CONTRIBUTION OF TOLL-LIKE RECEPTORS TO THE IMMUNE RESPONSE IN UPPER RESPIRATORY TRACT INFECTIONS

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Abstract: Upper respiratory tract infections (URTIs) are among the most common infectious diseases worldwide, caused primarily by viruses and, less frequently, by bacteria. Toll-like receptors (TLRs), as key components of the innate immune system, play a central role in recognizing pathogen-associated molecular patterns (PAMPs) and initiating immune responses in the respiratory epithelium. This article reviews the expression and function of TLRs in the upper respiratory tract, their involvement in pathogen detection, and the consequences of dysregulated TLR signaling. Understanding TLR-mediated mechanisms in URTIs may aid in developing novel immunomodulatory therapies.

1. Introduction

The upper respiratory tract serves as the primary entry point for a variety of airborne pathogens, including viruses such as rhinoviruses, influenza viruses, and coronaviruses, as well as bacteria like *Streptococcus pneumoniae*. Toll-like receptors (TLRs) are essential pattern recognition receptors (PRRs) that detect microbial components and trigger innate immune responses (Kawai & Akira, 2010). In the respiratory mucosa, epithelial cells and immune cells express TLRs that contribute to early pathogen recognition and shaping of adaptive immunity (Bals & Hiemstra, 2004).

2. Expression and Localization of TLRs in the Upper Respiratory Tract

Different TLRs are differentially expressed in various cell types of the upper airway, including nasal epithelial cells, macrophages, and dendritic cells. TLR3, TLR7, and TLR8 are primarily located in endosomes and recognize viral RNA, while TLR2 and TLR4, expressed on the cell surface, detect bacterial lipoproteins and lipopolysaccharides, respectively (Kaisho & Akira, 2006). For instance, nasal epithelial cells express high levels of TLR3 and TLR7, which are essential for recognizing influenza and rhinoviral infections (Hajjar et al., 2002).

The upper respiratory tract (URT), which includes the nasal passages, nasopharynx, and oropharynx, is the first line of defense against inhaled pathogens. **Toll-like receptors (TLRs)** are expressed by various cell types in the URT, including epithelial cells, dendritic cells (DCs), macrophages, and endothelial cells. Their expression patterns and cellular localization are essential for initiating immune responses against both bacterial and viral pathogens.

TLR Expression in Nasal and Respiratory Epithelium

The **respiratory epithelium**, which forms the mucosal barrier of the URT, expresses several TLRs, with the highest expression levels observed for **TLR2**, **TLR3**, **TLR4**, and **TLR5**. These TLRs are crucial for detecting pathogen-associated molecular patterns (PAMPs) such as lipoproteins, lipopolysaccharides, flagellin, and viral RNA.

- **TLR2** is highly expressed on the surface of nasal epithelial cells, particularly those in the upper respiratory tract. It recognizes components of **Gram-positive bacteria** (e.g., *Staphylococcus aureus*, *Streptococcus pneumoniae*) and activates inflammatory pathways (Toshchakov et al., 2002).
- **TLR3**, located predominantly in endosomal membranes, recognizes **double-stranded RNA (dsRNA)** produced by many viruses, including **influenza** and **rhinoviruses**. It is

expressed in epithelial cells and dendritic cells of the URT (Alexopoulou et al., 2001).

- **TLR4** is expressed on both the apical and basolateral surfaces of respiratory epithelial cells, where it plays a key role in detecting **lipopolysaccharides (LPS)** from **Gram-negative bacteria** like *Haemophilus influenzae* (Hoshino et al., 2002). It can also recognize viral glycoproteins, such as the **RSV fusion protein** (Kurt-Jones et al., 2000).
- **TLR5**, present on the apical surface of airway epithelial cells, is primarily involved in recognizing **flagellin**, a component of bacterial flagella. This TLR is involved in detecting motile bacteria, such as **Pseudomonas aeruginosa** (Hayashi et al., 2001).

TLR Expression in Dendritic Cells and Macrophages

Dendritic cells (DCs) are central to the immune response in the URT and play a critical role in TLR-mediated pathogen detection. **Plasmacytoid dendritic cells (pDCs)**, which are abundant in the nasal mucosa, express **TLR7** and **TLR9** and are crucial for the detection of **single-stranded RNA (ssRNA)** viruses like **influenza** and **rhinovirus** (Lund et al., 2004). These pDCs produce type I interferons (IFNs), which are essential for limiting viral replication.

Macrophages also express multiple TLRs, including **TLR2**, **TLR4**, **TLR5**, and **TLR9**. They play a key role in clearing pathogens and promoting inflammation in response to infection. These cells are involved in both **direct pathogen killing** and the **activation of adaptive immunity** via the secretion of cytokines such as TNF-α, IL-6, and IL-12 (Toshchakov et al., 2002).

Localization of TLRs in Upper Respiratory Tract Tissues

The localization of TLRs in the URT is important for the initial detection of pathogens that are inhaled. TLRs are localized not only in epithelial cells but also in immune cells in the **nasal mucosa**, **sinuses**, and **pharyngeal tissues**. This ensures that TLRs are in close proximity to pathogens entering the body through the airways.

- In the **nasal mucosa**, TLR2, TLR3, TLR4, and TLR5 are expressed on the apical surface of epithelial cells, which are the first line of defense against airborne pathogens. These receptors are particularly important for detecting **bacterial pathogens** and initiating the innate immune response.
- **Dendritic cells** in the mucosa express **TLR7** and **TLR9** in endosomal compartments, where they detect **viral RNA and DNA**. They migrate to local lymph nodes to activate adaptive immune responses (Lund et al., 2004).

In the **sinuses** and **pharyngeal tissues**, both epithelial cells and immune cells, including **macrophages** and **neutrophils**, express TLRs. These tissues are critical sites for pathogen recognition in **sinusitis** and **pharyngitis**, conditions often associated with both bacterial and viral infections.

Regulation of TLR Expression in the URT

The expression of TLRs in the URT is **regulated by environmental factors** such as infection, allergens, and inflammatory cytokines. For example, **interleukin-1β (IL-1β)** and **tumor necrosis factor-α (TNF-α)**, which are produced during infection, can upregulate the expression of TLRs on respiratory epithelial cells, enhancing the sensitivity to pathogens (Bazzoni et al., 1999). In contrast, chronic exposure to allergens or pollutants can alter the expression of TLRs and may contribute to conditions like **asthma** or **chronic rhinosinusitis** (Tsoyi et al., 2011).

In **chronic respiratory diseases**, such as **asthma** and **chronic obstructive pulmonary disease (COPD)**, TLR expression can become dysregulated. Overexpression of certain TLRs, particularly TLR2 and TLR4, has been observed in **smokers** and individuals with **COPD**, which may contribute to the **chronic inflammation** seen in these conditions (Tsoyi et al., 2011).

3. TLRs and Viral Upper Respiratory Tract Infections

Viral infections such as influenza, RSV (respiratory syncytial virus), and SARS-CoV-2 are major causes of URTIs. TLR3 recognizes double-stranded RNA, a viral replication intermediate, and activates interferon regulatory factors (IRF3/7), leading to the production of type I interferons (IFN-α/β) (Kurt-Jones et al., 2000). TLR7 and TLR8 recognize single-stranded RNA from viruses like RSV and SARS-CoV-2 and similarly trigger antiviral pathways (Diebold et al.,

2004). These responses help limit viral replication but can also cause excessive inflammation if not properly regulated.

Toll-like receptors (TLRs) are pivotal for initiating immune responses against viruses that infect the upper respiratory tract (URT), including **influenza viruses**, **respiratory syncytial virus (RSV)**, **rhinoviruses**, and **coronaviruses** (including SARS-CoV-2). Viral components such as **single-stranded RNA (ssRNA)**, **double-stranded RNA (dsRNA)**, and **viral proteins** are recognized by specific TLRs, triggering antiviral signaling pathways that culminate in the production of **type I interferons (IFNs)** and **proinflammatory cytokines** (Akira et al., 2006).

TLR3: Sensing Double-Stranded RNA

TLR3, expressed on endosomal membranes of epithelial cells, dendritic cells (especially CD103$^+$ respiratory DCs), and macrophages, recognizes **viral dsRNA**, an intermediate product during viral replication (Alexopoulou et al., 2001). Upon activation, TLR3 recruits the adaptor protein **TRIF**, leading to the induction of **IFN-β**, **IL-6**, and **TNF-α**, crucial for controlling infections such as **influenza A** and **rhinovirus** (Le Goffic et al., 2006). In murine models, TLR3-deficient mice exhibit reduced IFN responses and impaired viral clearance (Le Goffic et al., 2006).

TLR7 and TLR8: Detecting Single-Stranded Viral RNA

TLR7 and **TLR8**, primarily found in **plasmacytoid dendritic cells (pDCs)** and monocytes, are responsible for recognizing **ssRNA** from viruses such as **influenza**, **RSV**, and **coronaviruses** (Diebold et al., 2004). These TLRs signal via the **MyD88-dependent pathway**, resulting in strong production of **type I IFNs (IFN-α/β)**, which establish an antiviral state and modulate adaptive immunity (Kawai & Akira, 2010). Notably, pDCs are among the most potent producers of type I IFNs in response to influenza infection, largely via TLR7-mediated sensing (Lund et al., 2004).

TLR9: Recognizing DNA Viruses

Though less common in URT infections, **TLR9** detects **unmethylated CpG DNA** from **DNA viruses** such as **adenoviruses** and **herpesviruses** (Hemmi et al., 2000). TLR9 activation contributes to IFN production and can modulate the outcome of co-infections or secondary bacterial infections during viral URT illness (Kumagai et al., 2007).

TLR4 and TLR2: Non-Canonical Viral Sensing

While primarily involved in bacterial recognition, **TLR4** and **TLR2** also participate in immune responses to certain viral proteins. For example, **RSV fusion (F) protein** activates **TLR4**, enhancing cytokine production and neutrophil recruitment (Kurt-Jones et al., 2000). Similarly, **TLR2** can detect **viral envelope proteins**, contributing to inflammatory responses in **rhinovirus** and **coronavirus** infections (Triantafilou et al., 2004; Choudhury & Mukherjee, 2020).

Clinical Implications

Inappropriate or excessive TLR activation during viral URT infections can contribute to **immunopathology**. For instance, **overactive TLR3 or TLR7 signaling** has been implicated in tissue damage during severe influenza and COVID-19 infections due to exaggerated cytokine release (Totura et al., 2015; van der Made et al., 2020). Conversely, **loss-of-function mutations in TLR7** are associated with **severe COVID-19 in young males**, underlining its importance in early antiviral defense (van der Made et al., 2020).

TLR-targeted therapies, such as **TLR7/8 agonists**, are under investigation for their **adjuvant potential in intranasal vaccines**, enhancing mucosal immunity against viruses like influenza and SARS-CoV-2 (Kasturi et al., 2011). On the other hand, **TLR antagonists** may help **dampen hyperinflammatory responses** in cases of cytokine storm syndromes.

4. TLRs and Bacterial Infections of the Upper Respiratory Tract

TLR2 and TLR4 are primarily involved in detecting bacterial components. TLR2 recognizes lipoteichoic acid from gram-positive bacteria like *Streptococcus pneumoniae*, while TLR4 binds to lipopolysaccharides from gram-negative pathogens (Takeuchi et al., 1999). The activation of these receptors leads to NF-κB-dependent transcription of pro-inflammatory cytokines (IL-1β, TNF-α), facilitating neutrophil recruitment and bacterial clearance (Cohen et al., 2000).

The upper respiratory tract (URT) is frequently exposed to bacterial pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. The innate immune system relies heavily on Toll-like receptors (TLRs) for early recognition and response to these bacterial invaders. Among the most relevant TLRs in bacterial URT infections are TLR2, TLR4, TLR5, and TLR9.

TLR2 plays a crucial role in recognizing components of Gram-positive bacteria, such as lipoteichoic acid (LTA), peptidoglycan, and bacterial lipoproteins. It often forms heterodimers with TLR1 or TLR6 to expand its range of ligand recognition (Takeuchi et al., 2001). For instance, in infections with *S. pneumoniae*, TLR2 activation leads to the release of inflammatory cytokines like TNF-α, IL-6, and IL-1β, which recruit neutrophils and macrophages to the infection site (Zhang et al., 2007).

TLR4, primarily associated with Gram-negative bacteria, recognizes lipopolysaccharide (LPS) from organisms such as *Haemophilus influenzae*. It signals through both MyD88-dependent and TRIF-dependent pathways, leading to the production of pro-inflammatory cytokines and type I interferons (Beutler, 2000; Hoshino et al., 2002). Notably, TLR4 activation is crucial for defense against *H. influenzae*-induced otitis media and sinusitis (Leichtle et al., 2009).

TLR5 detects bacterial flagellin, a structural component of motile bacteria, including *Pseudomonas aeruginosa*, which can colonize the URT, especially in immunocompromised individuals. TLR5 is expressed on epithelial cells lining the nasal passages and sinuses and contributes to mucosal immune activation and neutrophil recruitment (Hayashi et al., 2001).

TLR9, located in endosomes, recognizes unmethylated CpG motifs in bacterial DNA, particularly from Gram-positive bacteria like *S. aureus*. Activation of TLR9 contributes to the production of interferon-α and the modulation of B-cell responses (Hemmi et al., 2000).

In addition to pathogen recognition, TLRs influence the **severity and duration** of bacterial infections. For example, impaired TLR2 or TLR4 signaling in knockout mice results in delayed bacterial clearance and prolonged inflammation during *S. pneumoniae* or *H. influenzae* infections (Albiger et al., 2005; Melhus & Ryan, 2000). Moreover, excessive TLR activation can contribute to tissue damage, indicating a need for tightly regulated signaling.

Furthermore, some bacteria have evolved mechanisms to **evade or manipulate TLR signaling**. *S. pneumoniae*, for instance, can alter its cell wall components to reduce TLR2 activation, while *H. influenzae* modifies its LPS structure to escape TLR4 detection (Weiser et al., 2018).

Understanding the role of TLRs in bacterial URT infections has **clinical implications**. For instance, TLR agonists (e.g., synthetic lipopeptides targeting TLR2) are being studied as vaccine adjuvants to boost mucosal immunity. Conversely, TLR antagonists may be considered to manage excessive inflammation in chronic conditions like recurrent sinusitis (de Vos et al., 2009).

5. Crosstalk and Regulation of TLR Signaling in URTIs

TLR activation is tightly regulated to prevent tissue damage from excessive inflammation. Negative regulators such as SIGIRR and IRAK-M help dampen TLR signaling after pathogen clearance (Kobayashi et al., 2002). Moreover, crosstalk among TLRs and other PRRs (e.g., RIG-I) modulates the intensity and specificity of the immune response. Dysregulation of TLR signaling is associated with chronic rhinosinusitis and increased susceptibility to secondary infections (Lane et al., 2006).

6. Clinical Implications and Therapeutic Potential

Targeting TLRs offers a promising strategy in managing URTIs. TLR agonists are being explored as adjuvants in intranasal vaccines, enhancing mucosal immunity (Matsuo et al., 2010). Conversely, TLR antagonists may help mitigate severe inflammatory responses in viral infections, such as during cytokine storms in COVID-19 (van der Made et al., 2020).

Toll-like receptors (TLRs) are fundamental in the detection of pathogens in the upper respiratory tract (URT), and their involvement in both protective immune responses and immunopathology has significant clinical implications. While TLRs provide critical early responses to viral and bacterial infections, dysregulation of their signaling pathways can contribute to excessive

inflammation, tissue damage, and chronic respiratory diseases. Understanding the roles of TLRs in these infections opens avenues for novel therapeutic strategies aimed at either enhancing TLR responses to clear infections more efficiently or modulating their activity to prevent tissue damage and inflammation.

1. TLR Agonists in Vaccine Development

TLR agonists have been explored as potential **adjuvants** in vaccines, particularly those aimed at protecting against respiratory infections such as **influenza**, **RSV**, and **SARS-CoV-2**. These agonists activate innate immune responses and enhance the **mucosal immunity** in the respiratory tract. **TLR7/8** agonists, for example, have been shown to improve **immunogenicity** and **protection** in preclinical models of respiratory infections (Kasturi et al., 2011). TLR-based vaccine adjuvants can stimulate the production of **type I interferons (IFNs)** and **cytokines**, which are essential for robust antiviral immunity and long-lasting protection.

A major advantage of TLR agonist-based vaccines is their ability to activate both the **innate** and **adaptive immune responses**. The TLR7/8 agonist **resiquimod**, for example, has demonstrated efficacy in enhancing immune responses when used alongside vaccines for **influenza** (Poo et al., 2015). Furthermore, **TLR9 agonists** have shown potential in the development of vaccines for **DNA viruses** such as **adenovirus** and **herpes simplex virus (HSV)** (Tregoning et al., 2018).

2. Targeting TLRs to Control Inflammation in Viral Respiratory Infections

While TLRs are crucial for initiating the immune response to respiratory pathogens, their activation can sometimes lead to **excessive inflammation**, contributing to **immunopathology**. For example, excessive activation of **TLR3** during **influenza** infection has been linked to **acute lung injury** and **cytokine storms** (Le Goffic et al., 2006). Similarly, **TLR7** activation has been implicated in **severe COVID-19**, where hyperactivation of the immune response leads to **acute respiratory distress syndrome (ARDS)** and **cytokine release syndrome** (van der Made et al., 2020).

Therefore, **TLR antagonists** or **modulators** may be beneficial in treating conditions with **excessive immune activation**. **TLR4 antagonists**, for example, have been shown to reduce inflammation and tissue damage in animal models of **bacterial pneumonia** (Tiwari et al., 2014). **TLR2 antagonists** may be useful in preventing **chronic inflammation** associated with diseases like **asthma** and **chronic rhinosinusitis** (Tsoyi et al., 2011).

3. Modulation of TLR Responses in Chronic Respiratory Diseases

Chronic respiratory diseases such as **asthma**, **chronic obstructive pulmonary disease (COPD)**, and **chronic rhinosinusitis** are often characterized by persistent inflammation in the respiratory tract. **Dysregulation of TLR signaling** plays a key role in the chronicity of these conditions. For example, **TLR2** and **TLR4** are upregulated in the airways of individuals with **COPD** (Tsoyi et al., 2011). **TLR5** has also been implicated in **sinusitis**, where its activation promotes **neutrophilic inflammation** (Han et al., 2014).

In these diseases, **modulating TLR activity** could help to restore immune homeostasis and reduce chronic inflammation. Therapies that target **TLR4** signaling have been considered for managing **COPD**, while **TLR9 agonists** could potentially be used in **chronic rhinosinusitis** to enhance immune responses (Tsoyi et al., 2011). **TLR2 inhibitors** may provide therapeutic benefits in treating **asthma**, where **TLR2 overexpression** exacerbates airway inflammation.

4. Personalized Medicine and TLR Polymorphisms

Another promising therapeutic approach involves **personalized medicine** based on an individual's genetic makeup, specifically **TLR polymorphisms**. Variations in **TLR genes** can affect the response to infection and influence the severity of diseases. For instance, individuals with **TLR4 polymorphisms** may have altered responses to **Gram-negative bacterial infections** such as **pneumonia** and **sepsis** (Hoshino et al., 2002). Similarly, **TLR3 polymorphisms** may affect susceptibility to viral infections like **influenza** and influence the **outcome** of the infection (Takeuchi & Akira, 2007).

In **COVID-19**, certain **genetic variants** of **TLR7** have been associated with **severe disease** (van der Made et al., 2020). **Personalized therapies** that consider these genetic differences could be

tailored to optimize treatment and minimize adverse outcomes, leading to more effective and targeted interventions.

5. Risks and Challenges in TLR-Based Therapies

Despite their potential, TLR-based therapies face several challenges. **Systemic TLR activation** can lead to unintended **side effects**, including **autoimmunity**, **chronic inflammation**, and tissue damage. The challenge lies in developing **safe TLR agonists** and **antagonists** that can specifically target the pathogen without inducing broad, unregulated immune activation.

Furthermore, **immune tolerance** or **immune evasion** by pathogens, especially **viruses**, could limit the effectiveness of TLR-targeted therapies. Some viruses, such as **influenza**, have evolved mechanisms to **evade TLR recognition** or dampen TLR signaling, making it more difficult to achieve a strong immune response (Barton et al., 2007).

7. Conclusion

TLRs play a central role in the immune response to upper respiratory tract infections, contributing to both effective pathogen clearance and inflammatory damage. Harnessing the power of TLRs through **agonists** can enhance vaccine responses and boost immunity, while **antagonists** may provide a therapeutic strategy for preventing excessive inflammation in viral and bacterial infections. Furthermore, **personalized approaches**, including targeting specific **TLR polymorphisms**, may lead to more tailored and effective treatments. However, careful modulation of TLR activity is necessary to balance immune activation with the risk of unwanted inflammation and tissue damage

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