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Targeting the TLR9-MyD88 pathway in the regulation of adaptive immune responses

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Abstract

IMPORTANCE OF THE FIELD—Toll-like receptors (TLRs) are innate immune receptors critical in the innate immune defense against invading pathogens. Recent advances also reveal a crucial role for TLRs in shaping adaptive immune responses, conferring a potential therapeutic value to their modulation in the treatment of diseases.

AREAS COVERED IN THIS REVIEW—The aim of this review is to discuss TLR9, the TLR9-MyD88 signaling pathway and its role in regulation of adaptive immune responses, as well as potential therapeutic implications by targeting this pathway.

WHAT THE READER WILL GAIN—This review shows that the TLR9-MyD88 signaling pathway plays a critical role in promoting adaptive immune responses and that modulation of this pathway may have enormous therapeutic potential in enhancing vaccine potency, controlling autoimmunity, as well as improving the outcome of viral vector-mediated gene therapy.

TAKE HOME MESSAGE—Although TLR9 agonists have been used as adjuvants for enhancing vaccine potency, further exploitation of the TLR9-MyD88 pathway and its dynamic interaction with the immune system in vivo is needed to provide more effective therapeutic inventions in the design of vaccines for infectious diseases, allergies and cancer, in the control of autoimmunity, as well as in the improvement of viral vector-mediated gene therapy.

1. Introduction

The innate immune system represents the first line of defense against invading pathogens through recognition of pathogen-associated molecular patterns (PAMPs) by a set of receptors called pattern recognition receptors (PRRs)¹. Among the PRRs identified to date, the best studied is the Toll-like receptor (TLR) family. So far, 13 TLRs have been identified in mammals, and each TLR appears to recognize a unique set of PAMPs with distinct chemical structure¹. TLR2 recognizes a variety of microbial components such as peptidoglycan found in Gram-positive bacteria²; TLR4 recognizes lipopolysaccharide (LPS), a component of the outer membrane unique to Gram-negative bacteria³; TLR3 recognizes double-stranded RNA during viral replication⁴; TLR7 and TLR8 recognize single-stranded viral RNAs^{5, 6}, and TLR9 recognizes unmethylated CpG DNA motif found in DNA viruses and prokaryotic genomes^{7, 8}. TLRs are expressed on various sentinel cells of the immune system such as macrophages and dendritic cells (DCs) including

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Declaration of interest

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plasmacytoid DCs (pDCs) and conventional DCs (cDCs)¹. TLRs 1, 2, 4, 5 and 6 are expressed on the cell surface, whereas TLRs 3, 7, 8 and 9 have been shown to be expressed almost exclusively in endosomes^{9–12}.

Upon recognition of PAMPs, TLRs trigger a series of signaling cascades leading to induction of antimicrobial genes and inflammatory cytokines, which results in direct killing of the invading pathogens¹³. For example, TLRs induce the secretion of type I interferons (IFNs) such as IFN- α and IFN- β , in response to viral DNA or RNA, which is required for effective antiviral response. TLRs also induce activation of cytokines such as IL-1 β , IL-6, and TNF, key mediators of the inflammatory response, as well as chemokines that collectively induce acute inflammatory responses to pathogens. Most TLRs signal through the MyD88-dependent pathway. Following TIR domain stimulation, it recruits the adaptor protein MyD88, forming TIR-domain – TIR-domain homodimers, initiating the downstream signaling cascade leading to the induction of proinflammatory response characterized by the production of cytokines and chemokines^{14, 15}. However, TLR3 utilizes a MyD88-independent, but TIR-domain-containing adaptor protein inducing IFN-beta (TRIF)-mediated signaling pathway for the production of IFN- β . For TLR4, both MyD88- and TRIF- dependent pathways are utilized for the production of inflammatory cytokines¹⁶.

During the course of evolution, in addition to the innate immunity, vertebrates have acquired a sophisticated form of self-defense system, the adaptive immunity, rendering the hosts the capability of clonal expansion and memory recall¹⁷. TLR signaling not only leads to the activation of the innate immunity but also plays a critical role in the development of the ensuing antigen-specific adaptive immune responses¹⁸. In this review, we focus on recent advances made in the understanding of TLR9 recognition of CpG DNA, the TLR9-MyD88 signaling pathway and its role in the regulation of adaptive immune responses, as well as potential therapeutic implications by targeting this pathway.

2. The TLR9 pathway

2.1 TLR9 recognition of CpG DNA

TLR9 recognizes synthetic CpG motif-containing oligodeoxynucleotides (ODN)^{7, 19}. It also recognizes unmethylated CpG motif-containing bacterial or viral DNA^{20, 21}. It has been shown that TLR9 is critical in the control of bacterial infections with *Brucella*²², *Streptococcus pneumoniae*²³, and *Mycobacterium tuberculosis*²⁴. TLR9 polymorphism has been associated with an increased risk for *Helicobacter* infection, suggesting that TLR9 is involved in recognition and clearance of *Helicobacter*²⁵. TLR9 also detects many DNA viruses including herpes simplex virus (HSV) type 1²⁶ and type 2⁸, murine cytomegalovirus (MCMV)²⁷, adenovirus²⁸, and recently adenovirus-associated virus (AAV)²⁹, leading to production of type I IFNs and anti-viral immune responses. In addition to CpG motifs, recent studies have indicated an important role of nucleotide derivatives and the DNA sugar backbone in determining TLR9-dependent immune stimulatory activities^{30, 31}. Thus, TLR9 recognizes CpG motifs as well as DNA itself with certain structures.

There are three structurally distinct classes of CpG ODN: CpG-A, CpG-B and CpG-C³². CpG-A preferentially stimulates pDCs to type I IFNs and maturation of antigen presenting cells (APC), but very little B cell activation. CpG-B induces very strong B cell proliferation and differentiation, and average levels of type I IFN production and APC maturation. CpG-C has combined activities of CpG-A and CpG-B. The optimal CpG motif in mice consists of an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines³³, whereas the optimal motif in humans is TCGTT and/or TCGTA³⁴. In addition, the cell types that express TLR9 differ between species. In mice, TLR9 is expressed in B cells, monocytes and virtually all DC subsets including cDCs³⁵. The expression profile of TLR9 is much

more restricted in humans than in mice. In human, TLR9 is predominantly expressed in pDCs, also known as professional type I IFN-producing DCs, and B cells among resting immune cells³⁵. TLR9 has also been shown to express in some non-immune cells, including intestinal epithelium³⁶, keratinocytes³⁷, as well as respiratory epithelial cells³⁸.

2.2 The intracellular TLR9-MyD88 signaling pathway

As most pathogens encounter the endocytic pathway upon entering and leaving cells³⁹, the endosomal compartment has evolved as a key intracellular structure in detecting pathogens. In the steady state, TLR9 is localized in the endoplasmic reticulum (ER) in association with Unc93b, an ER protein critical for the translocation of TLR9 to the endosome when cells are stimulated^{40, 41}. All TLRs are type I integral membrane glycoproteins and contain leucine-rich repeats (LRRs) in their extracellular domain and a cytoplasmic TIR signaling domain¹. In the acidic endosomal compartments, binding of CpG motif-containing DNA to TLR9 triggers dimerization, which results in allosteric conformational changes in the TLR9 cytoplasmic TIR domains, leading to the recruitment of MyD88^{12, 42}.

MyD88 contains a TIR domain and a death domain⁴³. The association of TIR domains of MyD88 and TLR9 in turn activates the interleukin-1 receptor-associated kinase-4 (IRAK-4) and IRAK-1 through the death domain of MyD88⁴³. IRAK-4 is essential for TLR9-mediated pro-inflammatory cytokine production⁴⁴. Activation of IRAK-4 subsequently recruits tumor necrosis factor receptor associated factor 6 (TRAF6)⁴⁵, leading to activation of transforming growth factor- β associated kinase 1 (TAK1). TAK1 then phosphorylates I κ B kinase (IKK) complex, which results in NF- κ B activation, as well as mitogen-activated protein kinases (MAPKs), leading to AP-1 activation⁴⁶. Transcription factors NF- κ B and AP-1 are responsible for the transcription of proinflammatory cytokine genes, including IL-6, IL-12 and TNF, and upregulation of co-stimulatory molecules such as CD80 and CD86¹³.

Activation of TLR9 in pDCs induces production of predominately type I IFNs⁴⁷. This is critically dependent on the interaction between MyD88 and the interferon regulatory factor-7 (IRF7) signaling complex, which consists of IRF7, IRAK 4, IRAK1, TRAF6, resulting in phosphorylation of IRF-7 and induction of type I IFNs⁴⁸. However, this pathway is not operative in non-pDCs such as cDCs, and TLR9 stimulation in cDCs leads to the activation of NF- κ B and the production of pro-inflammatory cytokines such as IL-6 and IL-12, but not IFN- α . Why pDCs couple TLR9 signaling to IRF7 remains to be defined. It has been speculated that this might be related to a higher level of IRF7 expression in pDCs than cDCs⁴⁹; or that it may be attributed to the ability of pDCs to retain TLR9 ligand, CpG DNA in the endosomal compartment together with the MyD88-IRF7 complex for long periods of time⁵⁰. In addition, a recent study has suggested that the differential use of cofactors such as osteopontin may contribute to the expression of IFNs in pDCs⁵¹.

3. Regulation of adaptive immune responses by the TLR9-MyD88 pathway

Early studies in TLR9-deficient mice revealed that TLR9 was not only required for the production of pro-inflammatory cytokines, but also for the induction of CD4 T helper 1 (Th1)-biased immune response and the proliferation of B cells⁷. Indeed, subsequent studies in other models of infection support a critical role for the TLR9-MyD88 pathway in the induction of adaptive immune responses in response to infections with *Mycobacterium tuberculosis*²⁴, *Leishmania major*⁵², mousepox virus⁵³, as well as AAV²⁹. How does the TLR9-MyD88 pathway promote adaptive immune responses?

Upon stimulation of TLR9 with CpG-DNA, murine cDCs and macrophages produce pro-inflammatory cytokines, such as TNF- α , IL-6 and IL-12, and upregulate surface expression of co-stimulatory molecules such as CD40, B7-1 (CD80) and B7-2 (CD86), and MHC class

II (Figure 1)⁵⁴. T cell activation and differentiation into effector cells occur after naïve T cells receive multiple signals from DCs including antigen stimulation ('signal 1') via T cell receptor (TCR), the co-stimulation ('signal 2'), and other signals such as the cytokine milieu. Thus, the enhanced DC maturation and the Th1-like cytokine and chemokine milieu induced by TLR9 stimulation result in efficient and robust activation of Th1 and CD8 cytotoxic T lymphocytes (CTL)^{55, 56}.

pDCs produce large amounts of type I IFNs upon TLR9 stimulation⁴⁷. In addition to direct anti-viral effects⁵⁷, type I IFNs are key molecules in regulating adaptive T cell responses and B cell responses⁵⁸. First, it has been shown that type I IFNs can induce DC maturation by upregulating the expression of co-stimulatory molecules, which in turn promotes CD8 and CD4 T cell responses⁵⁹. Second, DCs matured by type I IFNs promote cross-priming of virus-specific CD8 T cells⁶⁰. Third, type I IFNs can promote effector function of virus-specific T cells⁶¹, and enhance the survival of activated T cells⁶². Lastly, type I IFN signaling on DCs is important for the production of virus-specific IgM, whereas the generation of protective neutralizing antibodies to adenovirus critically depends on the type I IFN signaling on both CD4 T cells and B cells²⁸. Taken together, these observations suggest that type I IFNs play a pivotal role in promoting adaptive immune responses.

TLR9-activated DCs also activate natural killer (NK) cells, leading to NK cell cytotoxicity and production of IFN- γ . This activation is dependent on both cell-cell contact and paracrine action of type I IFNs, IL-12, and IL-15⁶³. In MCMV infection, NK cell activation requires TLR9 signaling and type I IFN production by pDCs²⁷. Since activated NK cells produce IFN- γ , which in turn activates DCs⁶⁴, TLR9 signaling may play a pivotal role in the crosstalk between DCs and NK cells to achieve maximal activation of both cell types and promoting adaptive immune responses.

CpG ODN can also directly induce the proliferation of B cells and promote their survival³³. In addition, there is synergy between TLR9 and the B cell receptor (BCR) in stimulating antigen-specific B cells³³. These processes are dependent on an intact TLR9-MyD88 signaling pathway⁷. TLR9 signaling also promotes Th1-biased Ig class switching in B cells^{65, 66}. Although it has been suggested that TLR signaling on B cells directly controls antibody production by B cells with the help of Th cells⁶⁷, a more recent report indicated that the role of intrinsic TLR signaling on antibody production by B cells remains controversial⁶⁸. Nevertheless, these results support a notion that CpG ODN can influence B cell response at multiple stages.

4. Targeting the TLR9-MyD88 pathway for the management of diseases

4.1 Development of TLR9 agonists as vaccine adjuvants for infectious and allergic diseases

As activation of the TLR9 pathway promotes antigen-specific adaptive T and B cell responses, TLR9 agonists such as CpG ODN have been developed and evaluated for use as vaccine adjuvants. Studies have shown that CpG ODN induce more potent Th1 immune responses than any other single vaccine adjuvant^{69, 70}. Thus, CpG ODN have become the adjuvant of choice in many vaccine formulations such as peptide or protein antigen, cell-based vaccines, live or inactivated recombinant viruses as well as dendritic cell vaccines⁷¹. It has been shown that CpG ODN delivered in a close association with the antigen-specific vaccine is critical for inducing optimal antigen-specific immune responses⁷¹. Although CpG ODN have been delivered via subcutaneous, intramuscular, or intravenous injection in most studies, they are also effective in inducing local and systemic humoral and cellular immunity when given through mucosal routes⁷².

Several CpG ODN have been evaluated in human clinical trials as vaccine adjuvants for hepatitis B virus (HBV), influenza virus, as well as bioterrorism agents such as anthrax. It has been shown that addition of CpG ODN to the commercial alum-absorbed HBV vaccine significantly enhances its potency. In a randomized, double-blind controlled phase I/II dose escalation study, healthy individuals were given three intramuscular injections of the alum-absorbed HBV vaccine either in saline or mixed with a CpG-B ODN, PF-3512676 (CpG 7909)⁷³. The subjects who received PF-3512676 as adjuvant developed protective anti-HBsAg specific antibody responses earlier (just within two weeks of vaccination) and had higher titers at all time points, compared to those individuals receiving the vaccine alone.

Addition of CpG ODN also reduces the vaccine dose required to induce protection against influenza virus. In a phase I randomized, double-blind clinical trial, individuals vaccinated with just one-tenth of the normal influenza vaccine dose had reduced levels of antigen-specific T cell response compared to those vaccinated with the full-dose vaccine alone⁷⁴. However, co-injection of CpG ODN, PF-3512676 with the one-tenth vaccine dose restored the antigen-specific T cell response to the level detected in subjects with full-dose vaccine alone. Co-administration of CpG ODN also accelerates the formation of protective antibody response. In a randomized controlled trial in healthy subjects, CpG ODN, PF-3512676 promoted seroconversion when used as an adjuvant with the approved anthrax vaccine⁷⁵. Furthermore, PF-3512676 induced significantly higher levels of neutralizing antibody titers.

The Th1-biased immune response induced by CpG ODN has also been utilized to suppress the pathogenic Th2 allergic response. In a murine model of asthma, CpG ODN reprogram the allergic Th2 response and prevent airway inflammation, leading to alleviation of the disease⁷⁶. Clinical trials have been conducted using CpG ODN in conjugation with the ragweed allergen as a vaccine. In these trials, selective and specific redirection of the allergic Th2 response towards a non-allergic and non-inflammatory Th1 response has been observed with apparent clinical benefit^{77, 78}.

It has recently shown that CpG ODN can induce TNF-dependent toxicity in rodents, but in human⁷⁹. This is consistent with the observations that CpG ODN are generally well tolerated in patients in clinical trials. The common adverse effects associated with CpG ODN are dose-dependent transient injection site reactions such as erythema and induration and less commonly mild flu-like symptoms⁸⁰. This differential toxicity profiles between rodents and humans could be due to TLR9 is predominantly expressed in pDCs, but not in other innate immune cells such as cDCs and macrophages³⁵, which produce mainly pro-inflammatory cytokines such as TNF upon TLR9 stimulation.

4.2 Targeting the TLR9 pathway in cancer immunotherapy

An ultimate goal of cancer immunotherapy is to eradicate pre-established tumors through therapeutic vaccinations⁸¹. However, most vaccine strategies have shown only moderate success in treating pre-established tumors in both animals and patients in clinical trials⁸². This modest success is likely due to tumor cells that have developed mechanisms to avoid recognition and elimination by the immune system. The mechanisms of immune evasion include down-regulation of components of the antigen processing and presentation machinery, production of cytokines that inhibit or divert productive effector responses, and induction of tumor antigen-specific T cell tolerance^{81, 82}. Thus, to elicit effective anti-tumor immunity, it is necessary to develop vaccine strategies capable of overcoming tumor-specific T cell tolerance.

Recent advances have revealed a critical role for TLRs in overcoming CD4⁺CD25⁺ regulatory T (T_{Reg}) cell-mediated T cell tolerance^{83, 84}. In a murine model of pre-established lymphoma, it has been shown that tumor-specific CD8 T cell tolerance is also

mediated by T_{Reg} cells and that DC vaccines co-administered with CpG ODN can effectively convert T_{Reg}-mediated tumor-specific CD8T cell tolerance into effector CD8 T cell response, leading to significant prolongation of tumor-free survival⁵⁶. In this study, CpG-induced type I IFNs by pDCs is critical for the reversal of tumor-specific T cell tolerance in vivo⁵⁶.

CpG ODN have also shown promise as cancer vaccine adjuvants in human clinical trials. In a study evaluating melanoma vaccines carrying the melanoma-associated antigen recognized by T cells (MART1), a protein expressed in most melanomas and some other types of tumors, vaccination with the MART1-derived peptide in incomplete Freund's adjuvant (IFA) combined with TLR9 agonist, PF-3512676 at a low dose resulted in about 10-fold increase in antigen-specific T cell frequency in patients than without the TLR9 agonist, and that ex vivo analyses showed these tumor-specific T cells have differentiated to effector cells to a level comparable to that seen in EBV and CMV specific T cells⁸⁵.

4.3 The TLR9 pathway and autoimmunity

Type I IFNs have been implicated in the pathogenesis of certain autoimmune diseases such as systemic lupus erythematosus (SLE)⁸⁶. Since TLR9 stimulation in pDCs induces the production of mainly type I IFNs, the TLR9-MyD88 signaling pathway is recently shown to play a critical role in autoimmunity, especially in SLE.

Although vertebrate DNA is methylated and contains DNA sequences that are suppressive to TLR9 activation, under certain disease conditions self DNA can activate the TLR9 signaling pathway, leading to the development of autoimmune diseases⁸⁷. SLE patients often have mutations in genes encoding DNA nucleases including DNase-I⁸⁸, DNase-II⁸⁹, and DNase-III⁹⁰, which result in accumulation of self DNA. In addition, anti-nuclear antibodies (ANA), an autoantibody for chromatin and ribonucleoprotein (RNP), are frequently detected in SLE patients. The ANA-DNA complex is internalized into the endosomal compartment in pDC through Fcγ receptors⁹¹. Activation of TLR9 in the endosomes by self DNA results in pDC activation and type I IFN production⁹¹. In B cells, self DNA binds to surface self DNA-specific IgM, and the DNA-IgM complex is internalized and recognized by TLR9 in the endosomes, which triggers the TLR9-MyD88 signaling pathway to facilitate autoimmunity⁹². TLR9 stimulation also enhances class switching in TLR9-MyD88 dependent manner and promotes the production of self DNA-specific IgG2a and IgG2b, which worsens the pathology of autoimmunity⁹³.

In order to suppress TLR9-dependent autoimmunity by self DNA in SLE, several suppressive ODN have been developed with potential therapeutic implications. These suppressive ODN can inhibit TLR-dependent activation induced by DNA- and RNA-immune complexes in vitro and are effective to treat lupus in an animal model^{94, 95}. Thus, suppressive ODN appear to be promising candidates for treating certain autoimmune diseases such as SLE.

4.4 Targeting the TLR9-MyD88 pathway in gene therapy

Recombinant viral vectors such as adenovirus and adenovirus-associated virus (AAV) have been used widely as vehicles for in vivo gene therapy because of the high efficiency with which they transfer genes into a wide spectrum of cells in vivo. However, the attendant host adaptive cellular and humoral immune responses to viral vectors have posed a significant hurdle in clinical application of these vectors. Recent advances have suggested a crucial role for the innate immune pathways in the induction of adaptive immune responses to viral vectors⁹⁶.

Several groups have recently demonstrated that the innate immune recognition of adenovirus is mediated by both TLR-dependent and -independent pathways. Sensing of adenovirus or adenoviral DNA by pDCs is mediated by TLR9 and MyD88, leading to production of type I IFNs^{28, 97}, whereas recognition of adenovirus by non-pDCs such as cDCs, macrophages and hepatic Kupffer cells is independent of TLR, also resulting in secretion of mainly type I IFNs^{28, 98}. The cytosolic sensing of adenoviral DNA is mediated by a yet unidentified DNA sensor and dependent on IRF3^{28, 98}.

The innate immune pathways involved in the detection of AAV are more restricted than those in adenovirus. A recent study shows that AAV mainly activates TLR9 on pDCs, leading to production of type I IFNs²⁹. In vivo, the TLR9-MyD88 pathway is crucial to the activation of CD8 T cell responses to both the transgene product and the AAV vector, leading to loss of transgene expression, and the generation of anti-transgene and AAV-neutralizing antibodies²⁹. This TLR9-dependent activation of adaptive immunity to AAV is mediated by type I IFNs²⁹. Furthermore, AAV also activates human pDCs to induce type I IFNs via TLR9²⁹. These results reveal an essential role for the TLR9-MyD88-type I IFN pathway in the induction of adaptive immune responses to AAV.

A critical role for the TLR9 signaling pathway in the induction of adaptive immune response to AAV suggests that strategies targeted to interfere with this signaling pathway in pDCs will likely minimize T cell responses to viral capsid antigen and the transgene product, thus lead to prolonged transgene expression and reduction in inflammation, improving the safety and efficacy of AAV vectors for gene therapy in humans. In addition, blockade of TLR9-MyD88-type I IFN signaling pathway will also diminish antibody responses to the transgene product as well as neutralizing antibody to AAV vector. Reduction of antibody to transgene product will prevent neutralization of secreted transgene-derived product, whereas reduction of antibody to AAV vectors will allow for re-administration of the vector if needed.

One way to interfere with the TLR9-MyD88-type I IFN signaling pathway is to use antagonists specific to TLR9 such as inhibitory ODN to block the TLR9-MyD88 pathway²⁹. As the activation of pDCs depends on an intact TLR9-MyD88 pathway, this will block the innate sensing of AAV by pDCs. Alternatively, blockade of type I IFNs by administering neutralizing antibodies to IFN- α and IFN- β may also prove effective as type I IFNs are required for adaptive immune responses to AAV²⁹.

5. Conclusion

In this review, we have emphasized on the innate immune recognition by TLR9, the TLR9-MyD88 signaling pathway, and the role of the TLR9 pathway in promoting the adaptive immunity. TLR9 agonists have been used as highly effective immune modulators with broad potential applications as vaccine adjuvants for the treatment of infectious diseases, allergic diseases and cancer. However, future research on the mechanisms of TLR9-mediated immunity, at both molecular and cellular levels, will help us develop more effective strategies to improve vaccine potency and to interfere with the TLR9 signaling pathway in favor of controlling autoimmunity and improving the outcome of viral vector-mediated gene therapy.

6. Expert Opinion

It is clear from the studies performed to date that the TLR9-MyD88 pathway plays a key role in the DNA-induced innate immune response, and is critical in promoting the ensuing adaptive T and B cell responses through the activation of various cell types including pDC, cDC, and B cells. The ligands for TLR9 have been identified and the TLR9 intracellular signaling pathway has been extensively studied at the molecular level. These critical

observations have led to the development of therapeutic strategies to enhance the potency of vaccines for infectious diseases, allergies and cancer, based on activation of the TLR9 pathway by its ligand, CpG ODN. Furthermore, TLR9 signaling has also been shown to play a role in some autoimmune diseases, as well as the unwanted acquired immunity against viral vectors for gene therapy.

Despite these advances, many questions remain. Although the TLR9-MyD88 intracellular signaling pathway has been well studied at the molecular level, precise knowledge about cell types, timing, and the location where TLR9 signaling takes place has yet to be fully defined. Elucidation of this will help design more effective strategies to modulate TLR9-dependent immunity in the treatment of infectious diseases, allergic diseases and cancer in vivo. Transgenic or knock-in mice with reporter gene in combination with an in vivo imaging system such as intravital two photon microscopy⁹⁹, may facilitate achieving this goal.

In addition to TLR9-dependent recognition of pathogen DNA in the endosomal compartment, dsDNA can also be recognized in the cytosol in a TLR-independent manner, which also induces production of type I IFNs^{100, 101}. Overstimulation of this pathway can lead to autoimmunity¹⁰². This cytosolic pathway is also involved in the sensing of adenoviral vector DNA^{28, 98}. The DNA-dependent activator of IFN-regulatory factors (DAI) was thought to be a potential cytosolic DNA sensor¹⁰³. Overexpression of DAI in mouse fibroblasts enhances the DNA-mediated induction of type I IFNs, whereas knockdown of endogenous DAI by siRNA inhibits it¹⁰³. However, recent generation of DAI-deficient mice showed that DAI was not essential for either innate or adaptive immune responses to DNA or DNA vaccination in vitro or in vivo¹⁰⁴. Therefore, the cytosolic sensor for dsDNA still remains elusive. Future studies are required to identify the cytosolic DNA sensor, which may shed light on the design of strategies to interfere with innate immune recognition of DNA in the cytoplasm in favor of suppressing autoimmunity as well as improving adenoviral vector-mediated gene therapy.

TLR9 agonists such as CpG ODN have been used widely as vaccine adjuvants to stimulate the TLR9 signaling pathway for the treatment of infectious diseases, allergic diseases and cancer. However, very few strategies have been developed to suppress the TLR9 pathway for the control of autoimmunity or to improve viral vector-mediated gene therapy. One way to inhibit the TLR9-MyD88 signaling pathway is to use antagonists specific to TLR9²⁹. Alternatively, since type I IFNs are the critical mediators that link the innate immunity to the adaptive immune responses, the development of strategies to interfere with type I IFN signaling pathway may also help control autoimmunity and improve the outcome of gene therapy with viral vectors.

Article highlights

- TLRs are crucial to both innate and adaptive immune responses.
- TLR9 recognizes CpG motif-containing DNA or ODN.
- Recognition of CpG by TLR9 in the endosome activates the adaptor MyD88, leading to production of type I IFNs and pro-inflammatory cytokines.
- The TLR9-MyD88 pathway play a critical role in regulating adaptive T and B cell responses
- Modulation of the TLR9-MyD88 pathway has important therapeutic potential in enhancing the potency of vaccines against pathogens and tumor, controlling autoimmunity and improving the outcome of viral-vector-mediated gene therapy.

- Further studies on the dynamic interaction of the TLR9-MyD88 pathway with the immune system in vivo are needed to provide more important insights into the design of effective therapeutic interventions.

Abbreviations

AAV	adenovirus-associated virus
ANA	anti-nuclear antibodies
BCR	B cell receptor
cDC	conventional DC
CTL	cytotoxic T lymphocyte
DAI	DNA-dependent activator of IFN-regulatory factor
DC	dendritic cell
ER	endoplasmic reticulum
HBV	hepatitis B virus
HSV	herpes simplex virus
IFN	interferon
IFA	incomplete Freund's adjuvant
IKK	I κ B kinase
IRAK	interleukin-1 receptor-associated kinase
IRF	interferon regulatory factor
LPS	lipopolysaccharide
LRR	leucine-rich repeat
MAPK	mitogen-activated protein kinase
MART1	melanoma-associated antigen recognized by T cells 1
MCMV	murine cytomegalovirus
MHC	major histocompatibility complex
MyD88	myeloid differentiating factor 88
NK cell	natural killer cell
ODN	oligodeoxynucleotide
PAMP	pathogen-associated molecular pattern
pDC	plasmacytoid DC
PRR	pattern recognition receptor
RNP	ribonucleoprotein
SLE	systemic lupus erythematosus
TAK	transforming growth factor- β associated kinase
TCR	T cell receptor
Th	T helper

TIR	Toll protein and the interleukin-1 receptor
TLR	Toll-like receptor
TRAF	TNF receptor associated factor
TRIF	TIR-domain-containing adaptor protein inducing IFN-beta
T _{Reg}	regulatory T cells

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Figure 1. The role of TLR9 in regulation of adaptive immunity and diseases

CpG DNA or ODN can activate TLR9 on pDC, cDC, and B cells. Activation of pDC leads to production of mainly type I IFNs such as IFN- α and IFN- β , which then act on various cell types, including NK cells, T cells, and cDC. Activated NK cells produce IFN- γ , which in turn activates cDC. Activation of cDC promotes their maturation and function in antigen presentation, as well as production of pro-inflammatory cytokines, both of which are critical for the activation of CTL and Th1 differentiation. cDC also activate NK cells and B cells by cell-cell contact or soluble factors. Th1 differentiation can promote both CTL and Th1-biased B cell response and suppress Th2 response. CTL exhibit cytotoxic response to combat against tumor and eliminate pathogens. Th1-biased B cell response leads to enhanced production of IgG2a, which increases neutralizing antibody titers against pathogens. Th1 also suppresses Th2 response, leading to reduced levels of IgE production, and thus inhibits allergic reactions.