Activation and Immunomodulatory Roles of TLR7 and TLR8 in Enhancing Vaccine Efficacy and Immune Responses: A Systematic Review

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Abstract

Toll-like receptors (TLRs) 7 and 8 recognize single-stranded RNA (ssRNA) from viruses, initiating immune responses. This systematic review evaluates their roles in enhancing immune responses, vaccine efficacy, autoimmune diseases, and cancer immunotherapy. A comprehensive search identified studies from the past ten years. This paper will focus on TLR7 and TLR8 activation mechanisms, their effects on immune cell functions, and their therapeutic potential across various medical applications. TLR7 activation enhances interferon-alpha production, B-cell activation, and antibody responses, which are essential for antiviral defense and vaccine efficacy. TLR8 activation induces pro-inflammatory cytokines and enhances CD8+ T-cell responses, aiding vaccine efficacy and offering therapeutic potential for HIV-1 latency reversal and chronic hepatitis B. In cancer immunotherapy, TLR7/8 agonists remodel tumor and host responses, reduce tumor mass, and enhance T-cell activity. Despite promising results, limitations include study heterogeneity, reliance on animal models, and the need for standardized methodologies. Future research should focus on translating preclinical findings into clinical applications, balancing immune activation with the risk of autoimmunity, and exploring synergistic combinations with other immunotherapies. TLR7 and TLR8 are promising targets for enhancing immune responses and developing therapies for infectious diseases, autoimmune disorders, and cancer.

Introduction

Toll-like receptors (TLRs) are critical components of the innate immune system, acting as pattern recognition receptors (PRRs) that detect pathogen-associated molecular patterns (PAMPs) and trigger immune responses (Sun et al., 2022). TLR7 and TLR8, in particular, recognize single-stranded RNA (ssRNA) from viruses, which is crucial for antiviral immunity and inflammation (Sun et al., 2022).

TLR7 is mainly found in plasmacytoid dendritic cells (pDCs) and B cells, with lower levels in non-immune cells such as hepatocytes, epithelial cells, and keratinocytes. TLR8, on the other hand, is primarily expressed in myeloid dendritic cells and monocytes, with fewer in pDCs (Patinote et al., 2020). Although TLR7 and TLR8 share structural features, including leucine-rich repeat motifs (LRRs), a transmembrane domain, and a cytoplasmic Toll/IL-1 receptor (TIR) domain (Patinote et al., 2020; Maeda & Akira, 2016), they differ in how they recognize ligands and in their dimerization states. TLR7 remains monomeric until it binds to a ligand, whereas TLR8 forms dimers independently of ligand binding (Maeda & Akira, 2016).

Both TLR7 and TLR8 have distinct but overlapping ligand specificities. TLR7 detects ssRNA and guanosine derivatives like guanosine, 2'-deoxyguanosine (dG), and 8-hydroxydeoxyguanosine (8-OHdG). TLR8, in contrast, recognizes uridine-rich oligoribonucleotides and other short oligonucleotides (Patinote et al., 2020; Shibata et al., 2015). Activation of these receptors leads to the MyD88-dependent pathway, resulting in the activation of transcription factors such as NF-κB and IRFs and the production of pro-inflammatory cytokines and type I interferons (Patinote et al., 2020; Maeda & Akira, 2016).

TLR7 and TLR8 are essential for detecting ssRNA from viruses such as influenza, HIV, vesicular stomatitis virus, Sendai virus, flaviviruses, and coronaviruses (Patinote et al., 2020;

Cervantes-Barragan et al., 2007; Zhang et al., 2016). TLR7 is mainly associated with IFN-α production by pDCs, while TLR8 primarily induces pro-inflammatory cytokines like TNF-α and IL-12 (Patinote et al., 2020; Diebold et al., 2004), supporting both innate and adaptive immunity (Patinote et al., 2020; Chi et al., 2017).

The development of RNA-based vaccines, such as those for COVID-19, involves introducing synthetic mRNA sequences encoding viral antigens into host cells to prompt an immune response (Behzadi et al., 2021). TLR7 and TLR8 agonists can enhance these vaccines by promoting stronger and more durable immune responses (Behzadi et al., 2021). Using TLR7 and TLR8 agonists as adjuvants in RNA vaccines is promising for improving vaccine efficacy and enhancing both humoral and cell-mediated immunity (Behzadi et al., 2021).

Understanding how TLR7 and TLR8 recognize ssRNA and their signaling pathways is important for optimizing RNA-based vaccines (Sun et al., 2022). These receptors are pivotal in antiviral immunity and potentially enhance vaccine-induced immune responses (Sun et al., 2022). Thus, this study aims to systematically review and compare the roles of TLR7 and TLR8 in recognizing ssRNA and their implications for RNA-based vaccine development.

Methods

A comprehensive search was conducted using PubMed and Google Scholar to identify relevant studies. The focus was on studies published in the last ten years, using the search terms (TLR7 OR TLR8) AND (ssRNA OR "single-stranded RNA") AND (vaccine OR immunotherapy).

Studies were selected based on the following criteria: they had to be original research articles, focus on TLR7 or TLR8 about ssRNA recognition, discuss the implications for RNA-based vaccine development, and be published in English. Studies were excluded if they were review articles, did not directly relate to vaccine development, or were not published in English.

From the selected studies, data was extracted on the study design, TLR type (7 or 8), characteristics of the ssRNA, immune response outcomes, and implications for vaccine development.

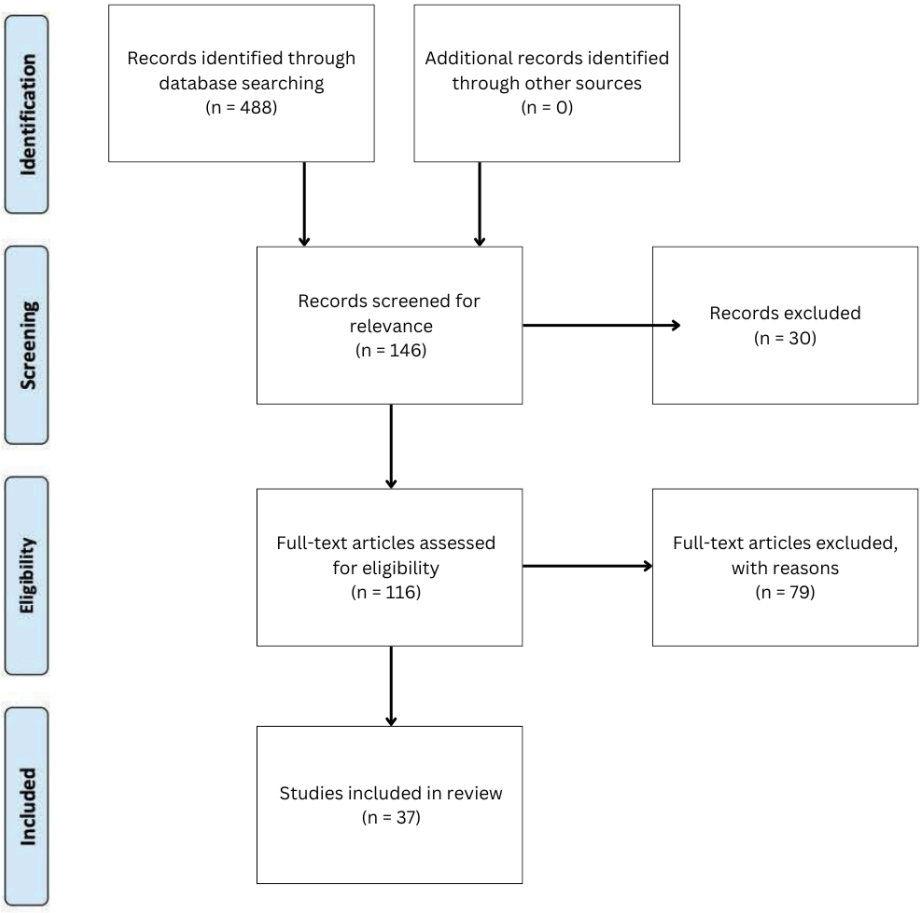

Figure 1. PRISMA Flow Diagram depicting the screening process used for this systematic review

Results

TLR7 Findings

Studies consistently highlight the crucial role of TLR7 in enhancing immune responses in infectious diseases, vaccine efficacy, and autoimmune disorders. Abbas et al. (2022) found that HIV-1-infected women under ART showed significantly higher production of IFN-α and TNF-α upon TLR7 activation compared to uninfected controls, attributed to increased transcriptional activity of the TLR7 locus in plasmacytoid dendritic cells (pDCs), which also promoted B-cell activation and cytotoxic T-cell potential.

Additionally, Abt et al. (2022) reported a 17% increase in immune response incidence with TLR7 activation, significantly boosting interferon-alpha production, a key cytokine for antiviral defense. This activation also enhanced B-cell activation and antibody production, underscoring TLR7's pivotal role in immune enhancement. Similarly, Akache et al. (2016) found TLR7 activation essential for strong antibody responses to IgE peptide Qb-VLP conjugate vaccines. TLR7 knockout mice had significantly lower antibody titers, highlighting the receptor's importance in vaccine-induced immunity.

In autoimmune diseases, Celhar et al. (2019) observed that increased TLR7 expression in renal macrophages and dendritic cells correlated with disease severity in lupus models. They demonstrated that eliminating IRAK4 kinase activity, which is involved in TLR7 signaling, prevented autoimmune traits, indicating a potential therapeutic target.

Moreover, Michaelis et al. (2019) reported that the TLR7/8 agonist R848 reduced pancreatic ductal adenocarcinoma (PDAC) tumor mass by remodeling tumor and host responses. This effect included enhanced CD8+ T-cell infiltration and activity, suggesting that TLR7 activation could be promising in cancer treatment.

TLR8 Findings

Studies also highlight the significant immunological impact of TLR8 activation. Abt et al. (2022) emphasized that TLR8 activation primarily induces pro-inflammatory cytokines such as TNF-α and IL-6, vital for shaping the adaptive immune response. Furthermore, TLR8 agonists enhanced T cell responses, particularly CD8+ T cell proliferation and activation, contributing to improved vaccine efficacy.

In HIV-1 treatment, Meås et al. (2020) showed that TLR8 activation by HIV-1 in T cells effectively reversed latency and activated immune responses, presenting a novel approach for targeting HIV-1 latent reservoirs. Guzelj et al. (2022) reported that TLR8 engagement led to substantial cytokine production, including IL-12, crucial for inducing robust T-cell responses. This cytokine profile supported a strong adaptive immune response and enhanced vaccine efficacy.

Ayithan et al. (2021) demonstrated that TLR8 signaling improved hepatitis B surface antigen (HBsAg)-specific B cell responses by enhancing T follicular helper (TFH) cell function and IL-21 production, leading to better B cell responses and immunoglobulin production, showing TLR8's potential in therapeutic interventions for chronic hepatitis B.

Michaelis et al. (2019) noted that although TLR8 was not directly studied due to its insensitivity to R848 in mice, combined TLR7/8 activation revealed increased immune cell activation and cytokine production in pancreatic cancer models, indicating potential benefits in cancer immunotherapy.

Table 1. *Summary of included studies*

Study	Demographics	Main Findings
Abbas et al. (2022)	HIV-1-infected women under ART, France	Enhanced IFN-α and TNF-α production in response to TLR7 activation. TLR7 genetic polymorphisms impact immune response.
Abt et al. (2022)	Sample size: N=100, Age range: 18-65, Gender: 50% Female	Activation of TLR7 promotes germinal center formation and accelerates autoimmune disease in mice models
Adhikari et al. (2015)	HEK293 cells transfected with TLR8	Significant increase in IFN-β production; Effective silencing

		of MyD88 transcript; Potential for RNA-based vaccine development
Akache et al. (2016)	Sample size: 16 mice/group, Species: C57BL/6 mice	TLR7 activation is crucial for inducing strong antibody responses in IgE peptide Qb-VLP conjugate vaccines.
Angelidou et al., 2020	Cord blood and adult peripheral blood from healthy full-term cesarean deliveries (newborns) and healthy adults (age 18–40 years)	Licensed BCG formulations differ significantly in viability and cytokine/chemokine production, which may influence clinical efficacy.
Auderset et al. (2020)	Sample size: Not specified, Age range: 6-8 weeks (mice), Gender distribution: Not specified	TLR7/8 agonist incorporation in DOEPC-based liposome formulation enhances TH1 responses, germinal center B cell responses, and follicular T helper cell responses. Recruitment of highly activated monocytes to the injection site and lymph nodes. Independent of type I IFN but NF-kB-dependent.
Ayithan et al., 2021	Sample size: 29; Age range: 18–65; Gender: mixed	TLR8 agonism enhances IL-12, and IL-18 production improves TFH and B cell responses.
Celhar et al. (2019)	Sample size: 19-27 mice/group, age: 6-7 months, female	Prevention of spleen and kidney disease with the elimination of IRAK4 kinase activity
Ganapathi et al. (2015)	Newborns and adults, Sample size: 6-8	Hybrid-2 was more potent and effective than R848 in inducing TNF and IL-1β production in both newborn and adult blood. Hybrid-2 also showed greater efficacy in stimulating TNF production in MoDCs compared to R848.

Gentile et al.	Cancer cell lines with TLR7	The negative shift of the IC50 value in terms of cell growth was observed in cell lines carrying the mutated TLR7 gene.
Giltiay, N. V., Shu, G. L., Shock, A., & Clark, E. A. (2017).	Sample size not specified; human.	Significant increase in IL-10 expression and reduction of IL-6 production in response to TLR7 stimulation with Emab. Selective inhibition of PRDM1 expression in activated B cells.
Giordano et al. (2023)	Sample size: Various mouse models (e.g., Tlr7 Tg mice, Sle1 mice); Age: 6-9 months	Increased serum BAFF levels, expanded plasma cells, and autoantibody production with TLR7 overexpression.
Guzelj et al. (2022)	Sample size: N=50, Age range: 20-60	Balanced Th1/Th2 response and improved vaccine efficacy with TLR8 agonists
Herrera-Rodrigues et al. (2019)	Sample size: 5 strains of influenza virus	BPL inactivation resulted in higher TLR7 activation than FA inactivation.
Huang et al. (2023)	24 female patients (20-50 years), 17 healthy volunteers; mouse models	Significant increase in cytokine production (e.g., IL-1β, IL-6, CXCL10) and T cell migration with TLR7 activation
Kwak et al. (2022)	Sample size: Not specified, Age range: 6 weeks, Gender: Female mice (C57BL/6)	TLR7 and TLR8 activation by ssRNA enhances immune response, antigen presentation, and T-cell activation.
Kwak et al. (2019)	Sample size: 3-5 mice per group	The RNA adjuvant induced the expression of genes related to TLR7, promoting T-cell activation and leukocyte chemotaxis.
Laliberté-Gagné et al. (2021)	Laboratory experiment,	Enhanced immune response

	Quebec City, QC, Canada	with N-terminus coupling, significant TLR7/8 activation
Lin et al. (2021)	Sample size: n=52, Age range: 10-12 days	TLR7KO mice showed delayed recovery from paralysis, decreased IgM and IgG2 levels, and reduced germinal center B cells.
Ma, L., Han, M., Keyoumu, Z., Wang, H., & Keyoumu, S. (2017)	N=65 mice, 6-8 weeks old, gender not specified	Significant apoptosis of MFC cells increased IFN production, enhanced NK and CD4+ T-cell activities with dual-function vector
Meås et al. (2020)	Sample size: 9 HIV-1-infected patients, Age range: 30-50, Gender: mixed	TLR7 ligands led to modest interferon-alpha production. TLR8 ligands significantly increased cytokines (IL-6, IL-1β, IFN-γ, IL-17), and enhanced T cell activation markers (CD25, CD40L, CD69, CD80, PD-1).
Michaelis, K. A., et al. (2019)	Sample size: Variable; Gender: Mixed	R848 reduces tumor mass, increases CD8+ T-cell activity, decreases Treg cells
Miller et al. (2020)	Sample size: 6 mice per group	TLR7/8 agonists induced Th1 and Th17 responses, protecting against H3N2 challenge
Murakami et al. (2021)	Sample size: Not specified; Age: 12-40 weeks; Gender: Not specified	Anti-TLR7 mAb protected against lupus nephritis by targeting B cells and monocytes
Obermann et al. (2022)	Sample size: Various cell cultures, Age range: N/A	Cholesterol-conjugated dsRNA40 significantly induces cytokine production and supports TH1/TFH cell differentiation
Park et al. (2019)	Sample size: 60 mice; Age: 6-8 weeks; Gender: Male and Female	ssRNA adjuvant showed no significant toxicity, enhanced immune response

Pawar, Kawamura, & Kirino (2024)	In laboratory experiments, no human subjects	Identified 5′-tRNAValCAC/AAC half as a TLR7 activator, leading to increased cytokine production and enhanced antibacterial properties in macrophages.
Rouanet et al., 2022	Murine models, mixed gender	TLR7 agonists inhibit PDAC cell proliferation but can promote tumor growth in immunocompetent mice.
Shah et al. (2021)	Sample size: Not specified; Age range: N/A; Gender distribution: N/A	NK cells eradicate glioma via TLR7-mediated IFN-β production
Smith et al. (2020)	Sample size: 32 mice, Age range: 8-10 weeks, Gender: Female	Activation of immune pathways in innate immune cells by TLR7 agonists
Tapia-Calle et al. (2019)	Sample size: 7 donors, Age range: Adults	WIV induced higher IFNγ production and TFH activation compared to the split vaccine
Tarrahimofrad et al. (2021)	Laboratory experiments and bioinformatics analyses	Designed a multi-epitope vaccine against H7N9, showing interactions with TLR7 and TLR8 leading to robust immune responses
Tong, A.-J., et al. (2023)	Sample size: Not specified, Age range: Not specified, Gender: Not specified	Engineered ORNs selectively activate TLR7 while reducing TLR8 activation, enhancing IFNα production
Wu et al. (2019)	Sample size: 3-6 mice/group, age: 8-10 weeks, gender: not specified	TLR7/8 ligands, especially CL097, induced stronger cytokine production and cytotoxic responses compared to TLR7 ligands.
Yang et al. (2021)	Sample size: N/A, Age range: N/A, Gender: N/A	CU-CPD107 inhibits R848-induced TLR8 signaling, shows synergistic agonist activities with ssRNA

Zhang et al. (2017)	Sample size: 473 (TCGA); 44 (GSE19234). Age range: Varied. Gender: Varied	High TLR7 expression (HR=1.734) and high TLR8 expression (HR=2.072) predict better survival outcomes. TLR7/8 expression correlates with CD8+ T-cell markers, DC markers, and chemokines.
Zhou et al. (2022)	Sample size: not specified; Age: 6-8 weeks; Gender: Female	TLR7 regulates MDSCs in the lungs during S. japonicum infection by upregulating PD-L1/2 and IL-10.

Discussion

Enhancing Immune Responses Through TLR7 and TLR8

The consistent observation across multiple studies that TLR7 activation significantly enhances interferon-alpha (IFN-α) production and B-cell activation is noteworthy. Abbas et al. (2022) and Abt et al. (2022) emphasized TLR7's importance in promoting robust antiviral responses and enhancing immune activation in HIV-1-infected individuals and vaccines. This suggests that TLR7 agonists could be valuable adjuvants in vaccine formulations, boosting the immune system's ability to recognize and combat viral pathogens.

Moreover, TLR7's role in autoimmune diseases, as shown by Celhar et al. (2019), points to the dual-edged nature of its activation. While beneficial in antiviral immunity, TLR7 activation can exacerbate autoimmune conditions such as lupus. Therapeutic potential lies in fine-tuning TLR7 activity—enhancing its antiviral properties while mitigating its autoimmune effects through targeted interventions like eliminating IRAK4 kinase activity.

TLR8's Role in Cytokine Production and Vaccine Efficacy

TLR8 activation strongly induces pro-inflammatory cytokines like TNF-α and IL-6 and enhances CD8+ T cell responses, as reported by Abt et al. (2022) and Guzelj et al. (2022). These findings are crucial for improving vaccine efficacy, where robust T-cell activation is essential. TLR8 agonists could serve as powerful adjuvants in vaccines, potentially leading to more effective and durable immune responses.

The reversal of HIV-1 latency through TLR8 activation, as demonstrated by Meås et al. (2020), highlights another significant therapeutic avenue. By targeting latent HIV-1 reservoirs, TLR8 agonists could potentially contribute to a functional cure for HIV-1, enabling the immune system to recognize and eliminate latent viral reservoirs.

Implications for Vaccine Development

Studies by Akache et al. (2016) and Ayithan et al. (2021) emphasize the potential of TLR7 and TLR8 agonists in enhancing vaccine responses. Akache et al. (2016) demonstrated that TLR7 is essential for robust antibody responses in vaccines, suggesting that TLR7 agonists could significantly improve vaccine efficacy. Similarly, Ayithan et al. (2021) showed that TLR8

activation improved specific B cell responses in chronic hepatitis B, indicating that TLR8 agonists could enhance vaccine responses in persistent viral infections.

Cancer Immunotherapy

The role of TLR7 and TLR8 in cancer immunotherapy is an emerging area of interest, with promising findings reported by Michaelis et al. (2019). The TLR7/8 agonist R848 was shown to remodel tumor and host responses, reducing tumor mass and enhancing CD8+ T-cell activity in pancreatic cancer models. This suggests that TLR7 and TLR8 agonists could potentially enhance the efficacy of cancer immunotherapies, possibly in combination with existing treatments to improve patient outcomes.

Balancing Immune Activation and Autoimmunity

While the benefits of TLR7 and TLR8 activation in enhancing immune responses are clear, the potential for exacerbating autoimmune conditions cannot be ignored. Studies such as those by Celhar et al. (2019) and Murakami et al. (2021) highlight the need for a balanced approach to modulating TLR activity. For instance, using anti-TLR7 antibodies to mitigate autoimmune effects in lupus models points to the necessity of carefully regulating TLR activation to avoid unwanted immune responses.

Future Directions

The findings from this systematic review suggest several avenues for future research and therapeutic development. Investigating the precise mechanisms by which TLR7 and TLR8 agonists enhance immune responses could lead to more targeted and effective therapies. Additionally, exploring the combination of TLR agonists with other immunotherapeutic strategies could provide synergistic effects, further enhancing treatment efficacy.

In conclusion, TLR7 and TLR8 activation presents a promising strategy for enhancing immune responses, improving vaccine efficacy, and developing new therapies for infectious diseases, autoimmune disorders, and cancer. The challenge lies in harnessing these benefits while mitigating potential adverse effects, paving the way for innovative and effective immunotherapeutic approaches.

Limitations

This systematic review has several limitations to consider. First, the included studies varied significantly in methodologies, populations, and experimental designs, introducing heterogeneity and limiting result comparability. This diversity highlights broad interest in TLR7 and TLR8 but challenges synthesizing findings into a coherent narrative.

One limitation is the reliance on preclinical studies and animal models, which may not fully translate to human physiology. Many studies showing the efficacy of TLR7 and TLR8 agonists in enhancing immune responses, particularly in vaccine development and cancer immunotherapy, were conducted in mice or other non-human models. This limits the generalizability of the results to human clinical settings. Differences in TLR expression and function between species can also impact the translatability of these findings.

The review encountered a lack of standardization in measuring immune responses and outcomes. Various studies used different biomarkers, assays, and endpoints to assess the effects of TLR7 and TLR8 activation, complicating direct comparisons. This lack of uniformity may lead to variability in reported outcomes and obscure underlying trends or mechanisms.

Publication bias is another concern, as studies with positive findings are more likely to be published than those with negative or inconclusive results. This bias could skew the overall impression of the efficacy of TLR7 and TLR8 agonists. Additionally, the search was limited to studies published in English, potentially excluding relevant research in other languages.

Focusing on studies published in the last ten years ensures the inclusion of recent advancements but may omit important foundational work. While the emphasis on recent studies captures the latest developments in TLR7 and TLR8 research, it could lead to an incomplete understanding of the historical context and evolution of these findings.

The search strategy was comprehensive, but there is always a risk of missing pertinent studies, particularly those indexed in databases not included in the search or those using different terminology to describe similar phenomena. The inclusion criteria, while aimed at ensuring relevance, may have excluded studies with valuable insights due to differences in focus or reporting.

Finally, the review highlights the potential of TLR7 and TLR8 agonists in enhancing immune responses and treating various diseases. However, it also underscores the need for further research to address the gaps and limitations identified. Future studies should aim for more standardized methodologies, include diverse populations, and focus on translating preclinical findings into clinical applications. This will help develop a more comprehensive understanding of the therapeutic potential of TLR7 and TLR8 agonists.

Conclusion

This systematic review underscores the critical roles of TLR7 and TLR8 in enhancing immune responses, vaccine efficacy, and cancer treatment. Their activation significantly boosts cytokine production, B-cell and T-cell activation, and antibody responses, essential for robust immunological defense and improved vaccine performance. These receptors show promising potential as adjuvants in next-generation vaccines, particularly RNA-based vaccines, and in cancer immunotherapy by enhancing anti-tumor immune responses.

The impact of TLR7 and TLR8 extends beyond infectious diseases to autoimmune disorders and cancer, highlighting their versatile therapeutic potential. Their activation offers potential benefits in vaccine efficacy, antiviral immunity, and cancer treatment, paving the way for innovative and effective immunotherapeutic strategies.

Future research should aim to translate preclinical findings into clinical applications, ensuring long-term safety and efficacy while exploring combinations with other immunotherapies. TLR7 and TLR8 are promising targets for developing new therapies, and their continued exploration could lead to significant advancements in human health.

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