Vaccine xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Review

Clinical evaluation of CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases and cancer

Q1 Julia Scheiermann, Dennis M. Klinman*

Cancer and Inflammation Program, National Cancer Institute, NIH, Frederick MD 21702, United States

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 28 May 2014
Accepted 12 June 2014
Available online xxx

Keywords:
CpG oligonucleotide
Adjuvant
Infection
Cancer
Toll-like receptor

ABSTRACT

Synthetic oligonucleotides (ODN) that express unmethylated "CpG motifs" trigger cells that express Toll-like receptor 9. In humans this includes plasmacytoid dendritic cells and B cells. CpG ODN induce an innate immune response characterized by the production of Th1 and pro-inflammatory cytokines. Their utility as vaccine adjuvants was evaluated in a number of clinical trials. Results indicate that CpG ODN improve antigen presentation and the generation of vaccine-specific cellular and humoral responses. This work provides an up-to-date overview of the utility of CpG ODN as adjuvants for vaccines targeting infectious agents and cancer.

1. Introduction

Vaccines are highly effective public health interventions, having saved millions of lives by preventing infectious diseases. The efficacy of a vaccine is determined by the magnitude, duration and quality of the immune response it induces. Depending upon the disease, both the innate and adaptive arms of the immune system may contribute to protection. The innate immune response involves the activation of multiple cell types, including dendritic cells, macrophages, monocytes, neutrophils, basophils, eosinophils, lymphocytes and/or NK cells. The innate immune system provides a rapid response to pathogens, initiates pathogen clearance, and helps in the healing of damaged tissue. Cells of the adaptive immune system include primarily B and T lymphocytes. Adaptive immune responses develop more slowly but are highly specific and therefore critical for providing sterilizing immunity and long-term memory.

Successful vaccination requires a complex series of immune interactions. Professional APCs (dendritic cells and macrophages) as well as certain other immune cells (e.g. B lymphocytes) take up the vaccine Ag. The Ag is then digested and fragments presented to T lymphocytes via cell–cell interactions that require additional surface receptors. Ag-activated CD4 T cells provide help to Ag-specific B cells, supporting their proliferation, switch recombination and somatic hypermutation, resulting in the production of high-affinity Ab (an outcome measured in many clinical trials) [1–3]. CD8 T cells are also stimulated to proliferate and mature into cytotoxic effectors. These are typically measured by their production of Th1 cytokines, such as IFN-γ. Over the course of the vaccine-elicited response, Ag-specific memory B and T lymphocytes arise that persist long-term and provide protection from subsequent challenge [4–7].

Toll-like receptors (TLR) belong to a family of pathogen recognition receptors that are triggered by pathogen-associated molecular patterns expressed by bacteria, viruses, fungi and protozoa. The basic structure of these receptors include an extracellular leucine-rich repeat region, a transmembrane domain and a cytosolic Toll/Interleukin-1 receptor domain. TLR stimulation contributes to the induction and maintenance of innate and adaptive immune pathways as well as memory function [3]. Eleven human and 13 mouse TLRs have been identified. These TLRs have been categorized based on their ligand specificity, signal transduction pathways, cellular expression profiles and subcellular localization. While human

Abbreviations: Ab, antibody; AE, adverse event; Ag, antigen; APC, Ag presenting cell; CTL, cytotoxic T cell; CR, complete response; DC, dendritic cell; GIA, growth inhibition assay; GMA, geometric mean Ab titer; HI, hemagglutinin inhibition; ID, intradermal; IM, intramuscular; IN, intranodal; IV, intravenous; ISS, immunostimmulatory DNA sequence; NA, not available; NK, natural killer cell; NSCLC, non-small cell lung cancer; OS, overall survival; PBMC, peripheral blood mononuclear cells; PD, progressive disease; PF, Plasmodium falciparum; PFS, progressive free survival; PMR, parasite multiplication rate; PR, partial response; SD, stable disease; SQ, subcutaneous; TNA, toxin neutralizing assay.

* Corresponding author at: Bldg 567, Rm 205, CCR, NCI in Frederick, Frederick, MD 21702, United States. Tel.: +1 301 228 4265; fax: +1 302 118 4281.
E-mail address: klinmand@mail.nih.gov (D.M. Klinman).

http://dx.doi.org/10.1016/j.vaccine.2014.06.065
0264-410X/

TLRs 1, 2, 4, 5 and 6 are localized on the cell membrane, TLRs 3, 7, 8, 9 and 10 are embedded in intracellular vesicles [8,9], although TLR9 was found on the cellular membrane of CD19+ B cells [10].

TLRs are expressed primarily on immune cells although other cells are known to contribute to host protection, such as barrier epithelial cells, can also express these receptors. Once triggered by pathogen, these cells produce pro-inflammatory cytokines and chemokines that support the recruitment of additional inflammatory mediators, type I interferon and antimicrobial peptides [11]. TLRs are also expressed on malignant cells and may play a role in oncogenesis and tumor progression by influencing the tumor microenvironment [9,12–14].

2. Toll-like receptor 9

TLR9 recognizes the unmethylated CpG motifs present at high frequency in bacterial but rare in mammalian DNA. The TLR9 receptor is localized to in the endoplasmatic reticulum, late endosomal and lysosomal compartments of the intracellular milieu. Thus, internalization of pathogen-derived DNA is required for TLR9 triggering, an outcome that results from either intracellular infection or uptake of bacterial/viral particles by immune cells [15]. Once stimulated, TLR9 initiates a response biased towards pro-inflammatory/Th1 biased immunity [16].

TLR9 molecules differ between species, with the structure of human versus mouse TLR9 varying by 24% [17]. There is also variation between species in terms of which cell types express TLR9. For example, the TLR9 receptor is present in rodent but not in primate macrophages and myeloid dendritic cells (mDC). In humans, TLR9 is expressed primarily by plasmacytoid DC and B cells [18–21]. Reflecting their utility as vaccine adjuvants, B lymphocytes exposed to TLR9 agonists become more susceptible to activation by Ag [22–24] while TLR9 stimulated plasmacytoid DC (pDC) produce type I interferons and more efficiently present Ag to T cells [25–27]. Non-human primates (NHP) also respond to the same CpG motifs as humans. Thus, results from NHP are better predictors of human immunological responses to CpG ODN than rodents.

3. TLR9-mediated signaling cascade

The binding of CpG DNA to TLR9 induces proteolytic cleavage of the receptor [28,29]. After exiting the ER, the TLR9 ectodomain is cleaved by asparagine endopeptidase and/or cathepsins [30,31]. This truncated (rather than full length) form of TLR9 recruits myeloid differentiation factor 88 (MyD88) such that if proteolysis of the receptor is prevented, it becomes non-functional. The requirement for ectodomain cleavage provides an ancillary mechanism to restrict receptor activation to endolysosomal compartments and further prevents TLR9 from responding to self DNA. The signaling pathway triggered by the interaction of CpG DNA with TLR9 proceeds through the recruitment of MyD88, IL-1R-associated kinase (IRAK) and tumor necrosis factor receptor-associated factor 6 (TRAF6), and subsequently involves the activation of several mitogen-activated kinases (MAPK) and transcription factors (such as NF-kB and AP-1) culminating in the transcription of proinflammatory chemokines and cytokines [32]. WhileTLR9 expressing cells recognize unmethylated CpG motifs, other molecules may support the signal transduction. For example DEC-205, a multi-lectin receptor that can bind CpG motifs and has been shown to facilitate the uptake of CpG ODN by DC and B cells [33].

4. CpG ODN

Bacterial DNA is the native ligand for TLR9 and synthetic oligonucleotides (ODN) that mimic the structure of bacterial DNA duplicate this activity. In humans, four distinct classes of CpG ODN have been identified based on differences in structure and the nature of the immune response they induce. Although each class contains at least one motif composed of a central unmethylated CG dinucleotide plus flanking regions, they differ in structure and immunological activity.

K-type ODNs (also referred to as B-type) contain from 1 to 5 CpG motifs typically on a phosphorothioate backbone. This backbone enhances resistance to nuclease digestion and substantially prolongs *in vivo* half-life (30–60 min compared with 5–10 min for phosphodiester) [34]. K-type ODNs trigger pDC to differentiate and produce TNF-α and stimulate B cells to proliferate and secrete IgM [35,36]. Extensive clinical trials involving K-type ODN have been conducted (as reviewed below).

D-type ODNs (also referred to as A-type) have a phosphodiester core flanked by phosphorothioate terminal nucleotides. They carry a single CpG motif flanked by palindromic sequences that enables the formation of a stem-loop structure. D-type ODN also has poly G motifs at the 3′ and 5′ ends that facilitate concatamer formation. D-type ODN trigger pDC to mature and secrete IFN-α but have no effect on B cells [35,37]. The distinct activities of K- versus D-type ODNs are largely due to differences in the retention times of CpG/TLR9 complexes in the endosomes of pDC [38,39]. Whereas K-type ODNs are rapidly transported through early endosomes into late endosomes, D-type ODNs are retained for longer periods in the early endosome. It is in the early endosomes that D-type ODNs interact with MyD88/IRF-7 complexes, triggering a signaling cascade that supports IFN-α production [39]. D-type ODN tend to form complex multimers in solution due to interactions between their poly-G tails, and thus has posed a barrier to their use in clinical trials. However D-type ODN were recently packaged into stable virus like particles (VLP) and used as adjuvants in preclinical and clinical studies [40–42].

C-type ODNs resemble K-type in being composed entirely of phosphorothioate nucleotides but resemble D-type in containing palindromic CpG motifs that can form stem loop structures or dimers. This class of ODN stimulates B cells to secrete IL-6 and pDC to produce IFN-α. C-type ODNs have activity in both early and late endosomes, and thus express properties in common with both K- and D-type ODNs [43,44]. Phosphodiester linkages can be introduced into the CG dinucleotides (referred to as semi-soft C), a modification reported to further enhance the activity of C-type ODN. P-Class CpG ODN contain double palindromes that can form hairpins at their GC-rich 3′ ends as well as concatamerize due to the presence of the 5′ palindromes. These highly ordered structures are credited with inducing the strongest type I IFN production of any class of CpG ODN [45,46].

Stimulation via TLR9 results in the rapid activation of the innate immune system that in turn supports the induction of an adaptive immune response. This series of effects provides a mechanism by which CpG ODN might be harnessed as a vaccine adjuvant. Extensive animal testing showed that CpG ODN could support the induction of Ag-specific immunity against co-administered peptides and vaccines [47,48]. Below, we review the literature by focusing on clinical studies that examined the utility of CpG ODN as an adjuvant for vaccines targeting infection (Table 1) and cancer (Table 2). Most clinical trials evaluated the activity of CpG ODN adjuvanted vaccines by monitoring specific responses such as Ab titers, cytokine levels, cell proliferation and changes in the frequency of CTL, NK cells, CD8 and CD4 T cells. For vaccines targeting infectious agents, the efficacy of adding CpG ODN was typically examined by comparison to a control group that received vaccine only. In cancer vaccine trials, this critical control group was often missing which complicates interpretation of the reported results.

Please cite this article in press as: Scheiermann J, Klinman DM. Clinical evaluation of CpG oligonucleotides as adjuvants for vaccines targeting infectious diseases and cancer. Vaccine (2014), http://dx.doi.org/10.1016/j.vaccine.2014.06.065