Adjuvant Activity of CpG Oligodeoxynucleotides

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Synthetic oligodeoxynucleotides (ODNs) containing unmethylated CpG motifs directly stimulate human B cells and plasmacytoid dendritic cells (pDCs), thereby promoting the production of Th1 and proinflammatory cytokines and the maturation/activation of professional antigen-presenting cells. These activities enable CpG ODNs to act as immune adjuvants, accelerating and boosting antigen-specific immune responses by 5- to 500-fold. The CpG motifs present in bacterial DNA plasmids may contribute to the immunogenicity of DNA vaccines. Ongoing clinical studies indicate that CpG ODNs are safe and well tolerated when administered as adjuvants to humans and can improve vaccine-induced immune responses.

Keywords: Adjuvant, CpG, innate, oligodeoxynucleotide, Th1

INTRODUCTION

The mammalian immune system uses two general strategies to combat infectious diseases. Pathogen exposure rapidly triggers an innate immune response characterized by the production of immunostimulatory cytokines, chemokines, and polyreactive IgM antibodies (reviewed in [1–3]). The innate immune system is activated by exposure to pathogen-associated molecular patterns (PAMPs) expressed by a diverse group of infectious microorganisms [3]. The resultant innate immune response limits the early proliferation and spread of infectious organisms in vivo. Subsequently, the host mounts an adaptive immune response directed against determinants uniquely expressed by the pathogen. The resultant antigen-specific immunity is characterized by the production of high-affinity Abs and the generation of cytotoxic T cells that provide long-lasting protection [4].

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The recognition of PAMPs is mediated by members of the Toll-like family of receptors (TLRs) [5,6]. Bacterial DNA is a PAMP: differences in the methylation pattern and utilization pattern of CpG dinucleotides results in unmethylated CpG motifs being present at much higher frequency in the genomes of prokaryotes than eukaryotes [7,8]. The innate immune system detects these unmethylated CpG motifs using TLR9 [9–11]. Exposure to unmethylated CpG DNA released during an infection provides a “danger signal” to the innate immune system, triggering a protective immune response that improves the host’s capacity to eliminate the pathogen [12].

Synthetic oligodeoxynucleotides (ODNs) expressing CpG motifs similar to those found in bacterial DNA stimulate a similar response [12–15]. These immunomodulatory ODNs have a variety of potential therapeutic uses. This review will focus on efforts to harness them as vaccine adjuvants to improve antigen-presenting cell function and promote the induction of an antigen-specific adaptive immune responses. CpG ODNs are also being evaluated for the immunotherapy of cancer, as treatment for allergic disorders, and as immune stimulants to improve host resistance to infection.

**ACTIVITY OF CpG ODNs**

CpG ODNs are rapidly internalized by immune cells, where they interact with intracellular TLR9 [9,16]. This is a highly specific interaction that is exquisitely sensitive to modifications of the CpG motif [9,10]. Cells lacking TLR9 do not respond to CpG DNA but can be made responsive by transfection to express that receptor [9,10].

B cells and plasmacytoid dendritic cells (pDCs) are the primary human cell types that express TLR 9 and respond to CpG stimulation [9,10,17,18]. Activation of these cells by CpG DNA initiates an immunostimulatory cascade that culminates in the maturation, differentiation, and proliferation of natural killer (NK) cells, T cells, and monocytes/macrophages [15,19,20]. Together, these secrete cytokines and chemokines that create a proinflammatory (IL-1, IL-6, IL-18, and TNF) and Th1-biased (IFN-γ and IL-12) immune milieu [9,10,12,15,16,21,22].

Due to evolutionary divergence among the TLR9 molecules expressed by different species, the sequence motif (unmethylated CpG dinucleotide plus flanking regions) that optimally stimulates cells from one species may be ineffective in another species [23]. For example, the amino acid sequence of murine and human TLR9 differ by 24% [9]. Whereas the optimal sequence motif in mice consists of two 5’ purines, the central unmethylated CpG, and then two 3’ pyrimidines [12,15,22,24], the
optimal motif in humans is TCGTT and/or TCGTA [11,21,23,25–27]. In addition, the cell populations that express TLR 9 differ among species. In mice, immune cells of the myeloid lineage (including monocytes, macrophages, and myeloid DCs) express TLR9 and respond to CpG stimulation, whereas in humans these cell types do not express TLR9 and are not directly activated by CpG ODNs [28–30].

At least three structurally distinct classes of synthetic CpG ODNs have been described that are capable of stimulating cells that express human TLR9 [26,27,31,32]. “K” type ODNs (also referred to as “B” type) encode multiple CpG motifs on a phosphorothioate backbone. “K” ODNs trigger pDCs to differentiate and produce TNF-α, and B cells to proliferate and secrete Ig [26,27,33] (Table I). “D” type ODNs (also referred to as “A” type) are constructed of a mixed phosphodiester/phosphorothioate backbone and contain a single hexameric purine/pyrimidine/CpG/purine/pyrimidine motif flanked by self-complementary bases that form

| Table I | Comparison of “D,” “K,” and “C” Type ODNs |
|---------|----------------------------------------|
| ODN type | “D” also referred to as “A” |
| Example  | GGTGCATCGATGCA0GGGGGG |
| Structural characteristics | Mixed phosphodiester/phosphorothioate backbone |
| | Single CpG motif |
| | CpG flanking region forms a palindrome |
| | Poly G tail at 3’ end |
| Immunomodulatory activity | APC maturation |
| | Preferentially stimulates pDC to secrete IFN-α |

| ODN type | “K,” also referred to as “B” |
| Example  | TCCATGGAGTTTCTGACGGTT |
| Structural characteristics | Phosphorothioate backbone |
| | Multiple CpG motifs |
| | 5’ motif most stimulatory |
| Immunomodulatory activity | pDC maturation |
| | Preferentially supports the production of TNF-α and IL-6 |
| | Triggers B cell activation, including the production of IgM |

| ODN type | “C” |
| Example  | TCGTCGTTCAACGACGTGTGAT |
| Structural characteristics | Phosphorothioate backbone |
| | Multiple CpG motifs |
| | TCG dimer at 5’ end |
| | CpG motif imbedded in a central palindrome |
| Immunomodulatory activity | Stimulates B cells and pDC |
| | Induces production of IL-6 and IFN-α |

CpG motifs are underlined; phosphorothioate nucleotides are capitalized; phosphodiester nucleotides are shown in italics.
a stem-loop structure capped at the 3' end by a poly G tail [26]. “D” type ODNs trigger pDC to mature and secrete IFN-α, but have no effect on B cells [26,27].

“C” type ODNs resemble “K” type in being composed entirely of phosphorothioate nucleotides. “C” type ODNs were originally described as expressing a TCGTCG at the 5’ end, and commonly contain an internal “K” type motif (such as GTGCGT) embedded in a palindromic sequence [34]. This class of ODNs is capable of stimulating B cells to secrete IL-6 and pDCs to produce IFN-α (thus combining some of the stimulatory properties of “D” and “K” type ODNs) [31,32].

Studies examining the activity of peripheral-blood mononuclear cells (PBMCs) from humans, macaques, chimpanzees, and orangutans indicate that primates respond to the same broad classes of CpG ODNs [35–38]. In contrast, rodents respond poorly to some [9,10] but not all [25,32] ODNs that are highly active in primates. Thus, studies designed to examine the therapeutic potential of CpG ODNs are typically initiated in mice and then followed by studies in which the specific ODN planned for clinical use are evaluated in non-human primates.

IMMUNOGENICITY OF DNA VACCINES

Considerable excitement was generated by the finding that antigen-encoding DNA plasmids could induce cellular and humoral immune responses against encoded antigens [39–41]. Although the nature, magnitude, and duration of the immune response elicited by DNA vaccines is influenced by multiple factors, intramuscular delivery stimulates a Th1-driven response characterized by cytotoxic T lymphocyte (CTL) induction and the release of IFN-γ and antigen-specific IgG2a antibodies [40,42]. Early studies in mice demonstrated that DNA vaccines could protect against pathogen challenge [40,41]. Unfortunately, follow-up studies in non-human primates and human clinical trials suggest that the magnitude of the immune response elicited by DNA vaccination is lower than that achieved with other types of vaccine [43,44]. Ongoing efforts are directed toward improving the immunogenicity of DNA vaccines intended for human use and combining DNA vaccines with other methods of antigen presentation to generate a maximally protective immune response.

DO CpG MOTIFS CONTRIBUTE TO THE IMMUNOGENICITY OF DNA VACCINES?

There are a number of reasons to believe that CpG motifs might contribute to the immunogenicity of DNA vaccines. In addition to their
ability to directly support cytokine and Ig secretion, CpG DNA could contribute to the development of an immune response by upregulating cell surface expression of MHC and other co-stimulatory molecules. Originally demonstrated in B cells, this effect has also been observed in professional antigen-presenting cells. Indeed, CpG ODNs upregulate the expression of a variety of co-stimulatory molecules in dendritic cells, including CD40 and CD86 [45,46], with the fraction of stimulated APCs rising as a function of CpG ODN concentration. Of particular importance, CpG ODN–mediated activation of these APCs increased their functional capacity, as reflected by an improved ability to stimulate alloreactive T cells [45].

A number of early studies suggested that CpG motifs present in the bacterial backbone of DNA vaccines might contribute to plasmid immunogenicity [24,47–49]. This included evidence that (i) reducing the number of such motifs (by CpG methylase treatment) significantly reduced vaccine immunogenicity [50]; (ii) increasing the number of motifs promoted the induction of vaccine-specific immune responses [24,47,49,51]; and (iii) the nature of the immune response induced by DNA vaccines was similar to that elicited by CpG DNA [47].

Additional studies showed that the response elicited by suboptimal amounts of DNA vaccine could be boosted by co-administering free CpG ODN [50]. However, if CpG ODN was administered with high levels of a DNA vaccine, uptake of the vaccine was inhibited and the resultant immune response reduced [52]. In this context, an excess of control ODNs also reduced DNA vaccine immunogenicity [52]. Similarly, dose-response studies indicate that the ability of CpG ODNs to stimulate spleen cells to secrete cytokine and Ig in vitro reaches a plateau and then begins to fall. In this context, there is evidence that adding too many CpG motifs to the backbone of a plasmid vector may reduce its immunogenicity. In one report, introducing 16 additional CpG motifs into a DNA vaccine improved the humoral immune response elicited in vivo, while introducing 50 such motifs reduced the response [51]. These inconsistent findings raised concern that the impact of CpG motifs on DNA vaccine immunogenicity might be modest.

Consistent with that interpretation, results from Spies et al. demonstrate that DNA vaccines remain immunogenic when administered to TLR 9 KO mice [53]. Because mice lacking TLR9 cannot respond to CpG motifs, this finding strongly suggests that vaccine immunogenicity is not dependent on CpG-mediated activation of the innate immune system [53]. Although that report does not examine low doses of vaccine (where CpG motifs have their greatest effect), the implication remains that CpG-driven immune activation accounts for only a modicum of DNA vaccine immunogenicity. It should be noted
that the number, placement, and sequence of CpG motifs may be important. Efforts are underway to determine if different motifs can preferentially induce specific types of immune response and to identify regions in the plasmid where the addition of CpG motifs provides the greatest benefit [48,49]. These efforts may yield vectors with significantly improved immunostimulatory capacity for clinical use.

**CpG ODNs AS ADJUVANTS FOR CONVENTIONAL VACCINES**

The ability of CpG DNA to promote the production of Th1 and proinflammatory cytokines and induce the maturation/activation of professional antigen-presenting cells suggests they might be useful as adjuvants for “conventional” vaccines. Consistent with such a possibility, studies involving “K” type ODNs in mice established that CpG-mediated stimulation boosted both humoral and cell-mediated responses to proteins such as ovalbumin and keyhole limpet hemocyanin [54,55]. The adjuvant properties of CpG ODNs were significantly improved when the ODN was kept in close contact with the antigen. Physically binding ODN to antigen, cross-linking the two with alum, or co-incorporating them in lipid emulsions or vesicles generated IgG responses 10- to 1000-fold greater than induced by antigen alone [55–57]. This adjuvant effect had three components: (i) a CpG-induced enhancement in APC function, (ii) a CpG-dependent induction of a cytokine/chemokine microenvironment supportive of antigen-specific immunity, and (iii) an improvement in Ag uptake mediated by DNA-binding receptors on APC (this final effect being CpG independent) [58].

Further study established that CpG ODNs could boost the immune response to co-administered vaccines. Adding “K” type ODNs to a variety of vaccines (including those targeting influenza virus, measles virus, hepatitis B virus surface Ag, and tetanus) increased antigen-specific Ab titers in mice by up to 3 orders of magnitude [57,59–63]. Consistent with their effects on Th1 cytokine production, CpG ODNs preferentially induced the production of IFN-γ, which supported the secretion of IgG2a Abs and facilitated the development of antigen-specific cytotoxic T lymphocytes (CTLs) [57,59–62,64].

**CpG ODNs IMPROVE THE RESPONSE TO MUCOSAL VACCINES**

Many pathogens gain access to the host through the respiratory, gastrointestinal, vaginal, or rectal mucosa. Thus, the ability of CpG ODNs to boost mucosal immunity was examined. Administering
CpG ODNs with formalin-inactivated influenza virus intranasally significantly increased flu-specific Ab levels in the serum, saliva, and the genital tract of mice [59]. Similarly, CpG ODNs delivered with vaccine or β-galactosidase stimulated strong antigen-specific IgA responses throughout the mucosal immune system and in the serum [65–67] (Table II). Spleen cells from intranasally immunized mice preferentially produced IFN-γ rather than IL-4 when re-exposed to antigen in vitro. They also generated MHC-restricted, Ag-specific CTLs, replicating the effects of parenterally injected CpG ODNs plus antigen [65].

**CpG ODN AUGMENTS THE IMMUNE RESPONSE OF IMMUNOCOMPROMISED ANIMALS**

Vaccination is most effective when “herd immunity” is achieved and the number of individuals remaining susceptible to infection minimized. Individuals whose immune system is compromised (due to age, disease, or immunosuppressive therapy) are both resistant to vaccination and more susceptible to infection. Such individuals thus place the broader community at risk. Studies were performed in animals with various types of immunodeficiency to determine whether CpG ODNs, by stimulating the innate immune system, could overcome defects in their adaptive immune response.
Due to immaturity of the neonatal immune system, newborns frequently mount an inadequate response to foreign pathogens [68,69]. For example, newborns respond poorly to immunization with HBsAg, attenuated measles virus, or tetanus toxoid [60,62]. Studies in mice indicate that both Ab and CTL responses by young mice are enhanced by co-delivery of CpG ODN with antigen [60,62]. Interpreting these findings is complicated, however, because animals were immunized repeatedly, obscuring the effect of a single early dose of CpG ODNs on subsequent immune responsiveness. At the other end of the age spectrum, very old animals develop defects in cell-mediated and humoral immunity [70,71]. Vaccines for the elderly must compensate for these alterations in immune function. Several recent reports indicate that adding CpG ODNs significantly boost vaccine immunogenicity in geriatric mice [72,73].

Co-existing infection can also reduce vaccine responsiveness. For example, HIV-infected patients experience a progressive deterioration in the number and functional activity of CD4$^+$ T cells and respond suboptimally to vaccination [74–76]. Yet, retrovirus infection has less effect on the innate than adaptive immune system [77]. Thus, PBMCs from HIV-infected subjects (and SIV-infected macaques) can respond to CpG ODN stimulation despite declines in antigen-specific immunity [77]. The ability of CpG ODNs to boost the immune response of SIV-infected macaques to a hepatitis B vaccine was therefore investigated. Unlike healthy macaques, SIV-infected animals were unable to mount a protective antibody response when repeatedly vaccinated with Engerix B (HIV patients show a similar loss of vaccine responsiveness) [74,75]. Only 20% of the SIV-infected macaques ever developed protective titers of antibody. By comparison, the addition of “K” or “D” ODNs to the vaccine boosted Ab titers to protective levels in all animals with viral loads $<$ 10$^7$ copies/ml [77]. Although the antibody levels achieved were significantly lower than that of similarly immunized uninfected animals, these findings indicate that inclusion of CpG ODNs can boost the immunogenicity of vaccines in both normal and immunocompromised hosts. Similarly, the innate hyporesponsiveness of orangutans to hepatitis B vaccination was overcome by co-administration of CpG ODN [37] (Table II).

**ADJUVANT ACTIVITY OF CpG ODNs IN NON-HUMAN PRIMATES**

Building on the observation that non-human primates respond to the same CpG ODNs that stimulate human cells and that CpG ODN boost immunity to the hepatitis B vaccine, studies evaluating their effect
with other vaccines were initiated. When “K” type ODNs were co-administered with a peptide-based malaria vaccine, the result was a significant increase in the antigen-specific serum IgG response of Aotus monkeys [78]. In contrast, inclusion of control ODNs had no effect on vaccine immunogenicity [78].

“K” ODNs not only increase the magnitude but also accelerate the development of antigen-specific immunity. For example, when co-administered with AVA (the licensed anthrax vaccine), these ODNs induced a six-fold higher Ab response in rhesus macaques than AVA alone [79]. This enhanced Ab response arose in less than 2 weeks and resulted in significantly greater protection against anthrax infection as monitored using a serum transfer system. Upon boosting, the avidity of the anti-anthrax antibodies elicited by “K” type ODNs plus AVA was significantly higher than that induced by AVA alone [79]. Of note, despite the ability of “K” type ODNs to improve humoral immune responses in vivo, there is as yet no convincing evidence that they significantly affect T-cell responses in primates.

In another model, rhesus macaques were immunized with a candidate leishmania vaccine (heat-killed leishmania vaccine, HKLV) plus either “K” or “D” ODNs. Animals vaccinated with HKLV alone and then challenged with L. major developed large cutaneous lesions [35]. Monkeys vaccinated with HKLV plus “K” ODNs also developed large lesions, although somewhat more slowly than controls. This result establishes that not all CpG ODNs improve vaccine-induced immunity. By comparison, animals immunized with HKLV plus “D” type ODNs had significantly smaller lesions consistent with a reduced parasite burden [35,80]. PBMCs from these animals also had a higher proportion of cells that were stimulated by leishmania antigens to secrete IFN-γ in vitro than did those immunized with HKLV alone [35].

HUMAN CLINICAL EXPERIENCE

CpG ODNs have been administered to hundreds of subjects in more than a dozen clinical trials. These were studies designed to explore the safety and immunomodulatory properties of CpG ODNs, delivered alone or in combination with various vaccines, antibodies, or allergens. Three clinical trials in which CpG ODNs were used as vaccine adjuvants have been described. The first was a double-blind study in which CpG ODN was co-administered multiple times with Engerix-B (the licensed hepatitis B virus vaccine). Healthy adult volunteers immunized with the vaccine plus CpG ODN developed serum IgG antibody responses more rapidly than those immunized with vaccine alone. The
mean Ab titer in subjects treated with CpG ODN plus Engerix B was 13- to 45-fold higher than in recipients of vaccine alone after both primary and secondary immunization [81,82].

In the second double-blind study, CpG ODN was co-administered with the Fluarix influenza vaccine. Inclusion of CpG ODN did not increase the Ab response of naive recipients when compared with Fluarix alone but did increase antibody titers among subjects with pre-existing anti-flu antibodies. PBMCs from CpG ODN vaccinated subjects responded to *in vitro* re-stimulation by secreting significantly higher levels of IFN-γ than did PBMCs from control vaccinates [82]. No serious adverse events attributed to the use of CpG ODNs were observed. None of the subjects exposed to CpG ODNs developed signs or symptoms of autoimmune disease [83].

In the third recently completed study, CpG ODNs were administered in combination with AVA, the approved anthrax vaccine. Inclusion of the ODN significantly accelerated the induction of a strong humoral immune response while boosting the peak anti-PA response by 6- to 8-fold (nearly identical to the effects observed in rhesus macaques) [84].

**CpG ODN SAFETY**

There are several safety concerns raised by the clinical use of CpG ODNs. These include the possibility that ODNs might enhance the immunogenicity of self proteins at the site of delivery, thereby triggering the development of organ specific or systemic autoimmune disease, or increase susceptibility of the host’s immune system to pathogenic agents that cause toxic shock.

The ability of CpG DNA to impact the development of autoimmune disease is supported by studies showing that high doses of bacterial DNA elicit the production of autoantibodies against double-stranded-DNA in normal mice and accelerate autoantibody production in lupus-prone animals [85–87]. Bacterial DNA also stimulates the production of IL-6 and blocks the apoptotic death of activated lymphocytes, functions that predispose to the development of autoimmune disease by facilitating the persistence of self-reactive lymphocytes [88–91].

To clarify the magnitude of this safety concern, *in vivo* experiments were conducted in which mice were repeatedly injected with immunostimulatory doses of CpG DNA. Although the number of IgG anti-DNA secreting B cells rose by 2- to 3-fold [92], and serum IgG anti-DNA antibody titers rose by up to 60%, the magnitude of these effects was insufficient to induce or worsen systemic autoimmunity [92–94].
The situation was somewhat more complex for organ-specific autoimmune diseases that are typically promoted by the type of Th1 response preferentially elicited by CpG DNA. In an IL-12 dependent model of experimental allergic encephalomyelitis (similar to multiple sclerosis), animals treated with CpG DNA and then challenged with autoantigen developed autoreactive Th1 effector cells that caused disease, whereas mice injected with autoantigen alone remained disease free [95,96]. In a molecular mimicry model, CpG DNA co-administered with Chlamydia-derived antigens promoted the induction of autoimmune myocarditis [97]. CpG ODNs also increased the susceptibility of mice to interventions that can induce arthritis [98]. These findings indicate that CpG motifs can promote the development of deleterious autoimmune reactions under certain circumstances.

A final concern is the possibility that CpG ODNs might facilitate the development of toxic shock. Agents such as lipopolysaccharide and D-galactosamine cause toxic shock by triggering the over-production of TNF-α. CpG DNA also stimulates the production of this cytokine. Indeed, when CpG ODN was co-administered with subpathologic doses of lipopolysaccharide and D-galactosamine, severe mortality and morbidity ensued [99–101].

To examine whether these toxicities are likely to occur under normal circumstances, CpG ODNs at doses equal to or exceeding those typically used in adjuvant experiments were injected weekly for 4 months into normal BALB/c mice. All of the animals remained physically fit and none showed macroscopic or microscopic evidence of tissue damage or inflammation [102]. Similarly, no adverse health effects were reported in studies involving the delivery of CpG ODNs to non-human primates, when hundreds of milligrams of antisense ODN were administered repeatedly to patients [103], or when DNA vaccines composed of bacterial plasmids that contain CpG motifs were administered to normal volunteers [77,104].

Adverse events (AEs) were observed among recipients of CpG ODNs in combination with various protein-based vaccines. These were predominantly injection site reactions (such as pain and erythema) and flu-like symptoms that were short lived and did not interfere with the activities of daily living. The intensity of the AEs was similar in recipients of vaccine alone vs vaccine plus ODN, although the frequency of AEs was higher among those co-vaccinated with CpG ODN versus vaccine alone [81,82,84]. Only in the trial involving the anthrax vaccine were any serious (grade 3) AEs observed, with preliminary data showing no significant increase in their frequency due to co-administration of CpG ODN [84]. There were no clinically relevant changes in hematocrit or white blood cell count among
immunized volunteers, nor were there any changes in liver or renal function. None of the subjects exposed to CpG ODNs developed signs or symptoms of autoimmune disease [83].

Thus, while concern remains that CpG ODNs might have adverse effects under certain conditions, there is no evidence that at the doses used as vaccine adjuvants that they are directly toxic to normal animals or humans. Clinical studies are thus proceeding, with subjects being monitored for the development of adverse reactions.

It should also be appreciated that CpG ODNs are not universally successful as vaccine adjuvants. Failures of both “D” and “K” type ODNs to promote immunity in non-human primates were noted above. Of greater concern is recent evidence that CpG-induced increases in immune activity do not necessarily correlate with improved vaccine efficacy. For example, rodents treated with CpG ODN plus an RSV protein-based vaccine were only modestly protected from viral challenge and were at increased risk of pulmonary pathology [105].

CONCLUSION

CpG ODNs stimulate cells that express TLR9, initiating an immunomodulatory cascade that culminates in the production of Th1, proinflammatory cytokines, and chemokines. There is consistent evidence that CpG ODNs function as adjuvants when co-administered with conventional protein-based vaccines, boosting antigen-specific Ab and cell-mediated immune responses. CpG ODNs can both accelerate and magnify vaccine-specific immunity. These effects could be of considerable benefit when the rapid induction of a protective immune response is required (e.g., when confronted by the release of a biopathogen) [106]. Yet the adjuvant effect of CpG ODNs is strongest when the amount of immunogen being administered is suboptimal. This is usually referred to as an “antigen-sparing effect.” When high doses of vaccine are administered, the magnitude of the immunologic boosting attributable to CpG ODNs is modest.

The utility of CpG ODNs is enhanced by their ability to promote mucosal as well as systemic immunity. This is of considerable importance for pathogens that gain access to the host through the respiratory, gastrointestinal, and reproductive tracts. Several studies document that co-administering CpG ODNs with vaccines significantly increases antigen-specific IgA levels at mucosal sites and IgG levels systemically [59,61,65]. An additional benefit of CpG ODNs is their ability to boost immunity in groups with reduced immune function that typically respond poorly to vaccination, such as newborns, the elderly, and the immunosuppressed. Eliminating reservoirs of
susceptible individuals reduces the likelihood of pathogen transmission and thus enhances the community’s resistance to infection.

Preclinical studies involving non-human primates confirm the expectation that CpG ODNs selected for their ability to stimulate human immune cells are active in vivo. Although the magnitude of this effect varies with the type of antigen and ODN utilized, studies involving HKLV indicate that CpG ODNs can convert an otherwise ineffective vaccine to one that provides significant protection from infection [35], and studies with Engerix B document that CpG ODNs facilitate the induction of protective immunity in immunocompromised hosts [77]. Experiments involving AVA demonstrate that CpG ODNs improve both the magnitude and rapidity with which protection is elicited [79].

Clinical studies designed to evaluate the safety and activity of CpG ODNs in humans are ongoing. Available results suggest that these agents are generally safe and can boost the immunogenicity of some vaccines [82,84]. Efforts continue to (i) identify ODNs of different classes that are optimally active in humans when co-administered with different vaccines, (ii) determine how these different classes of ODN regulate discrete elements of the immune response, (iii) monitor the long-term safety of CpG ODNs, and (iv) establish the optimal dose, duration, and site(s) of vaccine/ODN delivery. We expect these efforts to further improve the utility of CpG-based vaccines for the induction of protective immunity against infectious pathogens.

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