
CME review article

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DNA-based immunotherapy to treat atopic disease

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Objective: To review the current literature regarding DNA-based immunotherapy with respect to signaling mechanisms, cytokine profiles, and the applicability and success of this strategy to treat allergic disease.

Data Sources: English-language articles were identified from the PubMed database using both standard and clinical queries. Search terms included *CpG*, *allergy*, *atopic disease*, *immunotherapy*, *DNA vaccination*, *immunomodulation*, and *immunostimulatory DNA*. Other sources included bibliographies from relevant articles.

Study Selection: Recent studies that provide information about the mechanisms or applications of DNA-based immunotherapy with respect to atopic disease are included in this review.

Results: DNA-based immunotherapy composed of unmethylated CpG repeats is capable of inducing a shift in the cytokine profile and immune response that favors the T_H1 arm. This observation makes DNA-based immunotherapy a promising candidate for the treatment of atopic diseases, which are known to be mediated by T_H2 -based responses. Early animal and human trials of DNA-based immunotherapy have shown the strategy to be both safe and effective.

Conclusions: DNA-based immunotherapy, although still in the early stages of development, has thus far been shown to be both safe and effective for a variety of atopic diseases and offers the potential for significant improvements over current immunotherapy protocols.

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INTRODUCTION

DNA vaccination is an emerging technology with tremendous potential applications not restricted to those of classic vaccines. Since it is still in its infancy, much is unknown about DNA vaccination, including the mechanisms of protection, ideal delivery methods, and possible adverse effects. However, numerous laboratories have made substantial progress in elucidating not only these facets but more importantly how the technology can be used to treat a multitude of diseases. Two components are necessary for any vaccine to stimulate the desired immune response. The first is a nonspecific com-

ponent that acts as an adjuvant, which stimulates the immune system to react to the vaccine. The second is the specific component, which directs the immune system to create an antigen-specific response. In the case of DNA vaccination, the nonspecific component is provided by unmethylated CpG repeats in the DNA, acting as a powerful adjuvant. The part of the vaccine that generates antigen specificity can come from a number of sources. Some proven methods include antigen covalently complexed to the DNA, antigen given in parallel to the DNA adjuvant, and, perhaps most intriguing, the DNA vaccine itself, which contains a gene whose protein product serves as the antigen.

This review highlights the mechanisms by which unmethylated CpG repeats induce a T_H1 -biased immune response. Specifically, signal transduction mechanisms and cytokine elaboration are discussed. Different approaches to delivering

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the antigenic component of DNA-based immunotherapy are also addressed. Finally, the evidence that supports the efficacy and safety of DNA-based immunotherapy in the treatment of atopic disease is discussed.

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CpG-INDUCED SIGNAL TRANSDUCTION

The immunogenicity of bacterial DNA was first discovered in an attempt to isolate the active component within *Mycobacterium bovis* bacillus Calmette-Guérin, an agent used for its antitumor activity.¹ Although vertebrate DNA could not elicit an immune response, bacterial DNA was shown to activate natural killer cells, induce interferon- γ (IFN- γ), and activate B-cell proliferation and immunoglobulin secretion.¹⁻⁴ Krieg et al⁵ later showed that unmethylated CpG dinucleotide repeats present in bacterial but not vertebrate DNA were the minimal components necessary to account for the observations. Many studies have now shown that stretches of unmethylated CpG dinucleotide repeats, typically found in bacterial DNA, function as efficient stimulators of innate immunity by signaling the presence of pathogens. For this to occur, our immune systems must have mechanisms for distinguishing self DNA from foreign DNA, as well as the appropriate signal transduction machinery. On the matter of distinction, human CpG motifs are generally highly methylated at the cytosine nucleotide and statistically underrepresented in the genome.⁶ Consistent with this hypothesis, if bacterial DNA is methylated it loses its immunogenicity.⁵ Although no receptors have been identified that recognize DNA in a sequence-specific manner, there is a family of pattern recognition receptors that includes the toll-like receptors (TLRs).^{7,8} The ligands for the 10 known TLRs include a variety of microbial molecules, such as lipoproteins and peptidoglycan recognized by TLR-2, lipopolysaccharide by TLR-4, and CpG DNA by TLR-9.⁹⁻¹²

During the past few years, a substantial amount of data have been gathered regarding CpG-induced signal transduction pathways. DNA uptake into the cell by endocytosis is required.¹³⁻¹⁶ On endosomal maturation, CpG DNA interacts with its intracellular receptor, TLR-9, which then interacts with the adaptor protein, MyD88 (Fig 1).^{12,17,18} Within several minutes multiple intracellular signaling cascades are activated, including the MAP kinases, p38, and JNK1/2, leading to AP-1-mediated transcription of target genes.^{15,16} MyD88 also interacts with IRAK and TRAF6 to activate IKK, which in turn activates NF- κ B.^{19,20} NF- κ B then translocates to the nucleus, where it mediates transcriptional activation of target proinflammatory genes, such as tumor necrosis factor α (TNF- α), interleukin (IL) 12, and CD40.¹⁶

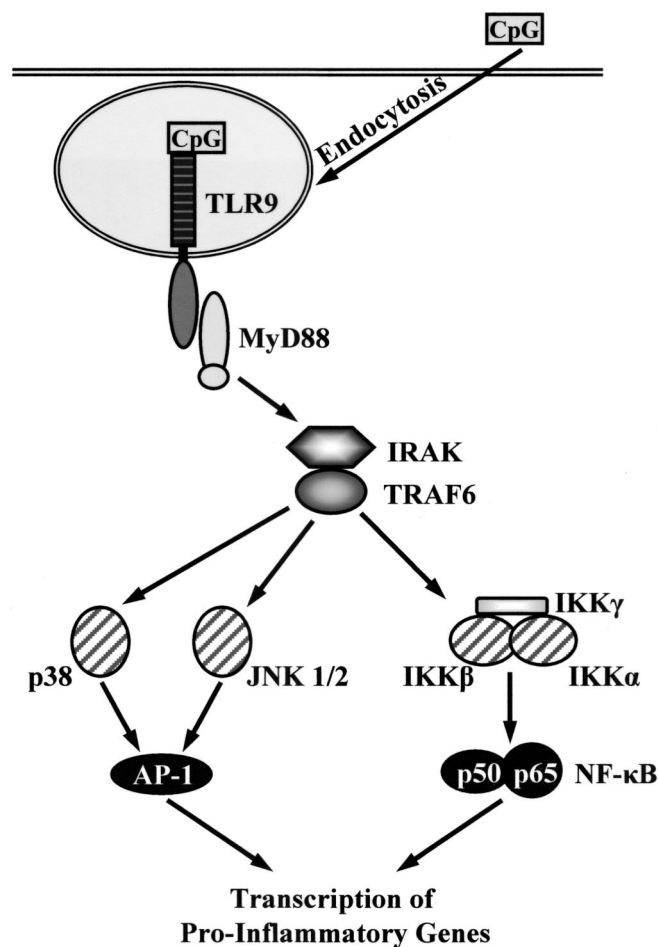


Figure 1. Toll-like receptor (TLR) signaling pathway. CpG DNA undergoes endocytosis and on vesicle maturation/acidification interacts with TLR-9, whose cytoplasmic portion associates with the adaptor protein, MyD88. IRAK is then recruited to the receptor-adaptor complex, where it in turn activates TRAF6. Through some additional downstream proteins such as TAK-1 (not shown), the complex then activates a number of downstream effectors, including the p38 and JNK1/2 MAP kinases and the I κ B kinase complex (IKK α , IKK β , and IKK γ). Subsequently, AP-1 and NF- κ B are activated and translocate to the nucleus to promote the transcriptional activation of proinflammatory genes.

The effects of CpG on mediators of the immune response vary, depending on the target cell. CpG acts as a potent T-cell-independent, B-cell mitogen.⁵ These activated B cells are induced to secrete IL-6, IL-10, and immunoglobulin and to express a number of antiapoptotic proteins, costimulatory molecules, and proinflammatory chemokines.²¹⁻²⁵ Of note is that the immune response is not generated against the adjuvant DNA but to the antigen delivered with it. CpG DNA also has direct stimulatory effects on monocytes, macrophages, and dendritic cells, which are induced to secrete IL-12, TNF- α , and IL-6 and up-regulate the expression of the costimulatory molecule B7.2 (Fig 2).²⁶⁻²⁸ Macrophage-secreted

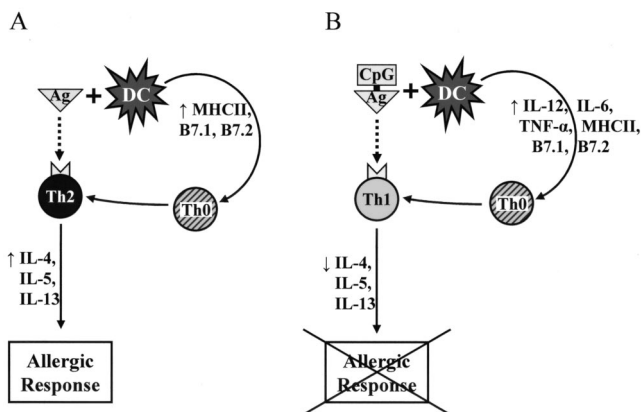


Figure 2. CpG oligostimulatory DNA induces shifts in the immune response. A, In atopic disease, the immune response is set up to favor the development of T_H2 cells. On allergen presentation, components of the innate immune system, such as dendritic cells (DCs), take up the antigen (Ag) and present it to naïve T cells (T_H0 cells), which are biased to develop into T_H2 cells in allergic individuals. On allergen reexposure (dotted line), these T_H2 cells trigger a cascade of events that lead to allergic symptoms. Mediators such as interleukin (IL) 4, IL-5, and IL-13 stimulate B-cell maturation with IgE production, mast cell activation, and eosinophil migration. B, In the presence of CpG oligostimulatory DNA sequences, cytokines such as IL-12, IL-6, and tumor necrosis factor α (TNF- α) are released by the DCs, which favor the maturation of naïve T cells into T_H1 cells. Consequently, there is a decrease in allergic symptoms. MHCII indicates major histocompatibility complex II.

IL-12 is capable of activating natural killer cells to have increased lytic ability and to secrete IFN- γ .^{27,29,30} Similarly, T cells are indirectly activated by the cytokine environment.^{31,32} The cytokines produced by dendritic cells on CpG activation act as potent inducers of a T_H1 cell fate.^{26,28,33,34} Consequently, DNA vaccines do not generally induce T_H0 cells to adopt a T_H2 fate. Interestingly, as the concentration of delivered CpG immunostimulatory DNA is increased, there appears to be an alteration in the generated immune response. On the administration of moderate concentrations of CpG DNA, the induction of T_H1 cytokines is heavily favored.³⁵ However, at high concentrations of administered CpG DNA, the primary effect is to suppress T_H2 cytokines. Since IL-10 has the ability to suppress the differentiation of both T_H1 and T_H2 cells, it is believed that this differential effect of CpG DNA based on the concentration administered is a result of the dose-dependent induction of IL-10.^{36,37} The importance of IL-10-mediated suppression as opposed to T_H1 cytokine elaboration on the protective effects of DNA-based immunotherapy in the treatment of atopic disease has yet to be determined.

ANTIGEN SPECIFICITY

These properties of bacterial DNA vaccination, as already mentioned, are not antigen specific. The adjuvant properties of CpG oligonucleotide DNA will enhance the immune response to whatever antigen is delivered with it. Traditionally, the antigenic component of vaccines has not been supplied by

DNA. Instead, the antigen itself, whether peptide or polysaccharide, has imparted specificity to the immune response. Some other ways of providing specificity to DNA-based immunotherapy include providing the allergen in parallel to the CpG DNA, in which case the 2 components are not covalently linked. Alternatively, the CpG DNA can be covalently conjugated to the allergen, which has been shown to increase efficacy perhaps by delivering the adjuvant and antigen to the same antigen-presenting cell population.^{38–42} These allergen-DNA conjugates have also been shown to be less allergenic, decreasing the risk of anaphylaxis. Perhaps the most intriguing and efficient way to provide specificity to the DNA vaccine is to have the gene that encodes the antigen itself serve as both the adjuvant and the antigen. Compared with what is known about CpG biology, far less is certain regarding how genes present in a vaccine can be transcribed and translated and have the protein product function as a specific immunogen. Briefly, what is suspected is that DNA injected into muscle is taken up by muscle cells.^{43–45} It is then expressed and the product secreted. Antigen-presenting cells in the draining lymph nodes take up the antigen and present it in association with major histocompatibility complex II to T cells. B cells also interact with the antigen, stimulating the humoral arm of the immune response.^{46,47} This process would be enhanced and directed by the immune stimulating effects of the CpG adjuvant inherent to the DNA vaccine. With respect to allergy immunotherapy, this option offers great utility, because plasmids that express the antigen of interest can be easily generated with recombinant techniques, can be stored indefinitely, and provide all necessary components of the treatment in one molecule. A more comprehensive discussion of the mechanisms underlying plasmid DNA uptake, expression, and immunogenicity is beyond the scope of this review. For additional information, several recent reviews are available on these topics.^{48–50}

PRINCIPLES OF DNA-BASED IMMUNOTHERAPY IN ATOPIC DISEASE

One of the primary reasons why DNA-based immunotherapy is so appealing is its ability to direct the immune reaction toward a T_H1 response as traditional immunotherapy does. The reason this is so advantageous is based on numerous lines of evidence that indicate that atopic diseases, such as atopic asthma, allergic rhinitis, and allergic conjunctivitis, are mediated by T_H2 cells and T_H2 -elaborated cytokines.^{51,52} Furthermore, insect allergy and food allergy are also amenable to DNA-based immunotherapy for similar reasons. The cytokines that have been shown to play important roles in the development and persistence of an allergic state include IL-4, IL-5, and IL-13, which are elaborated in a T_H2 -biased response.^{51,52} In addition, there appears to be a relative decrease in IL-10 action, which normally acts to down-regulate both T_H1 and T_H2 responses. Numerous lines of evidence in mouse models suggest that altering the biology of these cytokines can affect the allergic process. In light of the roles T_H2 -elaborated cytokines play in atopic disease, many efforts have

been made at altering their biology, although with limited results. Inhibiting IL-4 action in a mouse model of asthma failed to improve atopic inflammation and airway hyperresponsiveness.^{53,54} This lack of efficacy was also noted in a double-blind, placebo-controlled trial of nebulized IL-4.⁵⁵ In similar asthma models, IL-5 neutralization reversed airway eosinophilia but did not affect airway hyperresponsiveness.⁵⁶ Furthermore, a clinical trial of anti-IL-5 antibodies similarly reduced airway eosinophilia but had no effect on airway hyperresponsiveness.⁵⁷ Better results have been observed with IL-13 inhibition using a soluble IL-13R α 2-IgG fusion protein.⁵⁸ Specifically, airway hyperresponsiveness, eosinophilia, and mucous secretion were improved, although the early asthmatic response was unchanged. Rather than inhibiting T_H2-elaborated cytokines, increasing the levels of T_H1-elaborated cytokines has also been attempted and resulted in limited success. For example, a clinical trial of subcutaneous IL-12 administration in mild asthmatic patients demonstrated a decrease in eosinophilia but no significant improvement in the early or late asthmatic responses.⁵⁹ Of note, flu-like symptoms were experienced by most of the 19 participants who received IL-12 therapy. Four of these individuals withdrew from the study for reasons such as cardiac arrhythmias, abnormal liver function test results, and severe flu-like symptoms.

Based on such evidence, it appears that altering any single cytokine is unlikely to effectively treat atopic disease. This is logical, since the development, persistence, and manifestations of atopic disease are mediated by a multitude of cells, cytokines, chemokines, and other effectors. Given this fact, perhaps the most successful approach to treating the immunologic basis of atopic diseases is to alter the entire immune response. This is the mechanism by which both conventional and DNA-based immunotherapy have been proven to work. Therefore, CpG oligonucleotide administration, which can alter this T_H2-biased response in addition to up-regulating IL-10, is a prime candidate to treat the immunologic imbalance observed in allergy. As a demonstration of its efficacy, a number of studies have been performed in both mice and humans, indicating that DNA-based immunotherapy is not only safe but highly effective in converting the T_H2 allergic response to a nonpathologic T_H1 response. Specifically, CpG DNA has been shown to inhibit IL-4, IL-5,^{42,60–64} and IL-13.⁶⁵ Not only is CpG DNA capable of biasing toward a T_H1 response, but depending on the dose of administered DNA, it can also favor an increase in IL-10 with a subsequent reduction in both T_H1 and T_H2 responses, with a parallel improvement in allergic symptoms.^{35–37,62}

ANIMAL STUDIES OF DNA-BASED IMMUNOTHERAPY

Suggesting DNA-based immunotherapy can be used to prevent the onset of symptoms in high-risk individuals, several groups have demonstrated that CpG DNA administered before or concurrently with allergen sensitization can attenuate the allergic response in mice.^{60,64,66,67} Specifically, Kline et

al⁶⁰ sensitized mice in a model of atopic asthma and observed airway hyperreactivity, elevated serum IgE levels, and airway eosinophilia. If CpG immunostimulatory DNA was administered at the time of sensitization, all of these typical asthma characteristics were reduced in severity. Furthermore, T_H2-related cytokines, such as IL-4, were also reduced, whereas T_H1-related cytokines, such as IFN- γ and IL-12, were up-regulated. Similar results were obtained by other groups, including Broide et al.⁶³ who found that mucosal or systemic administration of CpG DNA before antigen challenge reduced airway eosinophilia in a murine model of asthma induced by ovalbumin. Sur et al⁶⁴ and Shirota et al⁶⁶ provided additional support for the efficacy of mucosal administration of CpG DNA in the prevention of asthma-related phenotypes such as airway eosinophilia and airway hyperresponsiveness. However, these studies primarily focused on the prevention of asthma. In the clinical setting, clinicians are instead responsible for treating established asthma. To this end, DNA-based immunotherapy has also demonstrated benefit in mouse models of established asthma. Serebrisky et al⁶⁵ administered CpG DNA 24 hours after each of 2 antigen challenges with conalbumin. The treated mice were found to have reduced antigen-specific IgE levels, airway eosinophilia, mucous production, and airway hyperreactivity. These clinical improvements correlated with increases in IFN- γ levels and decreases in IL-4, IL-5, and IL-13 levels. Santeliz et al⁶⁸ produced similar improvements in a model of established asthma in which CpG covalently conjugated to Amb a 1 was administered intradermally twice at 1-week intervals to mice previously sensitized and challenged with ragweed pollen extract. A more extensive immunotherapy protocol was studied by Kline et al⁶⁹ in which mice were sensitized to ovalbumin and 2 weeks later were treated with 4 biweekly intradermal injections of CpG oligostimulatory DNA and/or ovalbumin. Their findings were consistent with previous work that showed that airway eosinophilia, IgE levels, airway hyperreactivity, and levels of IL-5 were all reduced in conjunction with an increase in IFN- γ but only in those mice treated with CpG and ovalbumin. Administration of either CpG or ovalbumin alone did not result in clinical improvement.

A significant benefit of DNA-based immunotherapy is decreased allergenicity compared with allergen administration alone. Horner et al⁷⁰ synthesized ovalbumin-DNA conjugates and compared them to ovalbumin mixed with CpG DNA and ovalbumin alone. Compared with the ovalbumin-DNA mixture and ovalbumin alone, the conjugate bound significantly less to IgE- or to IgG-sensitized cells. Furthermore, induction of mast cell degranulation was significantly lower with the conjugates than with ovalbumin, again suggesting the conjugate is less allergenic. In particular, 100 ng of one such ovalbumin-DNA conjugate induced the same amount of mast cell degranulation as 1 ng of ovalbumin alone. The authors demonstrated that death due to anaphylaxis in ovalbumin-immunized mice was significantly lower when using the ovalbumin-DNA conjugate (17% and 0%

mortality with 2 different constructs) compared with ovalbumin alone (100% mortality) or to an ovalbumin-DNA mixture (100% mortality).

HUMAN STUDIES OF DNA-BASED IMMUNOTHERAPY

As with many promising therapeutics developed in animal models, translating these findings to humans is often wrought with difficulty. However, several human studies have already been performed that assess safety, tolerability, immunogenicity, and efficacy of CpG DNA coupled to allergen, all of which thus far suggest there is the potential for DNA-based immunotherapy to become an alternative to current immunotherapy protocols. Using Amb a 1 conjugated to CpG DNA, work by Creticos et al^{71,72} has demonstrated that the conjugate is 185-fold less reactive than a standard ragweed extract and therefore potentially safer and more tolerable to the patient. Interestingly, a subsequent blinded study in which patients were given a 6-dose regimen of the Amb a 1-DNA conjugate or placebo demonstrated lasting clinical benefit even into the following ragweed season with DNA-based immunotherapy.⁷³ After the 6-dose regimen, titers of anti-Amb a 1 IgG were comparable to those from patients who received standard immunotherapy for more than 1 year.

Similar clinical and biochemical improvements were observed in other clinical trials. Simons et al⁴² sought to determine whether DNA-based immunotherapy could redirect a ragweed-specific T_H2-biased response to a T_H1-biased response in human subjects with ragweed allergy. They administered 6 subcutaneous injections of placebo or Amb a 1 covalently conjugated to a 22-base-long CpG containing motif and quantified the immune responses to ragweed and other unrelated antigens at 2 and 16 weeks after treatment. On recall challenge, they found significant increases in IFN- γ , CXCL9, and CXCL10 and significant decreases in IL-5, CCL17, and CCL22, indicating a shift had occurred toward a T_H1-biased response. Cytokine profiles in response to challenge with unrelated antigens were unchanged from pretreatment levels, demonstrating the specificity of the immune redirection. Also of note, there were no identifiable adverse local or systemic reactions in those treated with the Amb a 1-DNA conjugate.

Despite these biochemical indicators of efficacy, it is the clinical effects that are among the most important factors in determining the success of a new therapy. Tulic et al⁷⁴ used a similar Amb a 1-immunostimulatory DNA conjugate to evaluate eosinophilia and cytokine messenger RNA (mRNA) expression in the nasal mucosa of patients allergic to ragweed. However, they also measured clinical outcomes as a result of the treatment. Twenty-eight patients received weekly escalating doses of the Amb a 1-DNA conjugate, whereas 29 control patients received placebo before the 2001 ragweed season. Although no difference was observed between the treatment and placebo groups in allergic symptoms or medication use during the first ragweed season, the subsequent ragweed season revealed a significant decrease in

chest symptoms and a trend toward reduced nasal symptoms in the treatment group. This clinical improvement was mirrored by a significant reduction in eosinophils and IL-4 mRNA-positive cells and increased number of IFN- γ mRNA-positive cells compared with the placebo group. The clinical and biochemical improvements observed in the treated group during the 2002 ragweed season were the result of only 6 injections given before the 2001 ragweed season. Although these early studies are highly promising, more investigative work must be performed into the optimal preparation, dosing, delivery, and duration of DNA-based immunotherapy. Furthermore, the potential long-term adverse effects of this type of therapy have yet to be determined.

CONCLUSION

Still in the early stages of development, DNA vaccination has proven itself in numerous animal studies. Models for prostate cancer, breast cancer, melanoma, parasitic infections, tuberculosis, and viruses such as human immunodeficiency virus and West Nile virus have all found protection from disease following DNA vaccination.^{75–80} Disease states that result from an insufficient immune response are clearly suitable to therapy with DNA vaccination. However, it is less clear how diseases of inappropriate immune responses such as allergy could benefit. Nevertheless, early animal and human studies of DNA vaccination as immunotherapy for atopic diseases have shown promise and, in some cases, superiority to current treatment standards.^{42,62,71–74} Undoubtedly, ongoing clinical trials will either validate or refute the potential for DNA vaccination as the new era in immunotherapy. Furthermore, with so many permutations on how exactly to induce the best clinical response, DNA-based immunotherapy will continue to undergo refinements based on findings in the laboratory and at the bedside.

REFERENCES

1. Tokunaga T, Yamamoto H, Shimada S, et al. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG, I: isolation, physicochemical characterization, and antitumor activity. *J Natl Cancer Inst.* 1984;72:955–962.
2. Yamamoto S, Kuramoto E, Shimada S, Tokunaga T. In vitro augmentation of natural killer cell activity and production of interferon-alpha/beta and -gamma with deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. *Jpn J Cancer Res.* 1988;79:866–873.
3. Yamamoto S, Yamamoto T, Shimada S, et al. DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. *Microbiol Immunol.* 1992;36:983–997.
4. Messina JP, Gilkeson GS, Pisetsky DS. Stimulation of in vitro murine lymphocyte proliferation by bacterial DNA. *J Immunol.* 1991;147:1759–1764.
5. Krieg AM, Yi AK, Matson S, et al. CpG motifs in bacterial DNA trigger direct B-cell activation. *Nature.* 1995;374:546–549.
6. Bird AP. CpG-rich islands and the function of DNA methylation. *Nature.* 1986;321:209–213.

7. Aderem A, Ulevitch JR. Toll-like receptors in the induction of the innate immune response. *Nature*. 2000;406:782–787.
8. Medzhitov R, Janeway C Jr. The Toll receptor family and microbial recognition. *Trends Microbiol*. 2000;8:452–456.
9. Poltorak A, He X, Smirnova I, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science*. 1998;282:2085–2088.
10. Brightbill HD, Libraty DH, Krutzik SR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science*. 1999;285:732–736.
11. Aliprantis AO, Yang RB, Mark MR, et al. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science*. 1999;285:736–739.
12. Hemmi H, Takeuchi O, Kawai T, et al. A Toll-like receptor recognizes bacterial DNA. *Nature*. 2000;408:740–745.
13. Yi AK, Tuetken R, Redford T, et al. CpG motifs in bacterial DNA activate leukocytes through the pH-dependent generation of reactive oxygen species. *J Immunol*. 1998;160:4755–4761.
14. Macfarlane DE, Manzel L. Antagonism of immunostimulatory CpG-oligodeoxynucleotides by quinacrine, chloroquine, and structurally related compounds. *J Immunol*. 1998;160:1122–1131.
15. Hacker H, Mischak H, Miethke T, et al. CpG-DNA-specific activation of antigen-presenting cells requires stress kinase activity and is preceded by non-specific endocytosis and endosomal maturation. *EMBO J*. 1998;17:6230–6240.
16. Hartmann G, Krieg AM. Mechanism and function of a newly identified CpG DNA motif in human primary B cells. *J Immunol*. 2000;164:944–953.
17. Schnare M, Holt AC, Takeda K, et al. Recognition of CpG DNA is mediated by signaling pathways dependent on the adaptor protein MyD88. *Curr Biol*. 2000;10:1139–1142.
18. Hacker H, Vabulas RM, Takeuchi O, et al. Immune cell activation by bacterial CpG-DNA through myeloid differentiation marker 88 and tumor necrosis factor receptor-associated factor (TRAF)6. *J Exp Med*. 2000;192:595–600.
19. Wesche H, Henzel WJ, Shillinglaw W, et al. MyD88: an adapter that recruits IRAK to the IL-1 receptor complex. *Immunity*. 1997;7:837–847.
20. Muzio M, Ni J, Feng P, Dixit VM. IRAK (Pelle) family member IRAK-2 and MyD88 as proximal mediators of IL-1 signaling. *Science*. 1997;278:1612–1615.
21. Yi AK, Klinman DM, Martin TL, et al. Rapid immune activation by CpG motifs in bacterial DNA: systemic induction of IL-6 transcription through an antioxidant-sensitive pathway. *J Immunol*. 1996;157:5394–5402.
22. Redford TW, Yi AK, Ward CT, Krieg AM. Cyclosporin A enhances IL-12 production by CpG motifs in bacterial DNA and synthetic oligodeoxynucleotides. *J Immunol*. 1998;161:3930–3935.
23. Macfarlane DE, Manzel L, Krieg AM. Unmethylated CpG-containing oligodeoxynucleotides inhibit apoptosis in WEHI 231 B lymphocytes induced by several agents: evidence for blockade of apoptosis at a distal signalling step. *Immunology*. 1997;91:586–593.
24. Yi AK, Krieg AM. CpG DNA rescue from anti-IgM-induced WEHI-231 B lymphoma apoptosis via modulation of I kappa B alpha and I kappa B beta and sustained activation of nuclear factor-kappa B/c-Rel. *J Immunol*. 1998;160:1240–1245.
25. Yi AK, Hornbeck P, Lafrenz DE, Krieg AM. CpG DNA rescue of murine B lymphoma cells from anti-IgM-induced growth arrest and programmed cell death is associated with increased expression of c-myc and bcl-xL. *J Immunol*. 1996;157:4918–4925.
26. Stacey KJ, Sweet MJ, Hume DA. Macrophages ingest and are activated by bacterial DNA. *J Immunol*. 1996;157(5):2116–22.
27. Chace JH, Hooker NA, Mildstein KL, et al. Bacterial DNA-induced NK cell IFN-gamma production is dependent on macrophage secretion of IL-12. *Clin Immunol Immunopathol*. 1997;84:185–193.
28. Jakob T, Walker PS, Krieg AM, et al. Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. *J Immunol*. 1998;161:3042–3049.
29. Ballas ZK, Rasmussen WL, Krieg AM. Induction of NK activity in murine and human cells by CpG motifs in oligodeoxynucleotides and bacterial DNA. *J Immunol*. 1996;157:1840–1845.
30. Cowdery JS, Chace JH, Yi AK, Krieg AM. Bacterial DNA induces NK cells to produce IFN-gamma in vivo and increases the toxicity of lipopolysaccharides. *J Immunol*. 1996;156:4570–4575.
31. Lipford GB, Bauer M, Blank C, et al. CpG-containing synthetic oligonucleotides promote B and cytotoxic T cell responses to protein antigen: a new class of vaccine adjuvants. *Eur J Immunol*. 1997;27:2340–2344.
32. Sun S, Zhang X, Tough DF, Sprent J. Type I interferon-mediated stimulation of T cells by CpG DNA. *J Exp Med*. 1998;188:2335–2342.
33. Schattenberg D, Schott M, Reindl G, et al. Response of human monocyte-derived dendritic cells to immunostimulatory DNA. *Eur J Immunol*. 2000;30:2824–2831.
34. Sparwasser T, Koch ES, Vabulas RM, et al. Bacterial DNA and immunostimulatory CpG oligonucleotides trigger maturation and activation of murine dendritic cells. *Eur J Immunol*. 1998;28:2045–2054.
35. Kitagaki K, Jain V, Businga T, et al. Immunomodulatory effects of CpG oligodeoxynucleotides on established Th2 responses. *Clin Diag Lab Immunol*. 2002;9:1260–1269.
36. Adkiss CA, Blaser K. Role of IL-10 in allergen-specific immunotherapy and normal response to allergens. *Microbes Infect*. 2001;3:891–898.
37. Jutel M, Akdis M, Budak F, et al. IL-10 and TGF-beta cooperate in the regulatory T cell response to mucosal allergens in normal immunity and specific immunotherapy. *Eur J Immunol*. 2003;33:1205–1214.
38. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity. *Eur J Immunol*. 2000;30:1939–1947.
39. Tighe H, Takabayashi K, Schwartz D, et al. Conjugation of immunostimulatory DNA to the short ragweed allergen Amb 1 enhances its immunogenicity and reduces its allergenicity. *J Allergy Clin Immunol*. 2000;106:124–134.
40. Marshall JD, Abtahi S, Eiden JJ, et al. Immunostimulatory sequence DNA linked to the Amb 1 allergen promotes Th1 cytokine expression while downregulating Th2 cytokine expression. *J Allergy Clin Immunol*. 2001;108:191–197.
41. Spiegelberg HL, Horner AA, Takabayashi K, and Raz E. Allergen-immunostimulatory oligodeoxynucleotide conjugate: a novel allergoid for immunotherapy. *Curr Opin Allergy Clin Immunol*. 2002;2:547–551.
42. Simons FE, Shikishima Y, Van Nest G, et al. Selective immune

- redirection in humans with ragweed allergy by injecting Amb 1 a linked to immunostimulatory DNA. *J Allergy Clin Immunol*. 2004;113(6):1144–1151.
43. Wolff JA, Malone RW, Williams P, et al. Direct gene transfer into mouse muscle *in vivo*. *Science*. 1990;247:1465–1468.
44. Wolff JA, Williams P, Acsadi G, et al. Conditions affecting direct gene transfer into rodent muscle *in vivo*. *Biotechniques*. 1991;11:474–485.
45. Satkauskas S, Bureau MF, Mahfoudi A, Mir LM. Slow accumulation of plasmid in muscle cells: supporting evidence for a mechanism of DNA uptake by receptor-mediated endocytosis. *Mol Ther*. 2001;4:317–323.
46. Doe B, Selby M, Barnett S, et al. Induction of cytotoxic T lymphocytes by intramuscular immunization with plasmid DNA is facilitated by bone marrow-derived cells. *Proc Natl Acad Sci U S A*. 1996;93:8578–8583.
47. Kim JJ, Trivedi NN, Nottingham LK, et al. Modulation of amplitude and direction of *in vivo* immune responses by co-administration of cytokine gene expression cassettes with DNA immunogens. *Eur J Immunol*. 1998;28:1089–1103.
48. Fattori E, La Monica N, Ciliberto G, Toniatti C. Electro-gene-transfer: a new approach for muscle gene delivery. *Somat Cell Mol Genet*. 2002;27:75–83.
49. Piccirillo CA, Prud'homme GJ. Immune modulation by plasmid DNA-mediated cytokine gene transfer. *Curr Pharm Des*. 2003;9:83–94.
50. Herweijer H, Wolff JA. Progress and prospects: naked DNA gene transfer and therapy. *Gene Ther*. 2003;10:453–458.
51. Jain VV, Kitagaki K, Kline JN. CpG DNA and immunotherapy of allergic airway diseases. *Clin Exp Allergy*. 2003;33:1330–1335.
52. Norman PS. Immunotherapy: 1999–2004. *J Allergy Clin Immunol*. 2004;113:1013–1023.
53. Corry DB, Folkesson HG, Warnock ML, et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med*. 1996;183:109–117.
54. Coyle AJ, Le Gros G, Bertrand C, et al. Interleukin-4 is required for the induction of lung Th2 mucosal immunity. *Am J Respir Cell Mol Biol*. 1995;13:54–59.
55. Borish LC, Nelson HS, Corren J, et al. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J Allergy Clin Immunol*. 2001;107:963–970.
56. Mathur M, Herrmann K, Li X, et al. TRFK-5 reverses established airway eosinophilia but not established airway hyperresponsiveness in a murine model of chronic asthma. *Am J Respir Crit Care Med*. 1999;159:580–587.
57. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet*. 2000;356:2144–2148.
58. Taube C, Duez C, Cui ZH, et al. The role of IL-13 in established allergic airway disease. *J Immunol*. 2002;169:6482–6489.
59. Bryan SA, O'Connor BJ, Matti S, et al. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet*. 2000;356:2149–2153.
60. Kline JN, Waldschmidt TJ, Businga TR, et al. Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol*. 1998;160:2555–2559.
61. Hussain I, Jain VV, Kitagaki K, et al. Modulation of murine allergic rhinosinusitis by CpG oligodeoxynucleotides. *Laryngoscope*. 2002;112:1819–1826.
62. Kline JN, Krieg AM, Waldschmidt TJ, et al. CpG oligodeoxynucleotides do not require Th1 cytokines to prevent eosinophilic airway inflammation in a murine model of asthma. *J Allergy Clin Immunol*. 1999;104:1258–1264.
63. Broide D, Schwarze J, Tighe H, et al. Immunostimulatory DNA sequences inhibit IL-5, eosinophilic inflammation, and airway hyperresponsiveness in mice. *J Immunol*. 1998;161:7054–7062.
64. Sur S, Wild JS, Choudhury BK, et al. Long term prevention of allergic lung inflammation in a mouse model of asthma by CpG oligodeoxynucleotides. *J Immunol*. 1999;162:6284–6293.
65. Serebrisky D, Teper AA, Huang CK, et al. CpG oligodeoxynucleotides can reverse Th2-associated allergic airway responses and alter the B7.1/B7.2 expression in a murine model of asthma. *J Immunol*. 2000;165:5906–5912.
66. Shirota H, Sano K, Kikuchi T, et al. Regulation of T-helper type 2 cell and airway eosinophilia by transmucosal coadministration of antigen and oligodeoxynucleotides containing CpG motifs. *Am J Respir Cell Mol Biol*. 2000;22:176–182.
67. Shirota H, Sano K, Kikuchi T, et al. Regulation of murine airway eosinophilia and Th2 cells by antigen-conjugated CpG oligodeoxynucleotides as a novel antigen-specific immunomodulator. *J Immunol*. 2000;164:5575–5582.
68. Santeliz JV, Van Nest G, Traquina P, et al. Amb 1-linked CpG oligodeoxynucleotides reverse established airway hyperresponsiveness in a murine model of asthma. *J Allergy Clin Immunol*. 2002;109:455–462.
69. Kline JN, Kitagaki K, Businga TR, Jain VV. Treatment of established asthma in a murine model using CpG oligonucleotides. *Am J Physiol Lung Cell Mol Physiol*. 2002;283:L170–L179.
70. Horner AA, Takabayashi K, Beck L, et al. Optimized conjugation ratios lead to allergen immunostimulatory oligodeoxynucleotide conjugates with retained immunogenicity and minimal anaphylactogenicity. *J Allergy Clin Immunol*. 2002;110:413–420.
71. Creticos PS, Eiden JJ, Balcer SL, et al. Immunostimulatory oligonucleotides conjugated to Amb 1: safety, skin test reactivity, and basophil histamine release [abstract]. *J Allergy Clin Immunol*. 2000;105(suppl):S70.
72. Creticos PS, Balcer SL, Schroeder JT, et al. Initial immunotherapy trial to explore the safety, tolerability and immunogenicity of subcutaneous injection of an Amb 1 immunostimulatory oligonucleotide conjugate (AIC) in ragweed allergic adults [abstract]. *J Allergy Clin Immunol*. 2001;107(suppl):S216.
73. Creticos PS, Eiden JJ, Broide D, et al. Immunotherapy with immunostimulatory oligonucleotides linked to purified ragweed Amb 1 allergen: effects on antibody production, nasal allergen provocation, and ragweed seasonal rhinitis [abstract]. *J Allergy Clin Immunol*. 2002;109:743.
74. Tulic MK, Fiset PO, Christodoulopoulos P, et al. Amb 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol*. 2004;113:235–241.
75. Kim JJ, Yang JS, Dang K, Manson KH, et al. Engineering enhancement of immune responses to DNA-based vaccines in a prostate cancer model in rhesus macaques through the use of cytokine gene adjuvants. *Clin Cancer Res*. 2001;7(3 suppl):882s–889s.
76. Cohen EP. DNA-based vaccines for the treatment of cancer—an experimental model. *Trends Mol Med*. 2001;7:175–179.

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77. Ayash-Rashkovsky M, Weisman Z, Zlotnikov S, et al. Induction of antigen-specific Th1-biased immune responses by plasmid DNA in schistosoma-infected mice with a preexistent dominant Th2 immune profile. *Biochem Biophys Res Commun*. 2001;282:1169–1176.
78. Lowrie DB. DNA vaccines against tuberculosis. *Curr Opin Mol Ther*. 1999;1:30–33.
79. Barouch DH, Letvin NL. DNA vaccination for HIV-1 and SIV. *Intervirology*. 2000;43:282–287.
80. Yang JS, Kim JJ, Hwang D, et al. Induction of potent th1-type

immune responses from a novel DNA vaccine for West Nile virus New York isolate (WNV-NY1999). *J Infect Dis*. 2001;184:809–816.

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CME Examination

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CME Test Questions

- What are the features that distinguish bacterial DNA from vertebrate DNA with respect to its immunogenic potential?
 - bacterial DNA lacks histones
 - CpG repeats are statistically underrepresented in human DNA
 - CpG islands in human DNA are more likely to be found in inaccessible heterochromatin
 - CpG repeats in human DNA are methylated, whereas bacterial repeats are not
 - a, b, and c
 - a and d
 - b and d
- Which of the following was NOT discussed in this review as a mechanism by which antigen specificity can be imparted to DNA-based immunotherapy?
 - Covalent conjugation of the antigen to the CpG immunostimulatory DNA
 - Administration of the antigen in parallel to the CpG immunostimulatory DNA
 - Oral administration of the antigen
 - The CpG immunostimulatory DNA encodes itself
 - All of the above were discussed in this review article
- A dose dependence of CpG-mediated inhibition of the allergic response has been demonstrated based on differential induction of which of the following cytokines?
 - Interleukin (IL) 1
 - IL-4
 - IL-5
 - IL-10
 - Tumor necrosis factor α
 - Interferon- γ (IFN- γ)
- A number of animal studies have demonstrated the effectiveness of CpG immunostimulatory DNA in inhibiting the allergic response. Which of the following cytokine changes was NOT associated with an improvement in allergic symptoms after DNA-based immunotherapy?
 - Decreases in levels of IL-4
 - Increases in levels of IL-5
 - Increases in levels of IL-12
 - Decreases in levels of IL-13
 - Increases in levels of IFN- γ
- Which of the following were observed in human trials of CpG-allergen conjugates as noted in this review?
 - Decreased eosinophilia in the nasal mucosa of treated patients
 - Local or systemic side effects were the same as in the placebo group
 - Significant improvements in allergic symptoms within 1 month of therapy
 - Clinical improvements in symptoms observed in the subsequent allergy season
 - a and b only
 - a and c only
 - a, b, and c only
 - All of the above

Answers found on page 451.