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~~***Mini-review***~~

Role of toll-like receptor 2 in immune response against *Pseudomonas aeruginosa* and its pathological characteristics

Shiva Ahmadishoar[1] & Ashraf Kariminik[2]*

[1]Department of Microbiology, Malekan Branch, Islamic Azad University, Malekan, Iran

[2]Department of Microbiology, Kerman Branch, Islamic Azad University, Kerman, Iran

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Pseudomonas aeruginosa is an important opportunistic bacterium, which its resistant form can induce some complications in immunocompromised patients. The bacterium uses several mechanisms to overcome immune responses and makes a resistant form. Toll like receptors (TLRs) are the most well-known innate immune receptors that play key roles in the induction and stimulation of immune responses against bacteria. Accordingly, it has been hypothesised that *Pseudomonas aeruginosa* may cause significant interactions with the receptors to be resistance to immune responses and make persistence infection. Thus, the main aim of this review article is to discuss the roles played by toll like receptors in the induction of appropriate immune responses and also induction of the bacterium complications.

Keywords: Bacteria, Cytokine, Inflammation, Innate immunity, Resistance, Signalling pathways

Introduction

Pseudomonas aeruginosa is an opportunistic bacterium which uses several mechanisms to regulate its gene expression and alter immune responses[1]. The main mechanisms and pathogen recognition receptors (PRRs) used by *P. aeruginosa* for induction of some compilations, such as pathological inflammation, are yet to be clarified. PRRs are the main innate immune receptors to recognise microbes and consist of cell membrane and intra-cytoplasmic forms. Toll like receptors (TLRs) are the most well-known PRRs that play significant roles against gram-positive and negative bacteria, including *P. aeruginosa*[2-4]. TLR2 is the unique member of the TLRs family that makes either homo, with itself, or heterodimers, with TLR1 and TLR6[5]. The molecule plays dual roles during induction of immune responses against foreign factors, from induction of immune responses to modulation of the system[5]. Therefore, it may play dual roles against *P. aeruginosa*. Recent information regarding the roles played by TLR2 against *P. aeruginosa* is presented in this review article.

P. aeruginosa; a pathogenic bacterium

P. aeruginosa is an opportunistic pathogen that can be found everywhere and is commonly associated with various hospital-acquired infections. These infections often occur in relation to medical devices and procedures, such as endovascular catheters, mechanical ventilation, surgical wounds, and burn infections[6]. It is also frequently implicated in chronic respiratory diseases, including cystic fibrosis, bronchiectasis, and chronic obstructive pulmonary disease (COPD). This particular type of Gram-negative bacteria, which does not ferment carbohydrates, is of great concern due to its increasing rates of drug resistance. This resistance is mainly attributed to a combination of factors, including reduced permeability of the outer membrane, active expulsion of drugs from the bacterial cell, and the acquisition of mobile genetic elements containing antibiotic-resistant genes[7,8]. *P. aeruginosa* has also been classified as part of the ESKAPE group, a group of six bacteria that require special attention due to their high levels of antimicrobial resistance[9]. Furthermore, *P. aeruginosa* possesses the ability to produce a wide range of virulence factors, such as biofilms, exotoxins, siderophores, and secretion systems. This versatility allows the pathogen to pose a significant threat and adapt effectively to its everchanging environment[10]. *P. aeruginosa* infections can be associated with some complications that are pro-inflammatory based disorders. Thus, it seems that pathological immune responses against the bacterium may be a reason for induction of the disorders.

*Correspondence:
Phone: +98-9133413556 (Mob.)
E-mail: a.kariminik@iauk.ac.ir

Toll-like receptor 2 and *P. aeruginosa*

TLR2 (TIL4 and CD282) is a highly conserved molecule and was characterised in 1998[5]. This is a type-I trans-membrane protein and recognises several pathogens associated molecular patterns (PAMPs), including bacterial peptidoglycan, lipopolysaccharide (LPS), lipoteichoic acid, lipoprotein, porins, and viral hemagglutinin/ glycoproteins, and damage associated molecular patterns (DAMPs), such as high mobility group box 1 (HMGB1), human glycosaminoglycan hyaluronan, heat shock proteins, and β-defensin-3[5]. This is the unique TLR that makes either homodimer or heterodimer, by TLR1 and TLR6[11]. Following TLR2/ligand interactions, inductions of two categories of internal Signalling pathways are plausible, MAPKs (Mitogen-activated protein kinase) and Myeloid differentiation primary response protein 88 (MyD88) dependent Signalling pathways as pro-inflammatory responses and the phosphoinositide 3-kinase (PI3K)/Akt pathway, as the plausible inducers of anti-inflammatory molecules, such as IL-10[5]. The Signalling pathways are presented in detail by Bagheri et al., 2014[5].

Due to the introduction regarding the functions of TLR2, it has been hypothesised that the molecule may play dual roles against *P. aeruginosa*. TLR2 may be a main factor against *P. aeruginosa* to control its infections and, accordingly, may participate in its pro-inflammatory related compilations. Indeed, the activation of Pattern Recognition Receptors (PRRs) can also induce immunologic tolerance against *P. aeruginosa* antigens. One of the mechanisms by which PRR activation contributes to immunologic tolerance is through the induction of regulatory immune responses. Upon recognition of *P. aeruginosa* antigens by PRRs, specific immune cells, such as regulatory T cells (Tregs), may be activated. Tregs play a crucial role in suppressing excessive immune responses and promoting immune tolerance. They can inhibit the activation and function of effector immune cells, such as T cells and macrophages, thereby preventing excessive inflammation and tissue damage[12,13]. To confirm the hypothesis, there are several investigations that confirmed the role played by TLR2 against *P. aeruginosa* in controlling its infection. Entezari *et al.*[12] showed that functional TLR2 Signalling pathways play key roles in the activation of macrophages phagocytosis against *P. aeruginosa* in a HMGB1 dependent manner . Shin *et al.*[13] revealed that *P. aeruginosa* infection is an important cause of TLR2 upregulation, and then subsequently enhances the pro-inflammatory innate immune responses against other infections. Another investigation showed that β-Glucan via interactions with TLR2 enhances phagocytosis, production of reactive oxygen species, glycolytic and oxidative metabolism, and mitochondrial mass in the macrophages against *P. aeruginosa*[14]. The significant roles played by TLR2 alone or in a synergistic manner with other PRRs have been demonstrated by Xaplanteri *et al.*[15]. TLR2 can also recognise the key *P. aeruginosa* quorum sensing signal molecule, N-3-oxododecanoyl-homoserine lactone (3OC12HSL), and then induction of IL-8 against the bacteria[16]. 3OC12HSL has various effects on the expression of TLR2, as Bao *et al.*[17] and Lu *et al.*[18]. reported that 3OC12HSL increases and decreases the expression of TLR2 in the positive and negative feedbacks, respectively.

Although TLR2 plays some roles in the induction of immune responses against *P. aeruginosa*, some investigations revealed that its roles may not be significantly necessary because in the mice lacking TLR2, but natural TLR4 and 5, there are normal immune responses and limited infections[19]. It was also documented by other investigators, which reported TLR2 lack was not associated with severe defect in normal immune responses against *P. aeruginosa* [20-22]. However, defections in other TLRs, such as TLR4 or TLR5, can be associated with defective immune responses against the bacterium[20]. Accordingly, Zhang *et al.*[23] revealed that the lack of TLR5 blocked the response to *P. aeruginosa*, however, it was not seen in the lack of TLR2 Signalling. Lack of TLR2 also was not associated with susceptibility to *P. aeruginosa* adhesion to the corneal epithelial cells[24]. Ramphal *et al.*[25] also demonstrated that TLR2 is not the main responsible PRR involved in hyper susceptibility to animal model of acute *P. aeruginosa* lung infections. Sun *et al.*[26] reported that although TLR2 is upregulated during *P. aeruginosa*-related keratitis, the PRR did not play a key role in decreasing proliferation of the bacterium. Another investigation revealed that although toll/IL-1R domain-containing adaptor protein (TIRAP), the adaptor protein for TLR2, is a critical factor for defence in the lung against Klebsiella pneumonia, it does not play significant roles against *P. aeruginosa*[27]. Due to the investigations, it appears

that there is controversial information regarding the roles played by TLR2 against *P. aeruginosa*. However, the most investigations were performed on the *P. aeruginosa*-derived molecules[28], but not on the live bacterium, so the studies on the live bacteria may be associated with different results. Accordingly, Lagoumintzis *et al.*[29] showed that *P. aeruginosa* viable bacterium TLR2 mediated immune responses against the bacterium more efficiently than the *P. aeruginosa* derived LPS[29]. In addition, the roles played by TLR2 against *P. aeruginosa* significantly rely on the bacterium's functions. Accordingly, *P. aeruginosa* growing in a medium of cystic fibrosis sputa leads to up-regulation of TLR2-related IL-8 and in phases of *P. aeruginosa* biofilm, the TLR pathway did not contribute to IL-8 production[30]. Coinfection with other bacteria may also alter the functions of TLR2 against *P. aeruginosa*. For example, Chekabab *et al.*[31] reported that *Staphylococcus aureus* inhibits production of TLR2 related IL-8 against *P. aeruginosa* in airway epithelial cells[31]. In contrast, in the coinfection of *Streptococcus pneumoniae* and *P. aeruginosa*, TLR2 expressions are upregulated in the corneal ulcers[32]. Additionally, *P. aeruginosa* may have anti TLR2 mechanism to regulate its function. For example, an investigation by Bertelsen *et al.*[33] revealed that immune responses dependent on TLR2 are more effective against *Pseudomonas nigrescens* than *P. aeruginosa*[33]. Zhang *et al.*[34] reported that *P. aeruginosa* encoded unknown proteins that are encoded by 3880 (PA3880) gene and down-regulates the functions of TLR2 via either direct interaction with the molecules or indirectly affecting its-related intracellular Signalling pathways. A study revealed that *P. aeruginosa* induces expression of miR-302b via interaction with TLR2 and through ERK-p38-NF-κB Signalling pathway[35]. miR-302b plays antiinflammatory roles during infection with the bacterium[35]. It has been documented that the functions of TLR2 on immune cells are dependent on the interaction with other cells. For instance, Martin and Prince[36] revealed that the gap junction channels between the airway epithelial cells participate in the movement of Ca (2^+) from cell to cell, the main inducers of TLR2 intracellular Signalling pathways. In addition, Ca (2^+) that is generated by *P. aeruginosa* induced TLR2 signals can activate calpains, the cysteine proteases, and then cleavage the transmembrane junctional proteins occludin and E-cadherin[37]. The cleavage leads to increased transepithelial migration of immune cells in response to TLR2 agonists in a mouse model of *P. aeruginosa* infection. Additionally, in the case of activation of several TLRs simultaneously, but not TLR2 lonely, antibacterial immune responses are plausible by TLR2 Signalling pathways[37]. Together, it appears that several factors determine the main roles played by TLR2 during *P. aeruginosa* infections, including interaction of TLR2 with a live bacterium, the bacterium phases and functions, coinfection with other bacteria, induction of tolerance by *P. aeruginosa*, interaction with other non-immune cells, and activation of several TLRs simultaneously. The plausible mechanisms are presented in figure 1.

Pathological roles played by TLR2 during *P. aeruginosa* infection

TLR2 (Toll-like receptor 2) may not have critical roles in the defence against *P.aeruginosa*, its involvement in the pathogenesis of infections caused by this bacterium and its associated complications, such as cystic fibrosis, has been previously established[38]. Accordingly, Jin *et al.*[39] showed that TLR2 does not participate in the induction of immune responses in the early phases of *P. aeruginosa* keratitis, it was upregulated in the late phases of the disease. In case of keratitis, the activation of TLR2 by PAMPs present on the invading pathogens triggers the release of pro-inflammatory cytokines like interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These cytokines attract immune cells to the cornea, stimulate the production of antimicrobial peptides, and promote the activation and migration of immune cells to the site of infection. The influx of immune cells, particularly neutrophils, helps to clear the infection but also contributes to the inflammatory response, leading to symptoms such as redness, swelling, and pain associated with keratitis. Overall, the activation of PRRs, particularly TLR2, in keratitis triggers an innate immune response characterised by the release of pro-inflammatory mediators, recruitment of immune cells, and the initiation of inflammation to combat the invading pathogens[39]. A study by Gally *et al.*[40] revealed that cigarette smoking was associated with decreased expression of fatty acid binding protein 5 (FABP5) in airway epithelial cells and promotes *P. aeruginosa* infection. Interestingly, down-regulation of FABP5 was associated with up-regulation of TLR2 and increased expression of IL-8, the most important chemokine for recruitment of

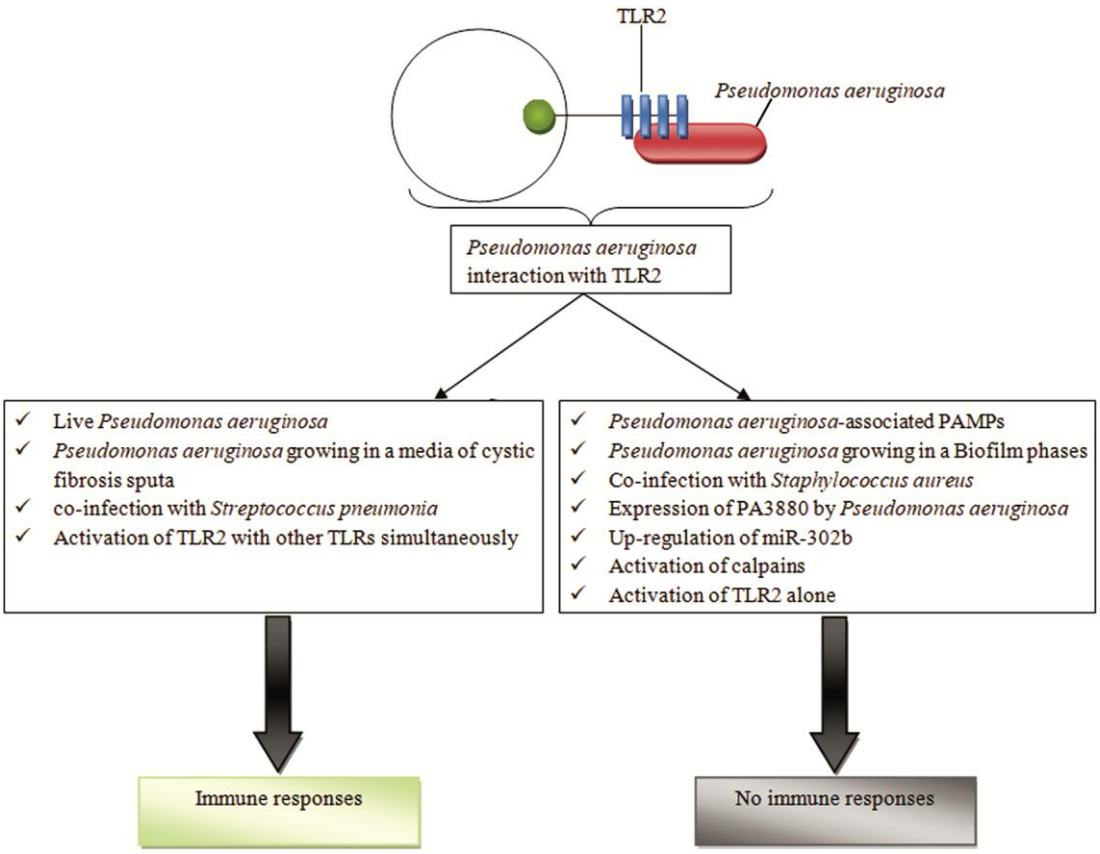

Fig. 1 — Outcome of *P. aeruginosa* and TLR2 interaction. Several factors determine the main roles played by TLR2 during *P. aeruginosa* infections. Interaction of TLR2 with live bacterium, *P. aeruginosa* growing in a media of cystic fibrosis sputa, coinfection with *Streptococcus pneumonia* and activation of TLR2 with other TLRs simultaneously lead to activation of immune cells, while interaction of TLR2 with *P. aeruginosa*-associated PAMPs, *P. aeruginosa* growing in a Biofilm phases, coinfection with *Staphylococcus aureus*, expression of PA3880 by *P. aeruginosa*, up-regulation of miR-302b, activation of calpains and activation of TLR2 alone are not associated with activation of immune cells.

neutrophils and inflammation[40]. TLR2 can increase expression of IL-8 in the pro-inflammatory compilations of *P. aeruginosa* via up-regulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[41]. Indeed, TLR2 activation can lead to increased expression of interleukin-8 (IL-8) in the pro-inflammatory responses associated with *P. aeruginosa* infections. This upregulation occurs through the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. When TLR2 recognises specific components of *P. aeruginosa*, it triggers intracellular Signalling pathways that result in the activation of NADPH oxidase. NADPH oxidase is an enzyme complex responsible for generating reactive oxygen species (ROS) within immune cells, such as neutrophils and macrophages. The activation of NADPH oxidase by TLR2 leads to the production of ROS, including superoxide anions. These ROS play a crucial role in Signalling cascade that leads to the increased expression of IL-8. The ROS generated by NADPH oxidase activate various downstream Signalling pathways, including the activation of transcription factors such as nuclear factor-kappa B (NF-κB).NF-κB, upon activation, translocates into the nucleus and binds to the promoter region of the IL-8 gene, promoting its transcription and subsequent expression. As a result, IL-8 is upregulated and released into the surrounding tissue. IL-8 is a potent chemokine that attracts and activates neutrophils, leading to their recruitment to the site of infection and further amplifying the inflammatory response. Therefore, TLR2 activation in the context of *P. aeruginosa* infections can stimulate NADPH oxidase activity, resulting in the generation of ROS and subsequent activation of NF-κB. This leads to the up-regulation of IL-8 expression, which plays a crucial role in the recruitment and activation of neutrophils, contributing to the pro-inflammatory response observed in *P. aeruginosa* associated

complications[41].Therefore, it appears that activation of TLR2 decreased immune defense against *P. aeruginosa* and increased the pro-inflammatory responses. The pro-inflammatory roles played by TLR2 in inducing injuries to the *P. aeruginosa* infected cells have also been documented by Sharma *et al.*[42]. They revealed that ODSH (2-O, 3-O-desulfated heparin), a heparin derivative with significant antiinflammatory properties, inhibits binding of HMGB1 to TLR2 and reduces the pathological pro-inflammatory responses against *P. aeruginosa*[42]. OprC is one of the bacterial porins that induces quorum sensing and attenuates immune responses against *P. aeruginosa*. However, the molecule can be recognised by TLR2 and it leads to inflammasome activation and increased production of pro-inflammatory cytokines[43]. The inflammatory condition can be associated with several pathological compilations of *P. aeruginosa* infection[43]. Paolillo *et al.*[44] revealed that the culture supernatants from *P. aeruginosa* containing lipase directly increased functions of mononuclear cells in the immunocompromised patients through interactions with TLR2. Additionally, it has been demonstrated that during cystic fibrosis, the presence of the mucoid phenotype of *P. aeruginosa* can be a marker for poor survival[45]. Interestingly, Beaudoin *et al.*[45] demonstrated that TLR2 crucially participates in the induction of mucoid phenotype in the *P. aeruginosa* infected patients who suffered from cystic fibrosis. The results were confirmed by Firoved *et al.*[46] who reported the main roles played by TLR2 against Pseudomonas lipoproteins to induce mucoid phenotype in the *P. aeruginosa* infected patients. Mizutani *et al.*[47] also proved the results and revealed that the intracellular Signalling of TLR2 is more resistant to the antiinflammatory effects of corticosteroids when compared to other TLRs and is more capable of inducing pathological inflammation in the cystic fibrosis mediated by *P. aeruginosa*. Farias and Rousseau[48] also demonstrated that pathological inflammation during infection with *Pseudomonas lipoproteins* in cystic fibrosis can be induced in TLR2-IL-33 mediated pathway[48]. In addition, exoenzyme S, as an important *P. aeruginosa* virulence factor, induces potent pro-inflammatory cytokines and chemokines via interactions with TLR2[49]. Another investigation on animal models revealed that using chicken-derived peptide cathelicidin-2 (CATH-2) can limit pathological inflammation by inhibiting TLR2, and hence increase survival of the *P. aeruginosa* infected animals[50]. YCG063, an inhibitor of reactive oxygen species, inhibits *P. aeruginosa* related pathological inflammation, which is mediated through the TLR2-mediated AKT/NF-κB pathway[51]. Accordingly, YCG063 either blocks the Signalling pathway or down regulates expression of TLR2[51]. The pathological roles played by TLR2 in the induction of pathological inflammation during *P. aeruginosa* infection have also been demonstrated by several investigations[52-54]. Thus, it may be hypothesised that TLR2 is a main factor that participates in the inflammatory complications of *P. aeruginosa* infection, and accordingly, can be considered for future immunotherapy against some disorders, like cystic fibrosis. In addition to induce pathological inflammation in the *P. aeruginosa* infected patients, TLR2 may also participate in other *P. aeruginosa* related compilations. For example, it has been reported that TLR2 can facilitate adhesion of *P. aeruginosa* to human cells in Lyn, a critical B cell Signalling kinase, dependent manner[55]. Another study revealed that TLR2 can inhibit wound healing via interaction with lipopolysaccharide (LPS) derived from *P. aeruginosa*[56]. TLR2 can also induce autophagy in the *P. aeruginosa* infected alveolar macrophages[57].

Collectively, there is a need for further exploration of the roles of TLR2 in the pathogenesis of Pseudomonas aeruginosa, as its utilisation and Signalling pathways appear to be complex and somewhat contradictory. Figure 2 illustrates the plausible pathological roles played by TLR2 in the *P. aeruginosa* infected patients. Due to the results authors suggest to explore more TLR2-ligands to manipulate *P. aeruginosa* infection and its complications. Indeed, the fact that TLR2 shares common intracellular Signalling molecules with other Toll-like receptors (TLRs) presents an opportunity for manipulating diseases by utilising other TLR ligands. By utilising ligands specific to other TLRs, it is possible to modulate the immune response and potentially manipulate the course of the disease. Additionally, the use of synthetic ligands or agonists specific to TLRs can also be explored. These ligands can selectively activate specific TLRs and modulate the immune response accordingly. Such approaches have shown promise in experimental studies and have the potential to be developed into therapeutic

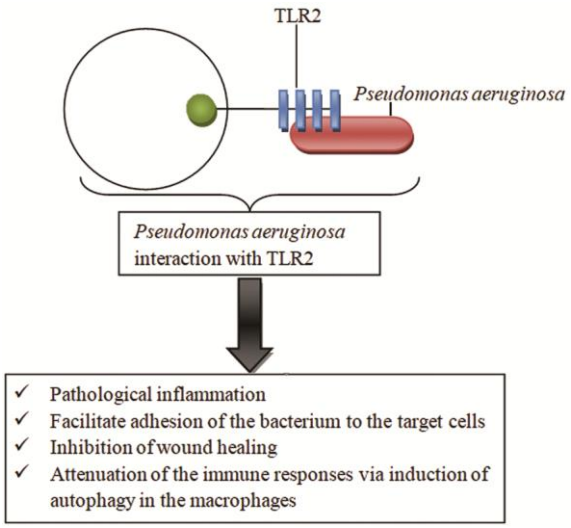

Fig. 2 — *P. aeruginosa* utilises TLR2 and its associated Signalling pathways to trigger certain complications. Pathological inflammation, facilitate adhesion of the bacterium to the target cells, inhibition of wound healing, and attenuation of the immune responses via induction of autophagy in the macrophages are the main pathological roles played by *P. aeruginosa*-TLR2 interactions.

strategies for manipulating diseases. However, it is essential to note that the manipulation of TLR Signalling for therapeutic purposes requires careful consideration and extensive research. Modulating the immune response can have both positive and negative effects, and the specific context of the disease and individual patient factors need to be taken into account. In summary, the utilisation of other TLR ligands presents an avenue for manipulating diseases by targeting common intracellular signalling pathways. Further research and understanding of the specific disease mechanisms are necessary to determine the potential efficacy and safety of such approaches.

Conflict of interest

Authors have no conflict of interest to declare.

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