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Activation of rabbit TLR9 by different CpG-ODN optimized for mouse and human TLR9

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ABSTRACT

Synthetic CpG-oligodeoxynucleotides (CpG-ODN) are potent adjuvants that accelerate and boost antigen-specific immune responses. Toll-like receptor 9 (TLR9) is the cellular receptor for these CpG-ODN. Previous studies have shown species-specific activation of mouse TLR9 (mTLR9) and human TLR9 (hTLR9) by their optimized CpG-ODN. The interaction between rabbit TLR9 (rabTLR9) and CpG-ODN, however, has not been previously investigated. Here, we cloned and characterized rabTLR9 and comparatively investigated the activation of the rabbit, mouse, and human TLR9 by CpG-ODN. The complete open reading frame of rabTLR9 encodes 1028 amino acid residues, which share 70.6% and 75.5% of the identities of mTLR9 and hTLR9, respectively. Rabbit TLR9 is preferentially expressed in immune cells rich tissues, and is localized in intracellular vesicles. While mTLR9 and hTLR9 displayed species-specific recognition of their optimized CpG-ODN, rabbit TLR9 was activated by these CpG-ODN without any preference. This result suggests that rabTLR9 has a broader ligand-recognition profile than mouse and human TLR9.

Abbreviations: TLR, toll-like receptor; CpG-ODN, CpG-oligodeoxynucleotides; NF-κB, nuclear factor-κB; MyD88, myeloid differentiation factor 88; IRAK, IL-1R associated-kinase; TRAF6, TNFR-activated factor 6; TAK1, TGF-β-activating kinase; HEK, human embryonic kidney; FCS, fetal calf serum; PBS, phosphate buffer saline; DAPI, 4′,6 diamidino-2-2 phenylindole; RACE, rapid amplification of cDNA end.

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1. Introduction

Toll-like receptors (TLRs) play a critical role in host defense to microbial infections by sensing a wide variety of microbial pathogens with diverse structures from lipids, lipoproteins, glycans, proteins to nucleic acids [1–5]. TLR9 belongs to a subfamily of these TLRs, comprising TLR3, TLR7, TLR8, and TLR9, that recognizes nucleic acids [5,6]. This subfamily of TLRs is distinct from other TLRs in their cellular localization. Whereas others are expressed on cell surface, these four TLRs are located in the intracellular vesicles [7,8].

Bacterial and viral DNA are potent stimuli to immune cells. This immunostimulatory activity is assigned to sequence motifs containing unmethylated CpG-dideoxynucleotides. Synthetic CpG-oligodeoxynucleotides (CpG-ODN) mimic the stimulatory effect of these microbial DNA in activation of immune cells. The activity of a CpG-ODN is determined by its length; the number of CpG motifs; and the spacing, position, and surrounding bases of these motifs. In addition, there are species-specific differences for these CpG-ODN. For example, CpG-ODN containing GTCGTT motif preferentially activates human cells, whereas CpG-ODN with GACGTT motif displays the greatest activity toward mouse cells [9–13].

TLR9 is the cellular receptor to mediate the function of these CpG-ODN [14–16]. Upon activation, this TLR recruits MyD88 to form a MyD88/IRAK1/IRAK4/TRAF6 complex. This in turn activates TAK1, leading to the activation of NF-κB and production of pro-inflammatory cytokines, including TNF-α, IL-6 and IL-12. In plasmacytoid dendritic cells, TLR9 activates IRF7 through the MyD88/IRAK1/IRAK4/TRAF6 complex, leading to the production of type I IFNs [17,18]. Because of the potent immune responses facilitate eradication of viral infected cells and cancer cells from bodies, CpG-ODN are investigated for their therapeutic application for treatment of infectious diseases and cancers [19,20]. In addition, these CpG-ODN are being developed as adjuvant to boost antigen-specific immune responses. In mice, they have been shown to be a very strong adjuvant to promote Th1 type of immune response [21,22]. The complete Freund's adjuvant (CFA) is commonly used for induction of cell-mediated immune responses in rodents. Nevertheless, CpG-ODN performing better in induction of Th1 type immune response, and appear to have an advantage over CFA in that they do not cause the severe local inflammation associated with the CFA [21,23,24].

Rabbits are commonly used for production of antibodies because they are relatively inexpensive and easy to handle. Although the safety and efficacy of using CpG-ODN as an immunological adjuvant to boost antibody production in rabbits have been investigated with favorable results [25], the function and interaction of rabbit TLR9 (rabTLR9) with CpG-ODN has not yet been investigated. In this study, we cloned and characterized rabTLR9 cDNA, and comparatively investigated the activation of rabbit, mouse, and human TLR9 by CpG-ODN.

2. Materials and methods

2.1. Reagents and antibodies

CpG-ODN, 4′,6 diamidino-2-2 phenylindole (DAPI) and Alexa-594-conjugated anti-mouse antibody were purchased from Invitrogen (Carlsbad, CA). FITC-conjugated CpG-2006 was purchased from Invivogen (San Diego, CA). Chloroquine and anti-flag M2 monoclonal antibody were purchased from Sigma (St. Louis, MO). Rabbit total RNAs from different tissues were purchased from Zyagen Laboratory (San Diego, CA).

2.2. Rapid amplification of cDNA ends

A modified rapid amplification of cDNA ends (RACE) method was performed using GeneRacer kit as described in the manual provided by the manufacturer (Invitrogen, Carlsbad, CA) to determine the 5′end cDNA sequence of rabTLR9. Briefly, rabbit spleen total RNA was treated with calf intestinal phosphatase and tobacco acid pyrophosphatase. After the selective ligation of GeneRacer RNA oligonucleotide to the 5′-ends of decapped mRNA, first strand cDNA synthesis was carried out with Superscript III reverse transcriptase. cDNA fragment encoding the 5′-end sequence of rabTLR9 was PCR amplified from the GeneRacer-generated first-strand cDNA library with a GeneRace 5′-primer and a primer specific to *rabTLR9* gene (5′-GGCAGGAACTGCGAGCCGTTGAC-3′) identified from nucleotide sequence of genomic DNA encoding *rabTLR9* (accession number: AAGW02044169). The generated cDNA containing 5′-end of rabTLR9 was ligated into a T/A cloning vector (Invitrogen, Carlsbad, CA) for sequencing.

2.3. Cloning and expression constructs for rabTLR9

Full-length rabTLR9 cDNA was cloned by PCR amplification from a rabbit first-strand cDNA library. This library was prepared from rabbit spleen total RNA using a Superscript preamplification kit (Invitrogen, Carlsbad, CA). PCR amplifications were performed using an Expand HI Fid PCR kit (Roche, Indianapolis, IN). The generated DNA fragment containing rabTLR9 was ligated into a T/A cloning vector (Invitrogen, Carlsbad, CA) for sequencing. To construct expression vector for rabTLR9, cDNA fragment was generated by PCR amplification from rabbit first-strand cDNA library with forward and reverse primers designed based on the DNA sequence for the complete coding region (amino acid residue 1-1028) of rabTLR9. The forward primer contains a KpnI site and has the sequence 5′-GGAAGGG TACCGCCACCATGTGTCCCTGTCGAGGAGCCC-3′. The reverse primer contains a SpeI site and has the sequence 5′-GGAAGACTAGTTTCAGCTGTGGGCCC CCGGCAG-3′. This PCR product was subcloned into a PEF6 expression vector (Invitrogen, Carlsbad, CA) containing a nucleotide sequence for expression of a Flag tag fusing to the c-terminal of the expressed rabTLR9.