26

TLR9 Agonists for Immune Enhancement of Vaccines

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INTRODUCTION

This review of the use of CpG oligodeoxynucleotide (ODN) agonists to toll-like receptor (TLR)9 as vaccine adjuvants briefly covers immune effects and mechanisms of CpG ODN and then provides more details as to how they can be used to enhance prophylactic and therapeutic vaccines against human or veterinary infectious diseases, cancer, and allergies.

Induction of adaptive immunity relies on simultaneous presentation of antigen to B and T cells, as well as activation of cells of the innate immune system including dendritic cells (DC), macrophages, and monocytes. Both B and T cells have highly specific receptors that recognize antigenic epitopes, and this in turn results in the development of antigen-specific antibodies and cytotoxic T-cell responses, respectively. In contrast, cells of the innate immune system lack highly specific antigen receptors but instead rely on a set of ''pattern recognition receptors'' (PRR), which have a general ability to detect ''pathogen-associated molecular patterns'' (PAMP) found in pathogens but not in self-tissues. Many of the PRR are found in the family of toll-like receptors (TLR), of which at least 10 types have been identified in humans. The immune system appears to use the presence of PAMP as a ''danger signal'' that indicates the presence of infection and activates appropriate defense pathways (1–3). Some TLR are located on the surface of immune cells and detect PAMP that would be present in the extracellular space. These include TLR2 and TLR6 that detect proteoglycans/peptidoglycans and bacterial lipopeptide (TLR2 only), TLR4 that detects lipopolysaccharide of gram-negative bacteria, and TLR5 that detects flagellin. Another group of TLR is located in the endosomal compartment of immune cells that detect nucleic acid–based PAMP that would be preferentially seen in the intracellular space. These include TLR3 that detects viral dsRNA, TLR7 and TLR8 that detect viral ss-RNA, and TLR9 that detects ''CpG motifs'' of bacterial and viral ssDNA (4). In some cases, the nucleic acid–binding TLR can also be activated by small-molecule mimics of their natural ligands, as is the case for imidazoquinolines that activate TLR7 and TLR8 (5,6); however, to date, there have been no reports of identification of small molecules that activate TLR9.

TOLL-LIKE RECEPTORS AND RECOGNITION OF CpG DNA BY TLR9

Recently there has been broad interest in testing and developing such danger signal ligands of PRR for immune stimulation, including use as adjuvants with vaccines to enhance antigen-specific responses. With respect to TLR9, synthetic ODN containing CpG motifs (CpG ODN) are being developed as immune therapy drugs and vaccine adjuvants.

There is an extensive literature regarding the molecular pattern in viral and bacterial DNA that activates TLR9, and the downstream signaling pathways. Since this chapter reviews the use of CpG ODN as vaccine adjuvants, only a brief outline on these aspects will be provided and more information can be obtained from other recent reviews (4). TLR9 is activated by CpG motifs that are unmethylated CpG dinucleotides within the context of certain flanking bases. These motifs are recognized as foreign, since mammalian DNA has suppression of CpG dinucleotides and the cytosine is usually methylated, which renders them nonimmune stimulatory. In humans, only B cells and plasmacytoid dendritic cells (pDC) express TLR9. Activation of other cell types results through indirect means, largely cytokine mediated. In mice the distribution of TLR9 is broader, including monocytes and myeloid-derived dendritic cells (mDC). CpG ODN enters immune cells after binding to cell surface DNA-binding proteins (non-sequence specific) and ends up within the endosomal compartment where it activates TLR9 (sequence dependent). There is some degree of species specificity with respect to optimal flanking sequences, with GACGTT being optimal in mice and GTCGTT being optimal in humans but also working in most species (4). Several different classes of CpG ODN have been described that differ largely in ability to form higher-ordered structures. These give different stimulatory profiles on human immune cells in vitro, but it is not clear whether these differences translate in vivo and in particular when used as vaccine adjuvants. Other factors that largely affect potency are the number and spacing of CpG motifs within the ODN and backbone modifications (4). Almost all vaccine data, heretofore, have been obtained with simple monomeric B-class CpG ODN.

NONCLINICAL STUDIES: GENERAL UTILITY OF CpG ADJUVANTS

Many of the identified direct and indirect effects of CpG ODN on immune cells could contribute to its efficacy as a vaccine adjuvant. Humoral responses are augmented because of CpG activation of B cells to secrete immunoglobulin and cytokines, aided by cross talk between the B-cell receptor and CpG signaling pathways. CpG also induces increased costimulatory molecule expression on B cells and other antigen presenting cells (APC). Furthermore, CpG inhibits B-cell apoptosis, contributing to a more sustained immune response (7,8). The most unique feature of CpG as an adjuvant is its outstanding ability