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Review article

# Clinical Pathogen *Stenotrophomonas maltophilia*: Role of Flagella in Immunostimulation of Respiratory Tract and Possibility of Using as an Adjuvant

Ayaid Khadem Zgair[1*], Sanjay Chhibber[2]

**ABSTRACT**

Finding a safe and cheap immune stimulant is one of the most important challenges facing immunologists working in vaccine production. The other challenge is finding a way to improve the immune response of people who suffer from an immune suppressive phenomenon. *Stenotrophomonas maltophilia* is considered one of the controversial pathogens because it is an opportunistic bacterium, but its infection rate increases over time. In the current study, the most important studies that dealt with pathological and immunological perspectives of *S. maltophilia* will be reviewed. Furthermore, it sheds light on flagella protein (flagellin) and its role in stimulating the innate immune response as well as the safe pro-inflammatory immune response. The current study showed that the flagella of *S. maltophilia* has the ability to stimulate the innate immune response, as well as the pro-inflammatory immune response, through binding to the toll-like receptor (TLR) 5 receptor, which in turn stimulates internal cellular interactions that result in the cells secreting immune mediators, including pro-inflammatory cytokines and chemokine. As well as activating various immune cells, especially phagocytic cells in the respiratory system, and increasing neutrophil infiltration to the mucosal layer of the respiratory tract system, which in turn increases the host response to other external pathogens. Studies have shown that administrating flagellin protein will stimulate the mucosal immune response in the respiratory system and help the host resist infectious diseases. These results pave the way for a belief that confirms the possibility of using this protein as an adjuvant in the future after conducting many in vivo experiments related to the evaluation toxicity of flagellin.

**Keywords:** Adjuvant, Innate immunity, Opportunistic pathogen, Respiratory tract, *Stenotrophomonas maltophilia.*

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## 1. INTRODUCTION

*Stenotrophomonas maltophilia* has emerged as an important opportunistic pathogen affecting primarily the hospitalized and debilitated hosts. It does not appear to be inherently virulent, and it is an uncommon cause of invasive infections [1]. In essence, *S. maltophilia* is an emerging human pathogen that increasingly poses a challenge to clinicians, microbiologists, and infection-control specialists [2]. The most common and severe clinical manifestations of *S. maltophilia* infection in humans include bacteremia, endocarditis, and respiratory tract diseases [3]. There are many factors that help bacteria to produce a strong immune response such as outer membrane protein, lipopolysaccharide, and flagellin. These antigens stimulate both the arms of immune response strongly [4]. Flagellin is commonly used to stimulate the immune response in different organs, esp-

* Correspondence: **Professor Ayaid K. Zgair**, E. mail: ayaid.zgair@sc.uobaghdad.edu.iq
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article.

ecially in the respiratory tract. Flagellin treatment stimulates the release of proinflammatory mediators and nitric oxide (NO) *in vitro* and *in vivo* [5]. However, the impact of flagellin on innate and adaptive immunity in the lung is clearly important, given the role of flagellin as a virulence factor [6]. TLRs are key components of the immune system that detect microbial infection and trigger antimicrobial host defense responses. TLR5 is a sensor for monomeric flagellin [7]. TLR5 engagement by bacterial flagellin activates the MyD88-dependent signaling pathway, which leads to the nuclear translocation of NF-KB and the activation of the MAPKs, ultimately inducing the maturation of antigen-presenting cells and the secretion of proinflammatory cytokines and chemokines. TLR5 is expressed by a variety of cells including monocytes, dendritic cells (DCs), epithelial cells, and mast cells [8]. The results of different studies suggest that flagellin recognition in different organs is mediated by distinct TLR5–expressing cells and provides insights into the cooperation of the TLR5 and TLR4 signaling pathways used by the innate immune system in the recognition of bacterial pathogens [9].

Flagellin has a good ability to activate the innate immune response mediated through the activation of antigen-presenting cells (APCs) directly, increase neutrophil infiltration, and elevate the level of all molecules that may be helpful in innate immune response [5]. Moreover, flagellin stimulates innate immunity, thereby promoting the development of subsequent adaptive immune responses. Dendritic cells are critical for the induction of an effective adaptive immune response, connecting the innate and adaptive immune systems by presenting antigens from the site of exposure to naive lymphocytes in secondary locations [10]. Thus flagellin administration may provide a clinically useful approach to prevent infections in patients treated with broad-spectrum antibiotics. Kinnebrew *et al.* (2010) proved that systemic administration of flagellin to antibiotic-treated mice dramatically reduced vancomycin-resistant enterococcus (VRE) colonization, by enhancing mucosal resistance due to the development of innate immunity against multi-drug-resistant organisms [11]. The previous study demonstrated that intranasal pretreatment of mice with purified *S. maltophilia* flagellin induced strong protection against intratracheal *S. maltophilia* and *staphylococcus aureus*, which was attributable to markedly improved bacterial clearance, reduced dissemination, and decreased alveolar permeability [13]. The present study highlighted the effect of *S. maltophilia* flagella on the stimulation of the innate immune response and the possibility of using flagellin (LPS free) to stimulate the pro-inflammatory immune response positively, which supports the hypothesis of using flagellin as an adjuvant in future.

## 2. Flagella

The most common and best-studied of all prokaryotic motility structures is the bacterial flagellum. It is composed of over 20 protein species with approximately 30 other proteins required for regulation and assembly, it is one of the most complex of all prokaryotic organelles. Well understood in its own right as a motility structure, it has become a model system for type III secretion systems in general [14]. The bacterial flagellum is a rotary structure driven by a motor at the base, with the filament acting as a propeller [15]. The flagellum consists of three major substructures: the filament, the hook, and the basal body. The filament is typically about 20 nm in diameter and usually consists of thousands of copies of a single protein called flagellin [16]. Less commonly the filament is composed of several different flagellins. At the tip of the flagellum is the capping protein. Hook-associated protein 2 (HAP2) connects the filament to the basal body. The junction of the hook and filament requires the presence of a small number of two hook-associated proteins called HAP1 and HAP3 [17]. The basal structure consists of a rod, a series of rings, the Mot proteins, the switch complex, and the flagellum-specific export apparatus. The rings anchor the flagellum to the cytoplasmic membrane (MS ring), the peptidoglycan (P ring), and the outer membrane (L ring). Gram-positive bacteria have flagella that lack the P and L rings [18].

The engine is powered by proton motive force. Motility protein A (MotA) and motility protein B (MotB) proteins form a channel through which the protons that power the rotation of the flagellum flow. Flagellin is a protein that arranges itself in a hollow cylinder to form the filament in the bacterial flagellum. It has a mass of about 30,000 to 60,000 Dalton. The structure of flagellin is responsible for the helical shape of the flagellar filament which is important for its proper function. The flagellin variable domain protrudes outwards and stacked together with other flagellin monomers, forms the external surface of the filament [19]. The surface exposed variable domain of the flagellin is antigenically diverse and forms the basis of a wide range of typing methods exploiting the diversity of the structural difference at the protein level for strain identification. The diversity in flagellin structure has been used as a new strategy for strain identification of *Sphingomonas* sp. strain A1 and *Clostridium botulinum* [20, 21]. Flagella are often involved in pathogenesis, with role in motility, adhesion, and in some cases the secretion of virulence factors [22].

## 3. *S. maltophilia* FLAGELLA

*S. maltophilia* is a polytrichious flagellated bacteria (Fig. 1). There are several studies available in the literature that monitored the characterization of *S. maltophilia* flagella and its flagellin protein. In 1983 Montie and Stover purified flagella from several *pseudomonas* species including *P. maltophilia* strain B69 (now referred to as *S. maltophilia*) by centrifugation method and found that B69 produced a flagellin that had a molecular mass of 33 kDa [23]. They found that anti-sera against flagella of *P. aeruginosa* and *P. cepacia* did not agglutinate *P. maltophilia* bacteria, suggesting the absence of antigenic cross–reactivity between these flagella. No further biochemical characterization of *S. maltophilia* flagella has been done. De Oliveira-Garcia *et al*. (2002) described the purification and characterization of *S. maltophilia* flagella but they used different methods of purification. The flagella produced by *S. maltophilia* strains are composed of a 38-kDa flagellin subunit. The identity of this polypeptide was demonstrated by N-terminal amino acid sequencing analysis. They also raised specific antibodies to study the production of flagella in a collection of clinical isolates, They found after electron microscopy that organisms had only one polar flagellum and others had several flagella structures, the flagella filaments were around 45 nm in width and >15 µm long [24]. In a previous study by Zgair and Chhibber (2009), they proved the The involvement of flagella in the adhesion process was evaluated by employing anti-flagellin antibodies or flagellin as inhibitors of adhesion. To achieve this, flagellin C was purified from wild-type clinical isolate (Sm2) that showed maximum adhesion *in vitro*. The molecular weight of pure preparation was 42 kDa on SDS-PAGE and antisera raised in rabbits against this preparation confirmed its purity on immunoblotting. The receptors were blocked by the treatment of bacteria either with anti-flagellin or pretreatment of mouse trachea or HEp-2 cells with purified flagellin. Reduced bacterial adherence in the presence of flagellin antisera and pure flagellin collectively

confirmed the role of flagella in adhesion to biotic surfaces. However, antiserum was more effective in reducing the adhesion probably due to its dual mode of action. It not only reduced the binding of adhesins on the flagella to specific receptors but also inhibited the motility of *S. maltophilia*. The inability of the bacterial colony to spread through agar containing antiserum confirmed the effect of antibodies on the motility of the bacterium [25]. Moreover, the same investigator proved that *S. maltophilia* has the ability to adhere to mouse tracheal mucus and that flagella plays an important role in this process. However, further studies using genetically defined mutants lacking flagella are needed to support this observation [26]. The same investigator proved that the instillation of experimental animals with purified (endotoxin-free) flagella generated a pro-inflammatory response and provided protection against different bacterial species [12].

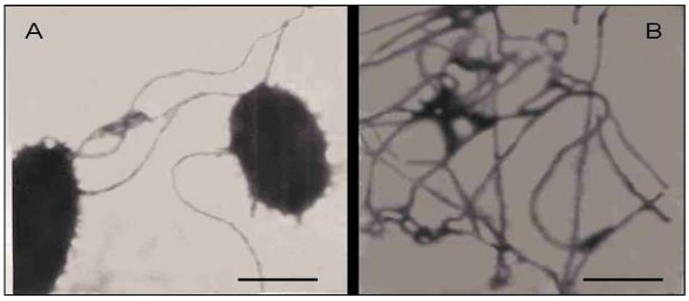


Fig. 1. Transmission electron micrographs showing the polar arrangement of flagella on *S. maltophilia* cells (a) and the purity of the flagellar preparation (b). The cells and polar flagellar preparation were negatively stained. Bars, 0.5 mm (a) and 0.25 mm (b) [26].

## 4. RESPIRATORY TRACT INFECTIONS

*S. maltophilia* has been reported to account for 5 % of nosocomial pneumonia. Nosocomial pneumonia has been observed during outbreaks of *S. maltophilia* infection, with several cases occurring over relatively short periods [27]. The respiratory tract is the most common site of isolation of *S. maltophilia* in hospitalized patients, accounting for the origin of 56 to 69 % of isolates, although the majority of patients (53 to 71%) with *S. maltophilia*-positive respiratory tract cultures are colonized rather than infected at this site [28]. More rigorous diagnostic criteria are therefore necessary if a distinction between colonization and true infection is to be made. *S. maltophilia* nosocomial pneumonia is associated with mechanical ventilation, tracheostomy, previous exposure to broad-spectrum antibiotics, use of respiratory tract equipment such as nebulizers [29] and therapy with aerosolized polymyxin. Respiratory tract involvement with *S. maltophilia* is associated with significantly increased mortality. It was reported that isolation of a “high-risk” pathogen such as *S. maltophilia* was the most important predictor of mortality in late-onset ventilator-associated pneumonia. The mortality rate in neutropenic patients with pneumonia is 40% [30]. In one *in vitro* study of *S. maltophilia* and influenza it was concluded that co-infection with *S. maltophilia* could enhance the pathogenicity of equine influenza virus [31].

## 5. FLAGELLIN STIMULATES IMMUNE SYSTEM

Flagellin, the major structural protein of bacterial flagella, signals via TLR5 and stimulates the immune system [8]. Flagellin treatment stimulates the release of proinflammatory mediators such as TNF-α, IL-1β, IL-6, IL-8, and nitric oxide (NO) *in vitro* and *in vivo* [5]. Although previous studies have established that flagellin induces systemic inflammatory responses when administered intraperitoneally or intravenously, the effects of flagellin on mucosal immunity in the lung have not been explored clearly. The impact of flagellin on innate and adaptive immunity in the lung is clearly important, given the role of flagellin as a virulence factor [6]. TLRs are key components of the immune system that detect microbial infection and trigger antimicrobial host defense responses. TLR5 is a sensor for monomeric flagellin [8]. TLR5 engagement by bacterial flagellin activates the MyD88-dependent signaling pathway, which leads to the nuclear translocation of NF-ΚB and the activation of the MAPKs, ultimately inducing the maturation of antigen-presenting cells and the secretion of proinflammatory cytokines and chemokines [8]. TLR5 is expressed by a variety of cells including monocytes, dendritic cells (DCs), epithelial cells, and mast cells [8]. The results of different studies suggest that flagellin recognition in different organs is mediated by distinct TLR5–expressing cells and provides insights into the cooperation of the TLR5 and TLR4 signaling pathways used by the innate immune system in the recognition of bacterial pathogens [7].

On the basis of the previous report, it was suggested that flagellin administration may provide a clinically useful approach to prevent infections in patients treated with broad-spectrum antibiotics. Kinnebrew *et al.* (2010) proved that systemic administration of flagellin to antibiotic-treated mice dramatically reduces vancomycin-resistant enterococcus (VRE) colonization, by enhancing mucosal resistance of innate immunity against multi-drug-resistant organisms. In a recent study, it has also been demonstrated that intranasal pretreatment of mice with purified *P. aeruginosa* flagellin induced strong protection against intratracheal *P. aeruginosa*-induced lethality, which was attributable to markedly improved bacterial clearance, reduced dissemination, and decreased alveolar permeability [11, 32]. Muñoz *et al.,* (2010) has also suggested that mucosal treatment with flagellin improved *S. pneumoniae* clearance in the lungs and promoted increased survival of infected host [33].

## 6. FLAGELLIN & INNATE IMMUNE SYSTEM

Flagellin plays a significant role in the mucosal innate immune system of the respiratory tract, primarily through its interaction with Toll-like receptor 5 (TLR5) and the activation of downstream signaling pathways. In present study we focused on some key aspects of the role of flagellin in the mucosal innate immune system of the respiratory tract such as detection of Flagellin [8]. Flagellin is a protein component of bacterial flagella, which are whip-like appendages that some bacteria use for motility. When bacteria bearing flagellin are inhaled or enter the respiratory tract, the mucosal innate immune system can detect flagellin as a potential sign of infection. The flagellin bind to TLR-5 which is a pattern recognition receptor (PRR) expressed on the surface of various cells, including epithelial cells lining the respiratory tract. TLR5 is responsible for recognizing and binding to flagellin, initiating the immune response. Upon binding to flagellin, TLR5 triggers intracellular signaling pathways, such as the NF-κB and MAPK pathways [34].

These signaling cascades lead to the production of proinflammatory cytokines and type I interferons. The activation of TLR5 by flagellin results in the release of proinflammatory cytokines like interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α). These cytokines recruit immune cells, such as neutrophils and macrophages, to the site of infection or

inflammation [35]. Neutrophils and macrophages are important components of the innate immune response in the respiratory tract. They help eliminate invading bacteria and contribute to the clearance of infection. Flagellin-induced innate immune responses can also influence the adaptive immune system. By activating antigen-presenting cells, such as dendritic cells, flagellin can promote the development of adaptive immune responses, including the production of specific antibodies and the activation of T cells [36]. Regulation of Tolerance, its role in host defense, and flagellin-TLR5 interactions can also play a role in regulating immune tolerance in the respiratory tract. Under cert-ain conditions, flagellin exposure can lead to immune tolerance, preventing excessive inflammation and allergic reactions [37].

*S. maltophilia* possesses flagellin as a structural component of its flagella. The role of *S. maltophilia* flagellin in the mucosal innate immune system of the respiratory tract can be understood as follows. When *S. maltophilia* or its flagellated forms enter the respiratory tract, flagellin can be recognized by the host's innate immune system as a potential pathogen-associated molecular pattern (PAMP). This recognition is typically mediated by Toll-like receptor 5 (TLR5), which is expressed on the surface of various cells in the respiratory mucosa. Binding of *S. maltophilia* flagellin to TLR5 initiates intracellular signaling cascades that lead to the activation of proinflammatory pathways, such as the NF-κB and MAPK pathways [34]. This activation triggers the production of proinflammatory cytokines, including interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) [12]. The pro-inflammatory cytokines produced in response to flagellin binding lead to the recruitment of immune cells to the site of infection or inflammation within the respiratory tract [12].

Neutrophils and macrophages, among other immune cells, are recruited to help combat the infection. Neutrophils and macrophages play a critical role in phagocytosing (engulfing) *S. maltophilia* and other invading bacteria [12]. They work to eliminate the bacteria, preventing further infection and spread [12]. Flagellin-induced innate immune responses can also influence the adaptive immune system. Dendritic cells, which are antigen-presenting cells can be activated by flagellin exposure. This can influence the development of adaptive immune responses, including the production of specific antibodies and the activation of T cells, which can enhance the immune response against *S. maltophilia* [12]. The interaction between *S. maltophilia* flagellin and the innate immune system may also lead to the modulation of immune responses [12]. In some cases, this modulation may promote tolerance, preventing excessive inflammation and potential damage to the respiratory mucosa. It's important to note that while the recognition of *S. maltophilia* flagellin by the mucosal innate immune system is a crucial aspect of host defense, the ability of *S. maltophilia* to cause infections, particularly in immunocompromised individuals, suggests that it has developed strategies to evade or overcome host immune responses in some cases. *S. maltophilia* flagellin can trigger innate immune responses in the respiratory tract, leading to the recruitment of immune cells, cytokine production, and potential modulation of adaptive immunity [39]. Understanding these interactions is essential for developing strategies to combat *S. maltophilia* infections and maintain respiratory health.

## 7. FLAGELLIN as an ADJUVANT

Understanding the role of flagellin in the mucosal innate immune system has led to research into its potential therapeutic applications. Flagellin and flagellin-derived peptides have been studied as vaccine adjuvants to enhance the immune response to vaccines and as potential treatments for respiratory infections. Flagellin serves as a crucial component in the mucosal innate immune system of the respiratory tract by triggering immune responses through TLR5 activation. This immune response helps protect against bacterial infections and influences the development of adaptive immunity while also contributing to the regulation of immune tolerance [39]. In a previous study by Zgair and Chhibber, (2012), they found that giving pure flagella through the nose of the mouse contributed to stimulating the safe primary inflammatory immune response, as histological examination experiments showed that there was no significant damage to the tissues, but rather the researchers found the filtration of immune cells responsible for the non-specific immune response, especially neutrophils. Moderate, in addition to the expansion of blood vessels, this coincides with an increase in the level of pro-inflammatory cytokines such as IL-1β and TNF-α. Furthermore, they found an increase in the ability of alveolar macrophages to engulf and kill bacteria nonspecifically. An increase in the level of nitric oxide was also reported. The researchers found that the stimulated immune response in these mice had contributed to increasing their resistance to infection with *S. maltophilia*, as well as another bacterium, *Staphylococcus aureus*. This confirms the ability of this antigen to stimulate the beneficial and harmless pro-inflammatory immune response because this response occurred for a short time and did not remain for a long time, If it remains for a long period, it will cause tissue damage [12]. The functional activation of conventional dendritic cells was independent of direct TLR5 signaling, thereby supporting the contribution of maturation signals produced by flagellin-stimulated airway epithelium. In conclusion, our results demonstrated that indirect TLR5-dependent stimulation of airway conventional dendritic cells is essential to flagellin's mucosal adjuvant activity [39].

## 8. CONCLUSION

The current study showed that the flagella of *S. maltophilia* can stimulate the innate immune response, as well as pro-inflammatory, through binding to the flagellin to TLR5 (onto immune cells), which in turn stimulates a series of internal biochemical reactions that result in the cells secreting immune mediators, including pro-inflammatory cytokines and chemokine. As well as activating various immune cells. The role of this protein in stimulating the non-specialized immune response (i.e. phagocytosis) paved the way for a belief that confirms the possibility of using this protein as an adjuvant in the future after conducting many experiments related to the safety of its use.

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**Conflict of interest**

The authors declare that they have no conflict of interests.

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*Author affiliation*

1. *Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.*
2. *Department of Microbiology, Panjab University, Chandigarh, India.*

ORCID ID

1. Ayaid K. Zgair: https://orcid.org/0000-0002-2356-3338
2. Sanjay Chhibber: https://orcid.org/0000-0003-2600-1450