, whereas *M. avium* was never studied in zebrafish before. Thus, we first developed a zebrafish larval infection model for studying *M. avium* infection. The results show that *M. avium* bacteria can infect zebrafish larvae effectively leading to the formation of granulomas. Moreover, we compared the innate immune response of zebrafish larvae to infection with these two different species of NTM, specifically with regard to the bacterial burden, granuloma-like cluster formation, and transcriptomic gene expression profiles. Subsequently, we utilized this model to study the function of *tlr2* in regulating leukocyte migration using a tail fin infection method.

In the last **Chapter 5**, we summarize the findings from the thesis and discuss the challenges and perspective for further research of TLR signaling by using the zebrafish larval model. In addition, we briefly discuss some unpublished results from ongoing studies into the function of TLR2 in system metabolism.

Table 1 Mycobacterial ligands of TLR2

Species	Ligand (s)	Ligand (s) abbreviation	PRRs	Accessory molecules	Observations	References
<i>Lipoproteins</i>						
<i>M. tuberculosis</i>	19-kDa lipoprotein (Rv3763)	<b>LpqH</b>	TLR2/1	CD14	Inhibits MHC- expression and antigen processing; IFN- $\gamma$ -induced genes is inhibited by prolonged LpqH stimulation	[77-79]
	24-kDa lipoprotein (Rv1270c)	<b>LprA</b>	TLR2/1	CD14/CD36	Induces cytokine response and regulate APC function	[79, 128]
	24-kDa lipoprotein (Rv1411c)	<b>LprG</b>	TLR2/1; TLR2	CD14	Long-term exposure of LprG inhibits the processing of MHC-II antigen; Short-term exposure of LprG induces the production of TNF- $\alpha$	[79, 80]
	24-kDa lipoprotein (Rv1016c)	<b>LpqT</b>	TLR2	Unknown	Induces TLR2 dependent apoptosis in macrophage and inhibits MHC- expression and antigen processing	[129]
	38-kDa glycolipoprotein	<b>PhoS1</b>	TLR2/1, TLR4	Unknown	Activates the ERK1/2 and p38 MAPK signaling, which in turn induce TNF- $\alpha$ and IL-6 expression	[79, 130]
	Lipoylated and glycosylated Mtb lipoprotein (Rv2873)	<b>MPT83</b>	TLR2	unknown	MPT83 induced cytokine production was decreased in the TLR2 defective mice	[131]
	<i>Lipoglycans/Glycolipids</i>					
	Lipoarabinomannan	<b>LAM</b>	TLR2/1; TLR2	CD14	Mtb LAM induces the production of pro- and anti-inflammatory cytokine to activate neutrophils	[75, 107]
	Arabinosylated lipoarabinomannan	<b>AraLAM</b>	TLR2	Unknown	Induces the pro-inflammatory responses	[132]
	Lipomannans	<b>LM</b>	TLR2/1; TLR2;	CD40/CD86	Induces TNF- $\alpha$ and NO secretion to activate macrophages	[133, 134]
	phosphatidylinositol dimannoside	<b>PIM2/6</b>	TLR2	Unknown	Induces the expression of TNF- $\alpha$ to activate macrophages	[75, 135]
	Trehalose dimycolate	<b>TDM</b>	TLR2	CD14/MARCO	Induces NF- $\kappa$ B signaling	[136]

Table 1 Continued. Mycobacterial ligands of TLR2

Species	Ligand (s)	Ligand (s) abbreviation	PRRs	Accessory molecules	Observations	References
<i>Others</i>						
<i>M. tuberculosis</i>	Heat shock protein 70	<b>HSP70</b>	TLR2	Unknown	Inhibits the secretion of IL-6 in TLR2-deficient macrophages	[137]
	55-kDa flavin containing monooxygenase (Rv3083)	<b>MymA</b>	TLR2	CD40/CD80/ CD86/HLA-DR	Upregulates the expression of TLR2 and its co-simulatory molecules. Activates macrophage by inducing TNF- $\alpha$ and IL-12.	[138]
	PE_PGRS proteins (Rv1818c)	<b>PE_PGRS33</b>	TLR2	CD14	Contributes to Mtb enter macrophage by interacting with TLR2	[139, 140]
	Secreted antigenic targets of 6-kDa (ESAT-6) family proteins (Rv1198)	<b>EsxL</b>	TLR2	Unknown	Induces TNF- $\alpha$ and IL-6 through TLR2 dependent NF- $\kappa$ B and MAPK signaling	[141]
	PE/PPE protein (Rv1196)	<b>PPE18</b>	TLR2	Unknown	Interacts with TLR2 to produce IL-10 and SOCS3 to in turn inhibit TLR2 signaling	[142, 143]
	PE/PPE protein (Rv1789)	<b>PPE26</b>	TLR2	CD80/CD86	Activates macrophage by inducing pro-inflammatory cytokine TNF- $\alpha$ , IL-6 and IL-12	[144]
	PE/PPE protein (Rv1808)	<b>PPE32</b>	TLR2	Unknown	Induces both anti-inflammatory cytokine IL-10 and pro-inflammatory cytokines TNF- $\alpha$ and IL-6	[145]
	PE/PPE protein (Rv3425)	<b>PPE57</b>	TLR2	CD40/CD80/ CD86	Activates macrophage by inducing pro-inflammatory cytokine TNF- $\alpha$ , IL-6 and IL-12	[146]
	Leucine-responsive regulatory protein	<b>Lrp</b>	TLR2	Unknown	Inhibit LPS-induced pro-inflammatory cytokine production IL-12 and TNF- $\alpha$	[147]
	Glycopeptidolipids	<b>GPLs</b>	TLR2, TLR4	Unknown	Promotes the activation of macrophages depended on a TLR2 and MyD88 manner. TLR2 senses GPLs needs its specific acetylation and methylation;	[148-150]
<i>M. abscessus</i>	TLR2-enriched fraction	<b>TLR2ef</b>	TLR2	Unknown	TLR2ef mildly protects Mab infected $\Delta$ F508 mice and its littermates	[151]
	Glycopeptidolipids	<b>GPLs</b>	TLR2	Unknown	The switch of Mab from the smooth to the rough morphotype depends on the present of bacterial surface GLPs.	[152]
<i>M. smegmatis</i>	Phosphoinositol-capped LAM	<b>PILAM</b>	TLR2/ TLR1	Unknown	High affinity binding to TLR2 and strong pro-inflammatory response	[133, 153]
	Arabinosylated lipoarabinomannan	<b>AraLAM</b>	TLR2	CD14?	The lung inflammation induced by AraLAM is diminished in TLR2 deficiency mice	[110]
	Dimannoside hosphatidyl-myo-inositol mannosides	<b>PIM2/6</b>	TLR2	Unknown	Induces the expression of TNF to activate primary macrophages	[135]

## Reference

1. Riera Romo M, Perez-Martinez D, Castillo Ferrer C. Innate immunity in vertebrates: an overview. *Immunology*. 2016;148(2):125-39. Epub 2016/02/16. doi: 10.1111/imm.12597. PubMed PMID: 26878338; PubMed Central PMCID: PMC4863567.
2. Hato T, Dagher PC. How the Innate Immune System Senses Trouble and Causes Trouble. *Clin J Am Soc Nephrol*. 2015;10(8):1459-69. Epub 2014/11/22. doi: 10.2215/CJN.04680514. PubMed PMID: 25414319; PubMed Central PMCID: PMC4527020.
3. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol*. 2011;30(1):16-34. Epub 2011/01/18. doi: 10.3109/08830185.2010.529976. PubMed PMID: 21235323.
4. Hara T, Nakashima Y, Sakai Y, Nishio H, Motomura Y, Yamasaki S. Kawasaki disease: a matter of innate immunity. *Clin Exp Immunol*. 2016;186(2):134-43. Epub 2016/06/28. doi: 10.1111/cei.12832. PubMed PMID: 27342882; PubMed Central PMCID: PMC45054572.
5. Hashimoto C, Hudson KL, Anderson KV. The Toll gene of *Drosophila*, required for dorsal-ventral embryonic polarity, appears to encode a transmembrane protein. *Cell*. 1988;52(2):269-79. Epub 1988/01/29. doi: 10.1016/0092-8674(88)90516-8. PubMed PMID: 2449285.
6. Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature*. 1997;388(6640):394-7. Epub 1997/07/24. doi: 10.1038/41131. PubMed PMID: 9237759.
7. Simpson ME, Petri WA, Jr. TLR2 as a Therapeutic Target in Bacterial Infection. *Trends Mol Med*. 2020;26(8):715-7. Epub 2020/06/22. doi: 10.1016/j.molmed.2020.05.006. PubMed PMID: 32563557; PubMed Central PMCID: PMC7845793.
8. Gopalakrishnan A, Salgame P. Toll-like receptor 2 in host defense against *Mycobacterium tuberculosis*: to be or not to be—that is the question. *Curr Opin Immunol*. 2016;42:76-82. Epub 2016/10/30. doi: 10.1016/j.coi.2016.06.003. PubMed PMID: 27326654; PubMed Central PMCID: PMC45086274.
9. Oliveira-Nascimento L, Massari P, Wetzler LM. The Role of TLR2 in Infection and Immunity. *Front Immunol*. 2012;3:79. Epub 2012/05/09. doi: 10.3389/fimmu.2012.00079. PubMed PMID: 22566960; PubMed Central PMCID: PMC3342043.
10. Moles A, Murphy L, Wilson CL, Chakraborty JB, Fox C, Park EJ, et al. A TLR2/S100A9/CXCL-2 signaling network is necessary for neutrophil recruitment in acute and chronic liver injury in the mouse. *J Hepatol*. 2014;60(4):782-91. Epub 2013/12/18. doi: 10.1016/j.jhep.2013.12.005. PubMed PMID: 24333183; PubMed Central PMCID: PMC3960359.
11. Chen L, DiPietro LA. Toll-Like Receptor Function in Acute Wounds. *Adv Wound Care (New Rochelle)*. 2017;6(10):344-55. Epub 2017/10/25. doi: 10.1089/wound.2017.0734. PubMed PMID: 29062591; PubMed Central PMCID: PMC5649397.
12. Di Lorenzo A, Bolli E, Tarone L, Cavallo F, Conti L. Toll-Like Receptor 2 at the Crossroad between Cancer Cells, the Immune System, and the Microbiota. *Int J Mol Sci*. 2020;21(24). Epub 2020/12/17. doi: 10.3390/ijms21249418. PubMed PMID: 33321934; PubMed Central PMCID: PMC7763461.

13. Rada I, Deldicque L, Francaux M, Zbinden-Foncea H. Toll like receptor expression induced by exercise in obesity and metabolic syndrome: A systematic review. *Exerc Immunol Rev.* 2018;24:60-71. Epub 2018/02/21. PubMed PMID: 29461969.
14. Mahfoud S, Petrova TV. The double life of TLR2. *Sci Signal.* 2021;14(666). doi: ARTN eabf4701 10.1126/scisignal.abf4701. PubMed PMID: WOS:000609622000004.
15. Li Y, Li Y, Cao X, Jin X, Jin T. Pattern recognition receptors in zebrafish provide functional and evolutionary insight into innate immune signaling pathways. *Cell Mol Immunol.* 2017;14(1):80-9. Epub 2016/10/11. doi: 10.1038/cmi.2016.50. PubMed PMID: 27721456; PubMed Central PMCID: PMC5214946.
16. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell.* 2010;140(6):805-20. Epub 2010/03/23. doi: 10.1016/j.cell.2010.01.022. PubMed PMID: 20303872.
17. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. The dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. *Cell.* 1996;86(6):973-83. Epub 1996/09/20. doi: 10.1016/s0092-8674(00)80172-5. PubMed PMID: 8808632.
18. Sahoo BR. Structure of fish Toll-like receptors (TLR) and NOD-like receptors (NLR). *Int J Biol Macromol.* 2020;161:1602-17. Epub 2020/08/07. doi: 10.1016/j.ijbiomac.2020.07.293. PubMed PMID: 32755705; PubMed Central PMCID: PMC7396143.
19. Imler JL, Hoffmann JA. Toll receptors in *Drosophila*: a family of molecules regulating development and immunity. *Curr Top Microbiol Immunol.* 2002;270:63-79. Epub 2002/12/07. doi: 10.1007/978-3-642-59430-4\_4. PubMed PMID: 12467244.
20. Satake H, Sekiguchi T. Toll-like receptors of deuterostome invertebrates. *Front Immunol.* 2012;3:34. Epub 2012/05/09. doi: 10.3389/fimmu.2012.00034. PubMed PMID: 22566918; PubMed Central PMCID: PMC3342246.
21. Meijer AH, Gabby Krens SF, Medina Rodriguez IA, He S, Bitter W, Ewa Snaar-Jagalska B, et al. Expression analysis of the Toll-like receptor and TIR domain adaptor families of zebrafish. *Mol Immunol.* 2004;40(11):773-83. Epub 2003/12/23. doi: 10.1016/j.molimm.2003.10.003. PubMed PMID: 14687934.
22. Blasius AL, Beutler B. Intracellular toll-like receptors. *Immunity.* 2010;32(3):305-15. Epub 2010/03/30. doi: 10.1016/j.immuni.2010.03.012. PubMed PMID: 20346772.
23. Pifer R, Benson A, Sturge CR, Yarovinsky F. UNC93B1 is essential for TLR11 activation and IL-12-dependent host resistance to *Toxoplasma gondii*. *J Biol Chem.* 2011;286(5):3307-14. Epub 2010/11/26. doi: 10.1074/jbc.M110.171025. PubMed PMID: 21097503; PubMed Central PMCID: PMC3030336.
24. 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):588-93. Epub 1998/01/22. doi: 10.1073/pnas.95.2.588. PubMed PMID: 9435236; PubMed Central PMCID: PMC18464.
25. Basto AP, Leitao A. Targeting TLR2 for vaccine development. *J Immunol Res.* 2014;2014:619410. Epub 2014/07/25. doi: 10.1155/2014/619410. PubMed PMID: 25057505; PubMed Central PMCID: PMC4098989.

26. Eriksson EM, Jackson DC. Recent advances with TLR2-targeting lipopeptide-based vaccines. *Curr Protein Pept Sci.* 2007;8(4):412-7. Epub 2007/08/19. doi: 10.2174/138920307781369436. PubMed PMID: 17696872.
27. Tjarnlund A, Guirado E, Julian E, Cardona PJ, Fernandez C. Determinant role for Toll-like receptor signalling in acute mycobacterial infection in the respiratory tract. *Microbes Infect.* 2006;8(7):1790-800. Epub 2006/07/04. doi: 10.1016/j.micinf.2006.02.017. PubMed PMID: 16815067.
28. Thoma-Uszynski S, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, et al. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science.* 2001;291(5508):1544-7. Epub 2001/02/27. doi: 10.1126/science.291.5508.1544. PubMed PMID: 11222859.
29. Santos-Sierra S, Deshmukh SD, Kalnitski J, Kuenzi P, Wymann MP, Golenbock DT, et al. Mal connects TLR2 to PI3Kinase activation and phagocyte polarization. *EMBO J.* 2009;28(14):2018-27. Epub 2009/07/04. doi: 10.1038/emboj.2009.158. PubMed PMID: 19574958; PubMed Central PMCID: PMC2718282.
30. O'Neill LA, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol.* 2007;7(5):353-64. Epub 2007/04/26. doi: 10.1038/nri2079. PubMed PMID: 17457343.
31. McClure R, Massari P. TLR-Dependent Human Mucosal Epithelial Cell Responses to Microbial Pathogens. *Front Immunol.* 2014;5:386. Epub 2014/08/28. doi: 10.3389/fimmu.2014.00386. PubMed PMID: 25161655; PubMed Central PMCID: PMC4129373.
32. Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity.* 2011;34(5):637-50. Epub 2011/05/28. doi: 10.1016/j.immuni.2011.05.006. PubMed PMID: 21616434.
33. Kang SS, Sim JR, Yun CH, Han SH. Lipoteichoic acids as a major virulence factor causing inflammatory responses via Toll-like receptor 2. *Arch Pharm Res.* 2016;39(11):1519-29. Epub 2016/08/09. doi: 10.1007/s12272-016-0804-y. PubMed PMID: 27498542.
34. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell.* 2006;124(4):783-801. Epub 2006/02/25. doi: 10.1016/j.cell.2006.02.015. PubMed PMID: 16497588.
35. Mukherjee S, Karmakar S, Babu SP. TLR2 and TLR4 mediated host immune responses in major infectious diseases: a review. *Braz J Infect Dis.* 2016;20(2):193-204. Epub 2016/01/19. doi: 10.1016/j.bjid.2015.10.011. PubMed PMID: 26775799.
36. Jimenez-Dalmaroni MJ, Xiao N, Corper AL, Verdino P, Ainge GD, Larsen DS, et al. Soluble CD36 ectodomain binds negatively charged diacylglycerol ligands and acts as a co-receptor for TLR2. *PLoS One.* 2009;4(10):e7411. Epub 2009/10/23. doi: 10.1371/journal.pone.0007411. PubMed PMID: 19847289; PubMed Central PMCID: PMC2760212.
37. Uematsu S, Akira S. Toll-Like receptors (TLRs) and their ligands. *Handb Exp Pharmacol.* 2008;(183):1-20. Epub 2007/12/12. doi: 10.1007/978-3-540-72167-3\_1. PubMed PMID: 18071652.
38. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol.* 2020;20(2):95-112. Epub 2019/09/29. doi: 10.1038/s41577-019-0215-7. PubMed PMID: 31558839.

39. Yu L, Wang L, Chen S. Endogenous toll-like receptor ligands and their biological significance. *J Cell Mol Med.* 2010;14(11):2592-603. Epub 2010/07/16. doi: 10.1111/j.1582-4934.2010.01127.x. PubMed PMID: 20629986; PubMed Central PMCID: PMCPCMC4373479.
40. Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. *Nat Rev Immunol.* 2010;10(6):427-39. Epub 2010/05/26. doi: 10.1038/nri2779. PubMed PMID: 20498669.
41. Bianchi ME. HMGB1 loves company. *J Leukoc Biol.* 2009;86(3):573-6. Epub 2009/05/06. doi: 10.1189/jlb.1008585. PubMed PMID: 19414536.
42. Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science.* 1999;285(5428):732-6. Epub 1999/07/31. doi: 10.1126/science.285.5428.732. PubMed PMID: 10426995.
43. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. *Clin Microbiol Rev.* 2009;22(2):240-73, Table of Contents. Epub 2009/04/16. doi: 10.1128/CMR.00046-08. PubMed PMID: 19366914; PubMed Central PMCID: PMCPCMC2668232.
44. Hossain MM, Norazmi MN. Pattern recognition receptors and cytokines in Mycobacterium tuberculosis infection--the double-edged sword? *Biomed Res Int.* 2013;2013:179174. Epub 2013/12/19. doi: 10.1155/2013/179174. PubMed PMID: 24350246; PubMed Central PMCID: PMCPCMC3844256.
45. Cook DN, Pisetsky DS, Schwartz DA. Toll-like receptors in the pathogenesis of human disease. *Nat Immunol.* 2004;5(10):975-9. Epub 2004/09/30. doi: 10.1038/ni1116. PubMed PMID: 15454920.
46. Pradhan VD, Das S, Surve P, Ghosh K. Toll-like receptors in autoimmunity with special reference to systemic lupus erythematosus. *Indian J Hum Genet.* 2012;18(2):155-60. Epub 2012/11/20. doi: 10.4103/0971-6866.100750. PubMed PMID: 23162288; PubMed Central PMCID: PMCPCMC3491286.
47. Netea MG, Van der Meer JW, Kullberg BJ. Toll-like receptors as an escape mechanism from the host defense. *Trends Microbiol.* 2004;12(11):484-8. Epub 2004/10/19. doi: 10.1016/j.tim.2004.09.004. PubMed PMID: 15488388.
48. Dasu MR, Thangappan RK, Bourgette A, DiPietro LA, Isseroff R, Jialal I. TLR2 expression and signaling-dependent inflammation impair wound healing in diabetic mice. *Lab Invest.* 2010;90(11):1628-36. Epub 2010/08/25. doi: 10.1038/labinvest.2010.158. PubMed PMID: 20733560.
49. Caplan IF, Maguire-Zeiss KA. Toll-Like Receptor 2 Signaling and Current Approaches for Therapeutic Modulation in Synucleinopathies. *Front Pharmacol.* 2018;9:417. Epub 2018/05/22. doi: 10.3389/fphar.2018.00417. PubMed PMID: 29780321; PubMed Central PMCID: PMCPCMC5945810.
50. Landen NX, Li D, Stahle M. Transition from inflammation to proliferation: a critical step during wound healing. *Cell Mol Life Sci.* 2016;73(20):3861-85. Epub 2016/05/18. doi: 10.1007/s00018-016-2268-0. PubMed PMID: 27180275; PubMed Central PMCID: PMCPCMC5021733.
51. Kondo T, Kawai T, Akira S. Dissecting negative regulation of Toll-like receptor signaling. *Trends Immunol.* 2012;33(9):449-58. Epub 2012/06/23. doi: 10.1016/j.it.2012.05.002. PubMed PMID: 22721918.
52. Yang L, Seki E. Toll-like receptors in liver fibrosis: cellular crosstalk and mechanisms. *Front Physiol.* 2012;3:138. Epub 2012/06/05. doi: 10.3389/fphys.2012.00138. PubMed PMID: 22661952; PubMed Central PMCID: PMCPCMC3357552.
53. LeBouder E, Rey-Nores JE, Rushmere NK, Grigorov M, Lawn SD, Affolter M, et al. Soluble forms of Toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk. *J*



Immunol. 2003;171(12):6680-9. Epub 2003/12/10. doi: 10.4049/jimmunol.171.12.6680. PubMed PMID: 14662871.

54. 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):6682-6. Epub 2000/12/20. doi: 10.4049/jimmunol.165.12.6682. PubMed PMID: 11120784.

55. Jeong E, Lee JY. Intrinsic and extrinsic regulation of innate immune receptors. *Yonsei Med J.* 2011;52(3):379-92. Epub 2011/04/14. doi: 10.3349/ymj.2011.52.3.429

10.3349/ymj.2011.52.3.379. PubMed PMID: 21488180; PubMed Central PMCID: PMC3101043.

56. Trengove MC, Ward AC. SOCS proteins in development and disease. *Am J Clin Exp Immunol.* 2013;2(1):1-29. Epub 2013/07/26. PubMed PMID: 23885323; PubMed Central PMCID: PMC3714205.

57. Ahmed S, Maratha A, Butt AQ, Shevlin E, Miggin SM. TRIF-mediated TLR3 and TLR4 signaling is negatively regulated by ADAM15. *J Immunol.* 2013;190(5):2217-28. Epub 2013/02/01. doi: 10.4049/jimmunol.1201630. PubMed PMID: 23365087.

58. Lee HJ, Chung KC. PINK1 positively regulates IL-1beta-mediated signaling through Tollip and IRAK1 modulation. *J Neuroinflammation.* 2012;9:271. Epub 2012/12/19. doi: 10.1186/1742-2094-9-271. PubMed PMID: 23244239; PubMed Central PMCID: PMC3533909.

59. Zhang G, Ghosh S. Negative regulation of toll-like receptor-mediated signaling by Tollip. *J Biol Chem.* 2002;277(9):7059-65. Epub 2001/12/26. doi: 10.1074/jbc.M109537200. PubMed PMID: 11751856.

60. Kobayashi K, Hernandez LD, Galan JE, Janeway CA, Jr., Medzhitov R, Flavell RA. IRAK-M is a negative regulator of Toll-like receptor signaling. *Cell.* 2002;110(2):191-202. Epub 2002/08/02. doi: 10.1016/s0092-8674(02)00827-9. PubMed PMID: 12150927.

61. Shio MT, Hassani K, Isnard A, Ralph B, Contreras I, Gomez MA, et al. Host cell signalling and leishmania mechanisms of evasion. *J Trop Med.* 2012;2012:819512. Epub 2011/12/02. doi: 10.1155/2012/819512. PubMed PMID: 22131998; PubMed Central PMCID: PMC3216306.

62. Kim EJ, Lee SM, Suk K, Lee WH. CD300a and CD300f differentially regulate the MyD88 and TRIF-mediated TLR signalling pathways through activation of SHP-1 and/or SHP-2 in human monocytic cell lines. *Immunology.* 2012;135(3):226-35. Epub 2011/11/03. doi: 10.1111/j.1365-2567.2011.03528.x. PubMed PMID: 22043923; PubMed Central PMCID: PMC3311045.

63. Liu Y, Yin H, Zhao M, Lu Q. TLR2 and TLR4 in autoimmune diseases: a comprehensive review. *Clin Rev Allergy Immunol.* 2014;47(2):136-47. Epub 2013/12/20. doi: 10.1007/s12016-013-8402-y. PubMed PMID: 24352680.

64. Skaug B, Chen J, Du F, He J, Ma A, Chen ZJ. Direct, noncatalytic mechanism of IKK inhibition by A20. *Mol Cell.* 2011;44(4):559-71. Epub 2011/11/22. doi: 10.1016/j.molcel.2011.09.015. PubMed PMID: 22099304; PubMed Central PMCID: PMC3237303.

65. Whitmore MM, Iparraguirre A, Kubelka L, Weninger W, Hai T, Williams BR. Negative regulation of TLR-signaling pathways by activating transcription factor-3. *J Immunol.* 2007;179(6):3622-30. Epub 2007/09/06. doi: 10.4049/jimmunol.179.6.3622. PubMed PMID: 17785797.

66. Kuwata H, Matsumoto M, Atarashi K, Morishita H, Hirotsu T, Koga R, et al. IkappaBNS inhibits induction of a subset of Toll-like receptor-dependent genes and limits inflammation. *Immunity*. 2006;24(1):41-51. Epub 2006/01/18. doi: 10.1016/j.immuni.2005.11.004. PubMed PMID: 16413922.
67. Carmody RJ, Ruan Q, Palmer S, Hilliard B, Chen YH. Negative regulation of toll-like receptor signaling by NF-kappaB p50 ubiquitination blockade. *Science*. 2007;317(5838):675-8. Epub 2007/08/04. doi: 10.1126/science.1142953. PubMed PMID: 17673665.
68. Koch BEV, Yang S, Lamers G, Stougaard J, Spaik HP. Intestinal microbiome adjusts the innate immune setpoint during colonization through negative regulation of MyD88. *Nat Commun*. 2018;9(1):4099. Epub 2018/10/07. doi: 10.1038/s41467-018-06658-4. PubMed PMID: 30291253; PubMed Central PMCID: PMC6173721.
69. Harding E. WHO global progress report on tuberculosis elimination (vol 8, pg 19, 2020). *Lancet Resp Med*. 2020;8(1):E3-E. doi: 10.1016/S2213-2600(19)30421-7. PubMed PMID: WOS:000503397100003.
70. Chakaya J, Khan M, Ntoumi F, Aklilu E, Fatima R, Mwaba P, et al. Global Tuberculosis Report 2020 - Reflections on the Global TB burden, treatment and prevention efforts. *Int J Infect Dis*. 2021. Epub 2021/03/16. doi: 10.1016/j.ijid.2021.02.107. PubMed PMID: 33716195.
71. Hoefsloot W, van Ingen J, Andrejak C, Angeby K, Bauriaud R, Bemer P, et al. The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples: an NTM-NET collaborative study. *Eur Respir J*. 2013;42(6):1604-13. Epub 2013/04/20. doi: 10.1183/09031936.00149212. PubMed PMID: 23598956.
72. Prevots DR, Marras TK. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. *Clin Chest Med*. 2015;36(1):13-34. Epub 2015/02/14. doi: 10.1016/j.ccm.2014.10.002. PubMed PMID: 25676516; PubMed Central PMCID: PMC4332564.
73. Saxena S, Spaik HP, Forn-Cuni G. Drug Resistance in Nontuberculous Mycobacteria: Mechanisms and Models. *Biology (Basel)*. 2021;10(2). Epub 2021/02/13. doi: 10.3390/biology10020096. PubMed PMID: 33573039; PubMed Central PMCID: PMC7911849.
74. Kilinc G, Saris A, Ottenhoff THM, Haks MC. Host-directed therapy to combat mycobacterial infections. *Immunol Rev*. 2021;301(1):62-83. Epub 2021/02/11. doi: 10.1111/imr.12951. PubMed PMID: 33565103; PubMed Central PMCID: PMC78248113.
75. Harding CV, Boom WH. Regulation of antigen presentation by Mycobacterium tuberculosis: a role for Toll-like receptors. *Nat Rev Microbiol*. 2010;8(4):296-307. Epub 2010/03/18. doi: 10.1038/nrmicro2321. PubMed PMID: 20234378; PubMed Central PMCID: PMC3037727.
76. Saraav I, Singh S, Sharma S. Outcome of Mycobacterium tuberculosis and Toll-like receptor interaction: immune response or immune evasion? *Immunol Cell Biol*. 2014;92(9):741-6. Epub 2014/07/02. doi: 10.1038/icb.2014.52. PubMed PMID: 24983458.
77. Pennini ME, Pai RK, Schultz DC, Boom WH, Harding CV. Mycobacterium tuberculosis 19-kDa lipoprotein inhibits IFN-gamma-induced chromatin remodeling of MHC2TA by TLR2 and MAPK signaling. *J Immunol*. 2006;176(7):4323-30. Epub 2006/03/21. doi: 10.4049/jimmunol.176.7.4323. PubMed PMID: 16547269.
78. Pai RK, Convery M, Hamilton TA, Boom WH, Harding CV. Inhibition of IFN-gamma-induced class II transactivator expression by a 19-kDa lipoprotein from Mycobacterium tuberculosis: a potential mechanism for

immune evasion. *J Immunol.* 2003;171(1):175-84. Epub 2003/06/21. doi: 10.4049/jimmunol.171.1.175. PubMed PMID: 12816996.

79. Drage MG, Pecora ND, Hise AG, Febbraio M, Silverstein RL, Golenbock DT, et al. TLR2 and its co-receptors determine responses of macrophages and dendritic cells to lipoproteins of *Mycobacterium tuberculosis*. *Cell Immunol.* 2009;258(1):29-37. Epub 2009/04/14. doi: 10.1016/j.cellimm.2009.03.008. PubMed PMID: 19362712; PubMed Central PMCID: PMCPCMC2730726.

80. Gehring AJ, Dobos KM, Belisle JT, Harding CV, Boom WH. *Mycobacterium tuberculosis* LprG (Rv1411c): a novel TLR-2 ligand that inhibits human macrophage class II MHC antigen processing. *J Immunol.* 2004;173(4):2660-8. Epub 2004/08/06. doi: 10.4049/jimmunol.173.4.2660. PubMed PMID: 15294983.

81. Texereau J, Chiche JD, Taylor W, Choukroun G, Comba B, Mira JP. The importance of Toll-like receptor 2 polymorphisms in severe infections. *Clin Infect Dis.* 2005;41 Suppl 7:S408-15. Epub 2005/10/21. doi: 10.1086/431990. PubMed PMID: 16237639.

82. Ogus AC, Yoldas B, Ozdemir T, Uguz A, Olcen S, Keser I, et al. The Arg753Gln polymorphism of the human toll-like receptor 2 gene in tuberculosis disease. *Eur Respir J.* 2004;23(2):219-23. Epub 2004/02/26. doi: 10.1183/09031936.03.00061703. PubMed PMID: 14979495.

83. Pattabiraman G, Panchal R, Medvedev AE. The R753Q polymorphism in Toll-like receptor 2 (TLR2) attenuates innate immune responses to mycobacteria and impairs MyD88 adapter recruitment to TLR2. *J Biol Chem.* 2017;292(25):10685-95. Epub 2017/04/27. doi: 10.1074/jbc.M117.784470. PubMed PMID: 28442574; PubMed Central PMCID: PMCPCMC5481573.

84. Sanchez D, Lefebvre C, Rioux J, Garcia LF, Barrera LF. Evaluation of Toll-like receptor and adaptor molecule polymorphisms for susceptibility to tuberculosis in a Colombian population. *Int J Immunogenet.* 2012;39(3):216-23. Epub 2012/01/10. doi: 10.1111/j.1744-313X.2011.01077.x. PubMed PMID: 22221660.

85. Jafari M, Nasiri MR, Sanaei R, Anoosheh S, Farnia P, Sepanjnia A, et al. The NRAMPI, VDR, TNF-alpha, ICAM1, TLR2 and TLR4 gene polymorphisms in Iranian patients with pulmonary tuberculosis: A case-control study. *Infect Genet Evol.* 2016;39:92-8. Epub 2016/01/18. doi: 10.1016/j.meegid.2016.01.013. PubMed PMID: 26774366.

86. Yim JJ, Kim HJ, Kwon OJ, Koh WJ. Association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and nontuberculous mycobacterial lung disease in a Korean population. *Hum Immunol.* 2008;69(9):572-6. Epub 2008/07/08. doi: 10.1016/j.humimm.2008.06.003. PubMed PMID: 18602432.

87. Feng CG, Scanga CA, Collazo-Custodio CM, Cheever AW, Hieny S, Caspar P, et al. Mice lacking myeloid differentiation factor 88 display profound defects in host resistance and immune responses to *Mycobacterium avium* infection not exhibited by Toll-like receptor 2 (TLR2)- and TLR4-deficient animals. *J Immunol.* 2003;171(9):4758-64. Epub 2003/10/22. doi: 10.4049/jimmunol.171.9.4758. PubMed PMID: 14568952.

88. Reiling N, Holscher C, Fehrenbach A, Kroger S, Kirschning CJ, Goyert S, et al. Cutting edge: Toll-like receptor (TLR)2- and TLR4-mediated pathogen recognition in resistance to airborne infection with *Mycobacterium tuberculosis*. *J Immunol.* 2002;169(7):3480-4. Epub 2002/09/24. doi: 10.4049/jimmunol.169.7.3480. PubMed PMID: 12244136.

89. Drennan MB, Nicolle D, Quesniaux VJ, Jacobs M, Allie N, Mpagi J, et al. Toll-like receptor 2-deficient mice succumb to *Mycobacterium tuberculosis* infection. *Am J Pathol.* 2004;164(1):49-57. Epub 2003/12/26. doi: 10.1016/S0002-9440(10)63095-7. PubMed PMID: 14695318; PubMed Central PMCID: PMCPMC1602241.
90. Carlos D, Frantz FG, Souza-Junior DA, Jamur MC, Oliver C, Ramos SG, et al. TLR2-dependent mast cell activation contributes to the control of *Mycobacterium tuberculosis* infection. *Microbes Infect.* 2009;11(8-9):770-8. Epub 2009/05/16. doi: 10.1016/j.micinf.2009.04.025. PubMed PMID: 19442756.
91. McBride A, Konowich J, Salgame P. Host defense and recruitment of Foxp3(+) T regulatory cells to the lungs in chronic *Mycobacterium tuberculosis* infection requires toll-like receptor 2. *PLoS Pathog.* 2013;9(6):e1003397. Epub 2013/06/21. doi: 10.1371/journal.ppat.1003397. PubMed PMID: 23785280; PubMed Central PMCID: PMCPMC3681744.
92. Konowich J, Gopalakrishnan A, Dietzold J, Verma S, Bhatt K, Rafi W, et al. Divergent Functions of TLR2 on Hematopoietic and Nonhematopoietic Cells during Chronic *Mycobacterium tuberculosis* Infection. *J Immunol.* 2017;198(2):741-8. Epub 2016/12/07. doi: 10.4049/jimmunol.1601651. PubMed PMID: 27920273; PubMed Central PMCID: PMCPMC5224966.
93. Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A. TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to *Mycobacterium tuberculosis*. *J Exp Med.* 2005;202(12):1715-24. Epub 2005/12/21. doi: 10.1084/jem.20051782. PubMed PMID: 16365150; PubMed Central PMCID: PMCPMC2212963.
94. Sugawara I, Yamada H, Li C, Mizuno S, Takeuchi O, Akira S. *Mycobacterial* infection in TLR2 and TLR6 knockout mice. *Microbiol Immunol.* 2003;47(5):327-36. Epub 2003/06/27. doi: 10.1111/j.1348-0421.2003.tb03404.x. PubMed PMID: 12825894.
95. BoseDasgupta S, Pieters J. Macrophage-microbe interaction: lessons learned from the pathogen *Mycobacterium tuberculosis*. *Semin Immunopathol.* 2018;40(6):577-91. Epub 2018/10/12. doi: 10.1007/s00281-018-0710-0. PubMed PMID: 30306257.
96. Kramarska E, Squeglia F, De Maio F, Delogu G, Berisio R. PE\_PGRS33, an Important Virulence Factor of *Mycobacterium tuberculosis* and Potential Target of Host Humoral Immune Response. *Cells.* 2021;10(1). Epub 2021/01/21. doi: 10.3390/cells10010161. PubMed PMID: 33467487; PubMed Central PMCID: PMCPMC7830552.
97. Bocchino M, Galati D, Sanduzzi A, Colizzi V, Brunetti E, Mancino G. Role of mycobacteria-induced monocyte/macrophage apoptosis in the pathogenesis of human tuberculosis. *Int J Tuberc Lung Dis.* 2005;9(4):375-83. Epub 2005/04/16. PubMed PMID: 15830742.
98. Sanchez D, Rojas M, Hernandez I, Radzioch D, Garcia LF, Barrera LF. Role of TLR2- and TLR4-mediated signaling in *Mycobacterium tuberculosis*-induced macrophage death. *Cell Immunol.* 2010;260(2):128-36. Epub 2009/11/19. doi: 10.1016/j.cellimm.2009.10.007. PubMed PMID: 19919859.
99. Lin J, Chang Q, Dai X, Liu D, Jiang Y, Dai Y. Early secreted antigenic target of 6-kDa of *Mycobacterium tuberculosis* promotes caspase-9/caspase-3-mediated apoptosis in macrophages. *Mol Cell Biochem.* 2019;457(1-2):179-89. Epub 2019/03/27. doi: 10.1007/s11010-019-03522-x. PubMed PMID: 30911956.
100. Ghorpade DS, Leyland R, Kurowska-Stolarska M, Patil SA, Balaji KN. MicroRNA-155 is required for *Mycobacterium bovis* BCG-mediated apoptosis of macrophages. *Mol Cell Biol.* 2012;32(12):2239-53. Epub

2012/04/05. doi: 10.1128/MCB.06597-11. PubMed PMID: 22473996; PubMed Central PMCID: PMCPMC3372268.

101. Ayelign B, Workneh M, Molla MD, Dessie G. Role Of Vitamin-D Supplementation In TB/HIV Co-Infected Patients. *Infect Drug Resist.* 2020;13:111-8. Epub 2020/02/06. doi: 10.2147/IDR.S228336. PubMed PMID: 32021325; PubMed Central PMCID: PMCPMC6959508.

102. Krutzik SR, Hewison M, Liu PT, Robles JA, Stenger S, Adams JS, et al. IL-15 links TLR2/1-induced macrophage differentiation to the vitamin D-dependent antimicrobial pathway. *J Immunol.* 2008;181(10):7115-20. Epub 2008/11/05. doi: 10.4049/jimmunol.181.10.7115. PubMed PMID: 18981132; PubMed Central PMCID: PMCPMC2678236.

103. Rivas-Santiago B, Hernandez-Pando R, Carranza C, Juarez E, Contreras JL, Aguilar-Leon D, et al. Expression of cathelicidin LL-37 during *Mycobacterium tuberculosis* infection in human alveolar macrophages, monocytes, neutrophils, and epithelial cells. *Infect Immun.* 2008;76(3):935-41. Epub 2007/12/28. doi: 10.1128/IAI.01218-07. PubMed PMID: 18160480; PubMed Central PMCID: PMCPMC2258801.

104. Lasunskaja EB, Campos MN, de Andrade MR, Damatta RA, Kipnis TL, Einicker-Lamas M, et al. *Mycobacteria* directly induce cytoskeletal rearrangements for macrophage spreading and polarization through TLR2-dependent PI3K signaling. *J Leukoc Biol.* 2006;80(6):1480-90. Epub 2006/09/29. doi: 10.1189/jlb.0106066. PubMed PMID: 17005905.

105. Eum SY, Kong JH, Hong MS, Lee YJ, Kim JH, Hwang SH, et al. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. *Chest.* 2010;137(1):122-8. Epub 2009/09/15. doi: 10.1378/chest.09-0903. PubMed PMID: 19749004; PubMed Central PMCID: PMCPMC2803122.

106. Borkute RR, Woelke S, Pei G, Dorhoi A. Neutrophils in Tuberculosis: Cell Biology, Cellular Networking and Multitasking in Host Defense. *Int J Mol Sci.* 2021;22(9). Epub 2021/05/06. doi: 10.3390/ijms22094801. PubMed PMID: 33946542; PubMed Central PMCID: PMCPMC8125784.

107. Hook JS, Cao M, Weng K, Kinnare N, Moreland JG. *Mycobacterium tuberculosis* Lipoarabinomannan Activates Human Neutrophils via a TLR2/1 Mechanism Distinct from Pam3CSK4. *J Immunol.* 2020;204(3):671-81. Epub 2019/12/25. doi: 10.4049/jimmunol.1900919. PubMed PMID: 31871022.

108. Gopalakrishnan A, Dietzold J, Verma S, Bhagavathula M, Salgame P. Toll-like Receptor 2 Prevents Neutrophil-Driven Immunopathology during Infection with *Mycobacterium tuberculosis* by Curtailing CXCL5 Production. *Infect Immun.* 2019;87(3). Epub 2018/12/19. doi: 10.1128/IAI.00760-18. PubMed PMID: 30559223; PubMed Central PMCID: PMCPMC6386542.

109. Andersson M, Lutay N, Hallgren O, Westergren-Thorsson G, Svensson M, Godaly G. *Mycobacterium bovis* bacilli Calmette-Guerin regulates leukocyte recruitment by modulating alveolar inflammatory responses. *Innate Immun.* 2012;18(3):531-40. Epub 2011/11/08. doi: 10.1177/1753425911426591. PubMed PMID: 22058091; PubMed Central PMCID: PMCPMC3548393.

110. Wieland CW, Knapp S, Florquin S, de Vos AF, Takeda K, Akira S, et al. Non-mannose-capped lipoarabinomannan induces lung inflammation via toll-like receptor 2. *Am J Respir Crit Care Med.* 2004;170(12):1367-74. Epub 2004/09/28. doi: 10.1164/rccm.200404-525OC. PubMed PMID: 15447943.

111. Tyne AS, Chan JGY, Shanahan ER, Atmosukarto I, Chan HK, Britton WJ, et al. TLR2-targeted secreted proteins from *Mycobacterium tuberculosis* are protective as powdered pulmonary vaccines. *Vaccine*. 2013;31(40):4322-9. doi: 10.1016/j.vaccine.2013.07.022. PubMed PMID: WOS:000324510500010.
112. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PEM, et al. Protection by BCG Vaccine Against Tuberculosis: A Systematic Review of Randomized Controlled Trials. *Clinical Infectious Diseases*. 2014;58(4):470-80. doi: 10.1093/cid/cit790. PubMed PMID: WOS:000331097800005.
113. Tran V, Liu J, Behr MA. BCG Vaccines. *Microbiol Spectr*. 2014;2(1):MGM2-0028-2013. Epub 2014/02/01. doi: 10.1128/microbiolspec.MGM2-0028-2013. PubMed PMID: 26082111.
114. Simpson ME, Petri WA. TLR2 as a Therapeutic Target in Bacterial Infection. *Trends in Molecular Medicine*. 2020;26(8):715-7. doi: 10.1016/j.molmed.2020.05.006. PubMed PMID: WOS:000561576400003.
115. Ahmed A, Dolasia K, Mukhopadhyay S. *Mycobacterium tuberculosis* PPE18 Protein Reduces Inflammation and Increases Survival in Animal Model of Sepsis. *Journal of Immunology*. 2018;200(10):3587-98. doi: 10.4049/jimmunol.1602065. PubMed PMID: WOS:000442364400028.
116. Nakajima H, Chiba A, Fukumoto M, Morooka N, Mochizuki N. Zebrafish Vascular Development: General and Tissue-Specific Regulation. *J Lipid Atheroscler*. 2021;10(2):145-59. Epub 2021/06/08. doi: 10.12997/jla.2021.10.2.145. PubMed PMID: 34095009; PubMed Central PMCID: PMCPCMC8159758.
117. van der Vaart M, Spaink HP, Meijer AH. Pathogen recognition and activation of the innate immune response in zebrafish. *Adv Hematol*. 2012;2012:159807. Epub 2012/07/20. doi: 10.1155/2012/159807. PubMed PMID: 22811714; PubMed Central PMCID: PMCPCMC3395205.
118. Li S, Yeo KS, Levee TM, Howe CJ, Her ZP, Zhu S. Zebrafish as a Neuroblastoma Model: Progress Made, Promise for the Future. *Cells*. 2021;10(3). Epub 2021/04/04. doi: 10.3390/cells10030580. PubMed PMID: 33800887; PubMed Central PMCID: PMCPCMC8001113.
119. Torraca V, Mostowy S. Zebrafish Infection: From Pathogenesis to Cell Biology. *Trends Cell Biol*. 2018;28(2):143-56. Epub 2017/11/28. doi: 10.1016/j.tcb.2017.10.002. PubMed PMID: 29173800; PubMed Central PMCID: PMCPCMC5777827.
120. Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nat Rev Drug Discov*. 2021. Epub 2021/06/13. doi: 10.1038/s41573-021-00210-8. PubMed PMID: 34117457.
121. Sieber S, Grossen P, Bussmann J, Campbell F, Kros A, Witzigmann D, et al. Zebrafish as a preclinical in vivo screening model for nanomedicines. *Adv Drug Deliv Rev*. 2019;151-152:152-68. Epub 2019/01/08. doi: 10.1016/j.addr.2019.01.001. PubMed PMID: 30615917.
122. Meijer AH, Spaink HP. Host-pathogen interactions made transparent with the zebrafish model. *Curr Drug Targets*. 2011;12(7):1000-17. Epub 2011/03/04. doi: 10.2174/138945011795677809. PubMed PMID: 21366518; PubMed Central PMCID: PMCPCMC3319919.
123. Torraca V, Cui C, Boland R, Bebelman JP, van der Sar AM, Smit MJ, et al. The CXCR3-CXCL11 signaling axis mediates macrophage recruitment and dissemination of mycobacterial infection. *Dis Model Mech*. 2015;8(3):253-69. Epub 2015/01/13. doi: 10.1242/dmm.017756. PubMed PMID: 25573892; PubMed Central PMCID: PMCPCMC4348563.

124. Hosseini R, Lamers GE, Soltani HM, Meijer AH, Spaink HP, Schaaf MJ. Efferocytosis and extrusion of leukocytes determine the progression of early mycobacterial pathogenesis. *J Cell Sci.* 2016;129(18):3385-95. Epub 2016/07/30. doi: 10.1242/jcs.135194. PubMed PMID: 27469488.
125. Clay H, Davis JM, Beery D, Huttenlocher A, Lyons SE, Ramakrishnan L. Dichotomous role of the macrophage in early *Mycobacterium marinum* infection of the zebrafish. *Cell Host Microbe.* 2007;2(1):29-39. Epub 2007/11/17. doi: 10.1016/j.chom.2007.06.004. PubMed PMID: 18005715; PubMed Central PMCID: PMCPMC3115716.
126. Yang CT, Cambier CJ, Davis JM, Hall CJ, Crosier PS, Ramakrishnan L. Neutrophils exert protection in the early tuberculous granuloma by oxidative killing of mycobacteria phagocytosed from infected macrophages. *Cell Host Microbe.* 2012;12(3):301-12. Epub 2012/09/18. doi: 10.1016/j.chom.2012.07.009. PubMed PMID: 22980327; PubMed Central PMCID: PMCPMC3638950.
127. Bernut A, Mai NC, Halloum I, Herrmann JL, Lutfalla G, Kremer L. *Mycobacterium abscessus*-Induced Granuloma Formation Is Strictly Dependent on TNF Signaling and Neutrophil Trafficking. *Plos Pathogens.* 2016;12(11). doi: ARTN e1005986  
10.1371/journal.ppat.1005986. PubMed PMID: WOS:000392193200021.
128. Pecora ND, Gehring AJ, Canaday DH, Boom WH, Harding CV. *Mycobacterium tuberculosis* LprA is a lipoprotein agonist of TLR2 that regulates innate immunity and APC function. *J Immunol.* 2006;177(1):422-9. Epub 2006/06/21. doi: 10.4049/jimmunol.177.1.422. PubMed PMID: 16785538.
129. Su H, Zhu S, Zhu L, Huang W, Wang H, Zhang Z, et al. Recombinant Lipoprotein Rv1016c Derived from *Mycobacterium tuberculosis* Is a TLR-2 Ligand that Induces Macrophages Apoptosis and Inhibits MHC II Antigen Processing. *Front Cell Infect Microbiol.* 2016;6:147. Epub 2016/12/06. doi: 10.3389/fcimb.2016.00147. PubMed PMID: 27917375; PubMed Central PMCID: PMCPMC5114242.
130. Jung SB, Yang CS, Lee JS, Shin AR, Jung SS, Son JW, et al. The mycobacterial 38-kilodalton glycolipoprotein antigen activates the mitogen-activated protein kinase pathway and release of proinflammatory cytokines through Toll-like receptors 2 and 4 in human monocytes. *Infect Immun.* 2006;74(5):2686-96. Epub 2006/04/20. doi: 10.1128/IAI.74.5.2686-2696.2006. PubMed PMID: 16622205; PubMed Central PMCID: PMCPMC1459749.
131. Chen ST, Li JY, Zhang Y, Gao X, Cai H. Recombinant MPT83 derived from *Mycobacterium tuberculosis* induces cytokine production and upregulates the function of mouse macrophages through TLR2. *J Immunol.* 2012;188(2):668-77. Epub 2011/12/17. doi: 10.4049/jimmunol.1102177. PubMed PMID: 22174456.
132. Das S, Bhattacharjee O, Goswami A, Pal NK, Majumdar S. Arabinosylated lipoarabinomannan (Ara-LAM) mediated intracellular mechanisms against tuberculosis infection: involvement of protein kinase C (PKC) mediated signaling. *Tuberculosis (Edinb).* 2015;95(2):208-16. Epub 2014/12/30. doi: 10.1016/j.tube.2014.11.007. PubMed PMID: 25544312.
133. Shukla S, Richardson ET, Drage MG, Boom WH, Harding CV. *Mycobacterium tuberculosis* Lipoprotein and Lipoglycan Binding to Toll-Like Receptor 2 Correlates with Agonist Activity and Functional Outcomes. *Infect Immun.* 2018;86(10). Epub 2018/07/25. doi: 10.1128/IAI.00450-18. PubMed PMID: 30037791; PubMed Central PMCID: PMCPMC6204744.



134. Gilleron M, Nigou J, Nicolle D, Quesniaux V, Puzo G. The acylation state of mycobacterial lipomannans modulates innate immunity response through toll-like receptor 2. *Chem Biol*. 2006;13(1):39-47. Epub 2006/01/24. doi: 10.1016/j.chembiol.2005.10.013. PubMed PMID: 16426970.
135. Gilleron M, Quesniaux VF, Puzo G. Acylation state of the phosphatidylinositol hexamannosides from *Mycobacterium bovis* bacillus Calmette Guérin and *mycobacterium tuberculosis* H37Rv and its implication in Toll-like receptor response. *J Biol Chem*. 2003;278(32):29880-9. Epub 2003/05/31. doi: 10.1074/jbc.M303446200. PubMed PMID: 12775723.
136. Bowdish DM, Sakamoto K, Kim MJ, Kroos M, Mukhopadhyay S, Leifer CA, et al. MARCO, TLR2, and CD14 are required for macrophage cytokine responses to mycobacterial trehalose dimycolate and *Mycobacterium tuberculosis*. *PLoS Pathog*. 2009;5(6):e1000474. Epub 2009/06/13. doi: 10.1371/journal.ppat.1000474. PubMed PMID: 19521507; PubMed Central PMCID: PMCPMC2688075.
137. Bulut Y, Michelsen KS, Hayrapetian L, Naiki Y, Spallek R, Singh M, et al. *Mycobacterium tuberculosis* heat shock proteins use diverse Toll-like receptor pathways to activate pro-inflammatory signals. *J Biol Chem*. 2005;280(22):20961-7. Epub 2005/04/06. doi: 10.1074/jbc.M411379200. PubMed PMID: 15809303.
138. Saraav I, Singh S, Pandey K, Sharma M, Sharma S. *Mycobacterium tuberculosis* MymA is a TLR2 agonist that activate macrophages and a TH1 response. *Tuberculosis (Edinb)*. 2017;106:16-24. Epub 2017/08/15. doi: 10.1016/j.tube.2017.05.005. PubMed PMID: 28802400.
139. Palucci I, Camassa S, Cascioferro A, Sali M, Anoosheh S, Zumbo A, et al. PE\_PGRS33 Contributes to *Mycobacterium tuberculosis* Entry in Macrophages through Interaction with TLR2. *PLoS One*. 2016;11(3):e0150800. Epub 2016/03/16. doi: 10.1371/journal.pone.0150800. PubMed PMID: 26978522; PubMed Central PMCID: PMCPMC4792380.
140. Zumbo A, Palucci I, Cascioferro A, Sali M, Ventura M, D'Alfonso P, et al. Functional dissection of protein domains involved in the immunomodulatory properties of PE\_PGRS33 of *Mycobacterium tuberculosis*. *Pathog Dis*. 2013;69(3):232-9. Epub 2013/10/10. doi: 10.1111/2049-632X.12096. PubMed PMID: 24106104.
141. Pattanaik KP, Ganguli G, Naik SK, Sonawane A. *Mycobacterium tuberculosis* EsxL induces TNF- $\alpha$  secretion through activation of TLR2 dependent MAPK and NF- $\kappa$ B pathways. *Mol Immunol*. 2021;130:133-41. Epub 2021/01/10. doi: 10.1016/j.molimm.2020.11.020. PubMed PMID: 33419561.
142. Nair S, Pandey AD, Mukhopadhyay S. The PPE18 protein of *Mycobacterium tuberculosis* inhibits NF- $\kappa$ B/rel-mediated proinflammatory cytokine production by upregulating and phosphorylating suppressor of cytokine signaling 3 protein. *J Immunol*. 2011;186(9):5413-24. Epub 2011/04/01. doi: 10.4049/jimmunol.1000773. PubMed PMID: 21451109.
143. Nair S, Ramaswamy PA, Ghosh S, Joshi DC, Pathak N, Siddiqui I, et al. The PPE18 of *Mycobacterium tuberculosis* interacts with TLR2 and activates IL-10 induction in macrophage. *J Immunol*. 2009;183(10):6269-81. Epub 2009/11/03. doi: 10.4049/jimmunol.0901367. PubMed PMID: 19880448.
144. Su H, Kong C, Zhu L, Huang Q, Luo L, Wang H, et al. PPE26 induces TLR2-dependent activation of macrophages and drives Th1-type T-cell immunity by triggering the cross-talk of multiple pathways involved in the host response. *Oncotarget*. 2015;6(36):38517-37. Epub 2015/10/07. doi: 10.18632/oncotarget.5956. PubMed PMID: 26439698; PubMed Central PMCID: PMCPMC4770718.



145. Deng W, Li W, Zeng J, Zhao Q, Li C, Zhao Y, et al. Mycobacterium tuberculosis PPE family protein Rv1808 manipulates cytokines profile via co-activation of MAPK and NF-kappaB signaling pathways. *Cell Physiol Biochem*. 2014;33(2):273-88. Epub 2014/02/15. doi: 10.1159/000356668. PubMed PMID: 24525621.
146. Xu Y, Yang E, Huang Q, Ni W, Kong C, Liu G, et al. PPE57 induces activation of macrophages and drives Th1-type immune responses through TLR2. *J Mol Med (Berl)*. 2015;93(6):645-62. Epub 2015/01/15. doi: 10.1007/s00109-014-1243-1. PubMed PMID: 25586105.
147. Liu Y, Li JY, Chen ST, Huang HR, Cai H. The rLrp of Mycobacterium tuberculosis inhibits proinflammatory cytokine production and downregulates APC function in mouse macrophages via a TLR2-mediated PI3K/Akt pathway activation-dependent mechanism. *Cell Mol Immunol*. 2016;13(6):729-46. Epub 2015/07/15. doi: 10.1038/cmi.2015.58. PubMed PMID: 26166760; PubMed Central PMCID: PMC45101441.
148. Sweet L, Zhang W, Torres-Fewell H, Serianni A, Boggess W, Schorey J. Mycobacterium avium glycopeptidolipids require specific acetylation and methylation patterns for signaling through toll-like receptor 2. *J Biol Chem*. 2008;283(48):33221-31. Epub 2008/10/01. doi: 10.1074/jbc.M805539200. PubMed PMID: 18824550; PubMed Central PMCID: PMC2586276.
149. Bhatnagar S, Schorey JS. Exosomes released from infected macrophages contain Mycobacterium avium glycopeptidolipids and are proinflammatory. *J Biol Chem*. 2007;282(35):25779-89. Epub 2007/06/27. doi: 10.1074/jbc.M702277200. PubMed PMID: 17591775; PubMed Central PMCID: PMC1836815.
150. Sweet L, Schorey JS. Glycopeptidolipids from Mycobacterium avium promote macrophage activation in a TLR2- and MyD88-dependent manner. *J Leukoc Biol*. 2006;80(2):415-23. Epub 2006/06/09. doi: 10.1189/jlb.1205702. PubMed PMID: 16760377.
151. Le Moigne V, Roux AL, Jobart-Malfait A, Blanc L, Chaoui K, Burlet-Schiltz O, et al. A TLR2-Activating Fraction From Mycobacterium abscessus Rough Variant Demonstrates Vaccine and Diagnostic Potential. *Front Cell Infect Microbiol*. 2020;10:432. Epub 2020/09/29. doi: 10.3389/fcimb.2020.00432. PubMed PMID: 32984067; PubMed Central PMCID: PMC7481331.
152. Roux AL, Ray A, Pawlik A, Medjahed H, Etienne G, Rottman M, et al. Overexpression of proinflammatory TLR-2-signalling lipoproteins in hypervirulent mycobacterial variants. *Cell Microbiol*. 2011;13(5):692-704. Epub 2010/12/15. doi: 10.1111/j.1462-5822.2010.01565.x. PubMed PMID: 21143571.
153. Briken V, Porcelli SA, Besra GS, Kremer L. Mycobacterial lipoarabinomannan and related lipoglycans: from biogenesis to modulation of the immune response. *Mol Microbiol*. 2004;53(2):391-403. Epub 2004/07/02. doi: 10.1111/j.1365-2958.2004.04183.x. PubMed PMID: 15228522.