

CpG DNA as a Th1 Trigger

Klaus Heeg Stefan Zimmermann

Institute of Medical Microbiology and Hygiene, Philipps University of Marburg, Marburg, Germany

Key Words

Bacterial DNA · Synthetic oligodeoxynucleotides · CpG · Th1 · Vaccination

Abstract

Over the last few years, it has been recognized that along with structural components and products of bacteria, bacterial DNA is also capable of signaling infectious danger to cells of the innate immune system. Particular DNA sequences (CpG motifs), which are abundant in prokaryotic (bacterial) but not in mammalian DNA, cause the activation and stimulation of immune cells. Research has been catalyzed by the finding that certain synthetic oligodeoxynucleotides mimic the action of bacterial DNA. Immunostimulation induced by bacterial DNA or synthetic oligonucleotides not only contributes to our knowledge of the pathogen-host interrelationship during infection, but can also be used therapeutically to condition or modify ongoing immune responses of the adaptive immune system. Accordingly, CpG motifs have been used as vaccine adjuvants as well as instructing agents to selectively induce Th1-dominated immune responses. Hence, CpG motifs might be used in the future as adjuvants and/or immunomodulatory agents to treat or prevent undesired Th2-dominated immune responses, such as allergy.

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Introduction

More than five decades have passed since DNA was recognized as the coding molecule of genetic information. Initially, the DNA molecule was considered to be immunologically inert, eliciting immune responses only in rare autoimmune diseases. This view of DNA has changed dramatically over the past years. It has been recognized that certain DNA sequences are sensed by cells of the innate immune system. These sequences are common in prokaryotic DNA, but less abundant in mammalian DNA. From these observations, the concept emerged that the recognition of prokaryotic DNA signals infectious danger to cells of the innate immune system, as other conserved bacterial products [e.g. endotoxin, lipopolysaccharide (LPS)] do via interaction with pattern recognition receptors. The presence of infectious danger is communicated to cells of the adaptive immune system and is a prerequisite for the initiation of adaptive immune responses (B and T cell responses). Bacterial DNA has the potency to initiate and bias these interactions, since it preferentially induces a Th1 type of reactivity within regulatory T cell circuits. The concept of the recognition of prokaryotic DNA has now been intensively investigated and holds the promise of being utilized in initiating and directing immune responses to antigens during vaccination and during the redirecting of undesired immune deviations, as

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Correspondence to: Dr. Klaus Heeg
Institute of Medical Microbiology and Hygiene, Philipps University Marburg
Pilgrimstein 2, D–35037 Marburg (Germany)
Tel. +49 6421 28 66453, Fax +49 6421 28 66420
E-Mail heeg@post.med.uni-marburg.de

with allergy. This review sums up the recent knowledge of bacterial DNA and synthetic oligodeoxynucleotides (ODNs) mimicking these effects. Special attention is paid to the influence of bacterial DNA and ODNs on the instruction and redirection of the balance between Th1 and Th2 cells.

Bacterial DNA and CpG Motifs: From Bacterial DNA to Specific Immunostimulatory DNA Sequence Motifs

Three different landmark observations led to the recognition of immunostimulatory DNA motifs. In the 1970s, the successful treatment of cancer in experimental animal models and also in humans with *Mycobacterium bovis* attracted attention [1]. Yamamoto et al. [2] identified a DNA-rich fraction as the active compound in *M. bovis* which they termed MY-1. MY-1 has been shown to cause tumor regression and induce interferon secretion from spleen cells and activated natural killer (NK) cells [3]. The effects of MY-1 are DNase sensitive and proteinase K insensitive. From randomly selected DNA sequences of mycobacteria, Yamamoto et al. [4, 5] identified a 45-mer ODN that was able to activate interferon secretion and NK cell activation. The ODN contained a palindromic sequence motif with a central C-G dinucleotide which was essential for the effects of ODN.

Patients suffering from systemic lupus erythematosus harbor in their sera anti-DNA antibodies which form immune complexes with DNA. From these complexes, it was possible to isolate DNA of bacterial origin [6]. Misena et al. [7] found that bacterial DNA was mitogenic for B lymphocytes in vitro, while DNA from vertebrates was not. Moreover, the methylation of cytosine residues of bacterial DNA has been found to completely abolish mitogenicity, indicating a DNA-sequence-dependent activity on B cells [8].

The third line of observations emerged from studies with antisense ODNs. These ODNs were introduced to specifically prevent the translation of target mRNA and thus prevent expression of the corresponding gene product [9]. Although this approach proved to be of value in preventing expression of the target protein, several investigators noticed unforeseen side effects of antisense and sense ODNs. In particular, it was observed that certain ODNs induced the proliferation of B lymphocytes, the upregulation of MHC molecules and splenomegaly [10–13]. All these effects were dependent on the particular sequence of the ODN [11]. Krieg et al. [14], managed to

define the motif underlying these effects on B lymphocytes. They reported that short (approximately 20 bases long) ODNs exhibited profound B cell activity provided they contained a central DNA motif comprising a central CpG flanked at the 5' end by two purines and at the 3' end by two pyrimidines (5'-purine-purine-C-G-pyrimidine-pyrimidine-3') [14]. Reversal of the central CpG dinucleotide to a GpC dinucleotide completely abrogated the mitogenic effect of ODN on B cells. In addition, methylation of the CpG dinucleotide had the very same inhibitory effect [14]. The immunostimulatory DNA motif defined by Krieg et al. [14] was almost identical to the motif derived from mycobacterial DNA known to trigger interferon production and NK cell activation [4, 5].

These three lines of observations together suggested a link between the recognition of bacterial CpG motifs and the CpG suppression observed in mammals [15]. Since the frequency of CpG dinucleotides is reduced in vertebrate DNA, and since most of the CpG dinucleotides are methylated in vertebrate DNA, the number of 'free' CpG motifs in bacterial DNA exceeds that in vertebrate DNA. These CpG motifs are sensed by cells of the innate immune system of vertebrates and signal infectious danger to the immune system [16–21]. Accordingly, CpG motifs in bacterial DNA represent a further primordial recognition system of prokaryotic danger, as with the pattern recognition system of the toll-like receptors which sense constitutive bacterial cell wall components [22].

CpG Motifs: Natural Occurrence and Synthetic Derivatives

CpG dinucleotides are abundant in all bacterial genomes and are used at expected frequencies [15]. In addition, some viral [23, 24] and even invertebrate eukaryotic genomes (e.g. drosophila [25]) contain CpG motifs. DNA derived from these sources is mitogenic for B lymphocytes and activates antigen-presenting cells (APCs) [20]. Plasmide often contain immunostimulatory CpG motifs which may even contribute to the efficacy of DNA vaccines in vivo [26–28]. In vertebrate genomes, CpG motifs can be found only at low frequency. However, vertebrate DNA has no mitogenic activity on immune cells. Thus, the rare CpG motifs in vertebrate genomes seem not to be accessible for immune recognition, probably due to the methylation of cytosine residues at position C-5 [15, 29].

Most of the DNA-sequence-specific information on immunostimulatory DNA has been derived from analyses with synthetic ODNs. Since ODN-containing phos-

phodiester linkages [P]ODNs are rapidly degraded *in vivo* and *in vitro* by the action of nucleases [30, 31], ODNs were made resistant to nucleases by chemically modifying the backbone of the DNA molecule. ODNs with phosphothioate linkages ([S]ODNs) are commonly used [30, 32]. However, due to the polyanionic property of [S]ODNs, DNA-sequence-independent nonspecific effects might be observed at high concentrations [33, 34]. [S]ODNs bind to many proteins with higher affinity than [P]ODNs, which might account for unexpected side effects [32]. At the same time, [S]ODNs are up to 100 times more efficient in eliciting CpG-dependent immune responses than [P]ODNs of the same sequence [35].

Using single-stranded synthetic ODNs, it was found that the immunostimulatory motif (5'-purine-purine-C-G-pyrimidine-pyrimidine-3') was necessary and sufficient to elicit a response by murine immune cells [14]. However, the 5' and 3' flanking regions influenced the intrinsic activity of the central CpG motif [14, 36]. The mechanisms behind these effects are not yet known. The optimal CpG motif for the stimulation of human immune cells has not yet been defined. ODN-activating human cells contain multiple CpG dinucleotides in certain repetitive cassettes. From this, it could be suggested that a repetitive CpG motif is required for human cell activation [37–39]. Nevertheless, synthetic [S]ODNs are widely used as a paradigm for bacterial DNA *in vivo* and *in vitro*. Synthetic ODNs can be produced in a sequence-specific manner in large quantities and with high purity. Thus, synthetic immunostimulatory ODNs might be suitable for use in humans in the future.

CpG DNA: Uptake and Mode of Action

In macrophages, the rate-limiting steps of the action of ODNs can be defined as cellular uptake, accumulation of ODN in early endosomes and subsequent activation of intracellular signal pathways. Since bacterial DNA and ODNs are polyanionic, they cross cell membranes rather inefficiently [40–43]. On the other hand, immunostimulatory ODNs provoke specific responses, and antisense ODNs specifically block translation within the cell. Therefore, uptake mechanisms or receptors for DNA must exist. Specific binding proteins for double-stranded DNA have been described for eukaryotic cells, but unfortunately these proteins have not been further characterized. Double-stranded DNA binding has been shown to be sensitive to heparin and antagonized by synthetic ODNs, independent of their sequence [40, 44]. It has fur-

ther been suggested that ODNs might be taken up via fluid-phase endocytosis [33]. Interestingly, after the uptake of ODNs by some model cell lines, subsequent exocytosis of ODNs has also been observed [33]. Blockade of the scavenger receptor system by dextran sulfate reduces the effects of palindromic BCG-derived DNA. The addition of poly-G sequences to CpG ODNs has been shown to enhance their cellular uptake [45]. It has been suggested that these sequences favor uptake via the scavenger receptor system [45, 46]. On the other hand, Mac-1, an integrin expressed on macrophages and NK cells, has been reported to function as a receptor for ODNs [47]. An increase in Mac-1 expression was accompanied by an increase in the internalization of ODNs. These results suggest multiple pathways/receptors for the uptake of ODNs which might be selective for certain DNA sequences.

For the classical CpG ODNs translocation into endosomes has been shown to be the prerequisite for the activation of macrophages [48–50]. It has been shown that the inhibition of uptake by competition with nonstimulatory ODNs completely blocked CpG effects [49]. However, for certain effects, ODNs might signal from the outside. Several ODNs exhibited mitogenicity for human B cells even after covalent coupling to sepharose beads [37]. In addition, G-rich ODNs might signal several cell types independently from uptake [45, 51]. Nevertheless, the immunostimulatory effects of CpG ODNs on macrophages require uptake and subsequent endosomal maturation, since lysosomotropic agents like bafilomycin A and chloroquine completely inhibit CpG-ODN-mediated downstream effects in these cell types [48–50].

Within minutes of the uptake of CpG ODNs in macrophages, mitogen-activated protein kinases are triggered, followed by the activation of jnk and p38 [49, 50]. Consequently, c-Jun and ATF2, components of the transcription factor AP-1, are phosphorylated and activated [49]. In addition, the triggering of macrophages with CpG ODNs also induces strong activation of the NFκB signal pathway [49, 52]. These signal pathways finally lead to the activation and cytokine secretion of CpG-triggered APCs [49, 53]. A model of these pathways is shown in figure 1. It should be emphasized that the intracellular adapter which links the CpG ODNs within the endosomes to the initiation of the kinase pathways has not been identified yet. It can be speculated that this protein might harbor the recognition sites for CpG DNA motifs.

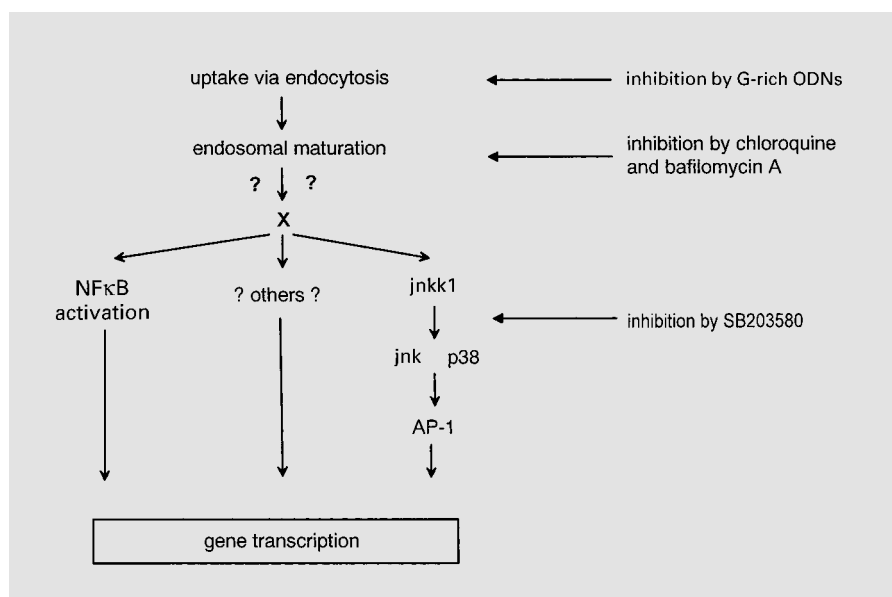


Fig. 1. Model of CpG-DNA-induced signal pathways, based on the report of Häcker et al. [49].

CpG DNA: Effects on Immune Cells in vitro

One of the most remarkable properties of CpG DNA is its ability to activate APCs. This activity of CpG is critical, since APCs represent the connection between innate immune cells and adaptive immunity [54]. After the uptake of CpG DNA, macrophages translocate NFκB and upregulate cytokine mRNA, such as TNF or IL-12 [55, 56]. Cytokine expression is paralleled by the induction of costimulatory molecules, including CD80, CD86 and CD40, as well as MHC class II molecules [55, 57]. Immature dendritic cells (DCs) grown from bone marrow cultures can be differentiated by CpG DNA to mature DCs which upregulate the expression of MHC class II, CD86 and CD40. It has been shown that CpG-DNA-treated DCs produce high amounts of IL-12 and IL-18, but only medium amounts of TNF or IL-6 [53]. In addition, these DCs were fully competent in eliciting mixed lymphocyte reactions in vivo and in vitro [53]. DCs derived from cultures of epidermal Langerhans' cells have been shown to behave similarly [58]. Thus, CpG DNA induces a milieu that favors the activation of T lymphocytes and which might guide the preferential development of Th1 cells, due to its profound ability to secrete Th1-inducing cytokines (IL-12 and IL-18).

CpG DNA induces the proliferation and IL-6 secretion of B cells [12, 14, 59], which in turn has been found to be responsible for IgM secretion by CpG-DNA-activated B lymphocytes [60–62]. The activation of B cells has been

shown to be accompanied by the initiation of reactive oxygen-dependent intracellular signal pathways [61], and the protection of B cells from anti-Ig-induced apoptosis [63, 64]. B cells activated by CpG DNA have been shown to upregulate costimulatory molecules and to serve as costimulatory cells during the activation of peptide-specific T cells [65]. Under certain experimental conditions, CpG effects on B cells have been augmented by simultaneously cross-linking the surface immunoglobulins [14, 34]. This indicates that CpG DNA, besides its direct effect on B cells might also deliver costimulatory signals to B lymphocytes.

As opposed to B cells and APCs, T cells do not respond to CpG DNA directly. After cross-linking of their T cell receptor by monoclonal antibodies, CpG DNA has been shown to costimulate T cells and induce their activation, IL-2 secretion and differentiation into cytolytic effector cells [66, 67]. However, T cell costimulation violates several rules of CpG activity on APCs or B cells. Firstly, G-rich ODN motifs which were not stimulatory for APCs were effective on T cells [67]. Secondly, the immunostimulatory CpG core motif was not mandatory for activity on T cells [51, 67]. And thirdly, T cell costimulation was chloroquine resistant [67]. The costimulatory activity of ODNs on T cells might have important implications. In the case of tumor cells lacking costimulatory molecules, ODNs could substitute for a costimulatory signal and thus may allow productive T cell activation.

In contrast to the costimulatory activity on T cells, ODNs further influence T cell reactivity indirectly via cytokines and costimulatory molecules. One important pathway in that respect is the induction of bystander T cell proliferation by CpG DNA. Accordingly, CpG DNA induces type I interferon secretion and in turn the secretion of IL-15, which controls bystander proliferation of memory-type T cells [68]. Interestingly, this effect can be observed *in vivo*; after an injection of CpG ODNs, T cells upregulate CD69 but not CD25 within 20 h [Lipford, unpubl. observations]. CpG DNA rapidly induces cytolytic activity and IFN- γ secretion by NK cells [4, 36, 69, 70]. *In vitro* activation of NK cells by CpG DNA requires IL-12 and TNF from macrophages [70, 71]. Thus, NK cells seem to be activated indirectly and thus behave similarly to T cells. Interestingly, G-rich ODNs might be active also on NK cells [72].

CpG DNA: Effects *in vivo*

After subcutaneous or intraperitoneal challenge, CpG DNA causes the activation of APCs followed by the secretion of proinflammatory cytokines [55, 73]. Mice sensitized with *D*-galactosamine to the lethal action of TNF succumb to lethal cytokine syndrome [55, 73]. Thus, CpG-DNA-induced TNF may cause harmful effects *in vivo*. In nonsensitized normal mice, CpG DNA causes local lymphadenopathy which lasts at least 1–2 weeks. The number of lymph node cells increases dramatically due to an influx of B cells, T cells and APCs. APCs derived from local lymph nodes spontaneously produce IL-12 and IFN- γ *in vitro* [74]. During this window, a sustained polarization of immune responses towards Th1 profiles can be observed. In addition, mice acquire a status of local resistance towards infection with certain intracellular pathogens, such as *Listeria* [75, 76] or *Leishmania* [74].

Coinjection of CpG DNA together with antigen discloses a powerful activity of CpG ODNs as adjuvant. This adjuvanticity includes antigen-specific B cell and T cell responses [25, 77–81]. Up to now, antibody responses against a number of different antigens, including tumor antigens, have been analyzed. The data underline the impressive potency of CpG DNA as adjuvant. In some reports, CpG DNA (ODNs or drosophila DNA) even surpassed the activity of Freund's complete adjuvant [25]. Interestingly, the immunoglobulin isotype response induced in the presence of CpG DNA has been found to be biased towards a Th1-type profile [78, 79, 82, 83]. Following intranasal application together with antigen, CpG

DNA has been shown to promote production of local anti-influenza-specific antibodies [84]. Thus, CpG DNA might be a candidate adjuvant for the induction of mucosal immunity [85, 86].

Under normal conditions, exogenous antigen does not enter the MHC-class-I-restricted antigen presentation pathway and thus fails to induce MHC-class-I-restricted CD8+ cytolytic T cells [87]. However, exogenous antigen entrapped in vehicles such as liposomes might enter this pathway and prime CD8+ T cells [88–90]. DCs play a pivotal role in this process [91, 92]. It was therefore intriguing to analyze whether CpG DNA would induce a milieu that favors the activation of class-I-restricted T cells *in vivo*. The use of CpG DNA as adjuvant permitted CD8+ T cell responses to soluble proteins and also to MHC-class-I-binding peptides [79].

In one study, the application of CpG DNA was shown to induce splenomegaly, which peaked at day 6 [93]. Detailed analyses revealed an increase in the numbers of GM-CFU, BFU-E and CFU. Accordingly, CpG DNA causes the accumulation of hematopoietic stem cells in the spleen and thus facilitated extramedullary hemopoiesis [93]. CpG DNA was successfully used to accelerate the recovery of the lymphohematopoietic system after sublethal irradiation [93]. It has also been shown that G-rich ODNs induce the proliferation of macrophage precursors in cultures of murine bone marrow cells [94]. Thus, ODN could be useful in restoring lymphohemopoiesis.

CpG DNA: A Th1 Trigger

CpG DNA induces the activation of APCs, accompanied by the secretion of cytokines such as IL-12 [53, 55, 71, 95]. In addition, CpG DNA induces *in vivo* a transient state of lymphadenopathy accompanied by a sustained secretion of IL-12 and IFN- γ [74]. Since IL-12 and IL-18 critically control the induction of Th1 cells, and since IFN- γ suppresses the development of Th2 cells [96, 97], it is suggested that CpG DNA might provide a milieu that favors the activation of Th1-type immune responses. This assumption was first supported by the observation that CpG DNA as adjuvant augmented and preferentially induced the immunoglobulin isotype pattern characteristic for Th1 responses [25, 78, 79, 82]. Even when mixed with alum, CpG DNA was shown to trigger primarily IgG2a antibodies and to activate CD8+ T cells *in vivo* [80]. Moreover, it has been found that CpG motifs in plasmid DNA suppress IgE induction and bias Th1-type isotypes during vaccination with naked DNA [98].

The Th-1-promoting activity of CpG DNA has been analyzed in vitro. Using an induction scheme that favors the differentiation of IL-4-producing Th2 cells, it has been shown that CpG DNA prevented the generation of Th2 cells, yet dramatically augmented the induction of IFN- γ -producing Th1 lymphocytes [99]. The efficacy of CpG ODNs even surpassed anti-IL-4 supplementation of the cultures known to bias Th1 development. Not only was the Th phenotype changed by CpG DNA, but also the number of growing T lymphocytes was markedly enhanced [99]. Thus the supplementation of cultures in vitro with CpG DNA could be used to generate and expand large numbers of antigen-specific Th1 cells. It is obvious that such cell lines might be of value for in vivo adoptive transfer experiments with antigen-specific Th1 cells, as in experimental antitumor or antiallergy approaches in mice [Röcken, pers. commun.].

The first in vivo probe for Th1 instruction facilitated by CpG DNA was performed in a Th2-driven disease model. The infection of BALB/c mice with *Leishmania major* causes the preferential induction of Th2 cells which fail to control the infection. As a consequence, BALB/c mice succumb to lethal generalized leishmaniasis [100]. In contrast, C57B1/6 mice mount a Th1 response and control *Leishmania* infection in an NO-dependent manner [101]. In one study, when Th2-prone BALB/c mice were injected with *Leishmania* and CpG DNA simultaneously, they controlled the size of the lesions and triggered a Th1 response [99]. Th1 lymphocytes could be isolated from these mice and characterized according to their cytokine and cytokine receptor profiles [99]. Immunity was long-lasting, protective towards a second challenge with *Leishmania* and controlled infection in an NO-dependent manner [99]. These results have been recently confirmed [102]. In addition, it has been shown that the Th1-promoting activity in this model was dependent on endogenous IL-12, since blockade of IL-12 prevented Th1 induction [102].

In the study discussed above, CpG ODNs not only prevented lethal Th2-dominated leishmaniasis when given to mice at the time point of infection, but were also able to cure BALB/c mice infected with *Leishmania* up to 3 weeks after infection [99]. For this effect, multiple injections of CpG DNA were needed. The cytokine profile of T cells recovered from cured mice indicated a Th1 phenotype. Moreover, the infection was controlled by an NO-dependent mechanism [99]. In addition, CpG DNA has effects when given before infection with *Leishmania*. It has been found that CpG ODNs induce a sustained milieu for Th1 polarization that is associated for up to

2 weeks [74] or even longer [103] with a state of local resistance towards lethal *Leishmania* infection in susceptible BALB/c mice.

In a murine model of asthma, CpG DNA was given during the sensitization phase to the eggs of *Schistosoma mansoni* [104]. This regimen lessened airway eosinophilia, prevented Th2 cytokine and IgE induction and prevented bronchial hyperreactivity, yet induced Th1-type cytokines [104]. Interestingly, Th1-promoting activity has been shown to be still operative in IL-12 $-/-$ and IFN- γ $-/-$ mice [105]. Moreover, in a mouse model of ovalbumin-induced eosinophilic airway inflammation, CpG DNA induced Th1 cytokine production and inhibited eosinophilic inflammation. Although CpG DNA has been found to be effective when given at the point of allergen challenge, it is also effective when given up to 6 days prior to allergen challenge [106, 107]. Furthermore, it has been shown that CpG DNA is able to bring about the secretion of IL-12, IL-18 and IFN- γ in vitro in peripheral blood mononuclear cells from allergy patients [108], indicating that this novel mode of influencing an induced Th2 response might also be operative in humans. Figure 2 gives an overview of the various effects of CpG DNA on the immune system.

CpG DNA: An Ideal Adjuvant for the Prevention and Treatment of Th2-Dominated Diseases?

From the data available, one could conclude that CpG DNA represents an ideal adjuvant for the prevention and treatment of Th2-dominated diseases. However, CpG DNA may cause harmful side effects which would need to be controlled properly. As mentioned, CpG ODNs induce a burst of cytokine secretion which may cause lethal shock in experimental mice [73]. This effect is mainly due to TNF. Obviously, CpG ODNs with reduced TNF-inducing potency, yet retained ability to induce IL-12 or IL-18, could circumvent these problems. CpG ODNs with these properties have been described already [95]. CpG DNA might enhance sensitivity to the toxicity of endotoxins, possibly mediated by IFN- γ [69]. Furthermore, CpG DNA might cause inflammatory lung disease when given intratracheally [109]. This effect seems to occur only after local administration, but not after systemic or subcutaneous injection of CpG ODNs. More serious might be the ability of bacterial DNA and CpG ODNs to induce reactive arthritis [110] or to (re)activate autoimmune responses, as shown in certain models of experimental allergic encephalomyelitis [111, 112]. These side effects may

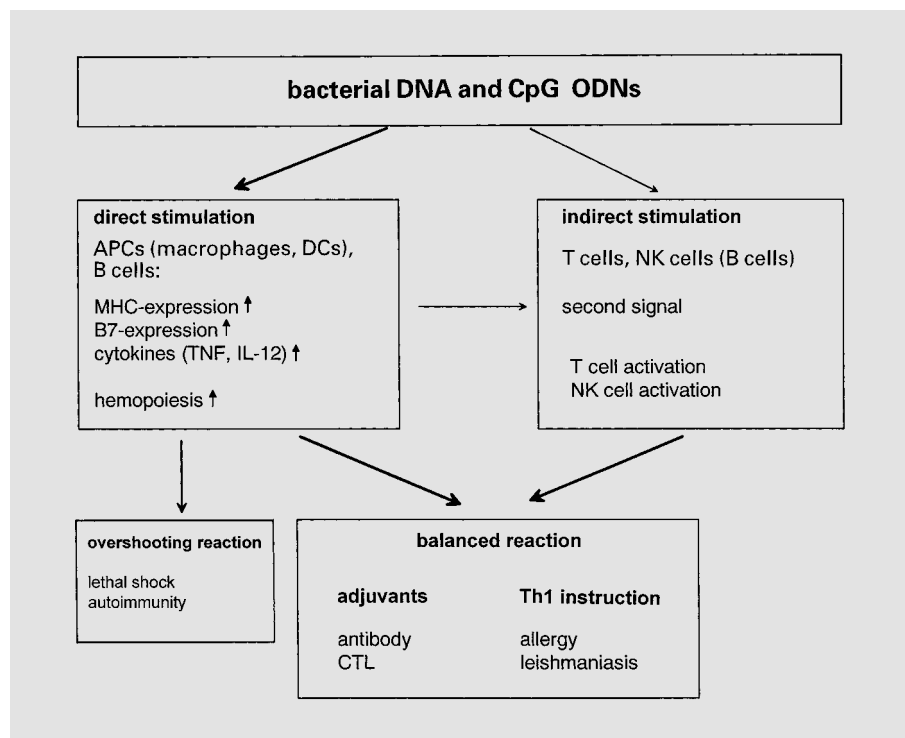


Fig. 2. Overview of the action of CpG DNA on the immune system. CTL = Cytotoxic T lymphocytes.

be directly linked to their ability to induce long-lasting local effects, including lymphadenopathy and constitutive secretion of proinflammatory cytokines [74, 75, 103, 113]. Obviously, it would be of value to control these effects through the termination of the action of CpG DNA via specific inhibition.

The latter approach might be feasible. ODNs derived from adenoviral DNA have been shown to specifically block the action of CpG ODNs [114]. There, analyses of the DNA sequences revealed that the suppressive motifs contained clusters of CG dinucleotides which were not found in the canonical stimulative CpG motif [114]. ODN motifs containing stretches of poly-G residues (G-rich ODNs) have also proved to be inhibitory [49, unpubl. data]. It has been found that G-rich ODNs blocked the cellular uptake of CpG ODNs, and thus their activity via competition [49]. G-rich ODNs have been found in vivo to prevent cytokine release and lethal shock induced by bacterial DNA or CpG ODNs but did not interfere with endotoxin- or superantigen-mediated cytokine secretion [unpubl. data]. Thus, inhibitory DNA sequences might be useful in controlling the in vivo action of CpG ODNs and in bypassing undesired side effects of CpG ODNs.

Another open question is whether the experiments performed in mice may find their counterpart in humans. At

present, information on the action of CpG ODNs on human cells is limited. In addition, an optimal CpG motif for human cells has still not been defined. One critical obstacle is the fact that human cells are much more sensitive to LPS than murine cells. Therefore, minute LPS contamination in ODN preparations which cannot be detected in standard assays might lead to false-positive results. It has been reported that ODNs induced the proliferation of human B cells, yet the ODN sequence requirements were different to those in mice [115]. CpG-dinucleotide-containing ODNs have been found to stimulate human monocytes and DCs [39], and to induce the upregulation of costimulatory molecules and the secretion of IL-12 and IL-6, but only very low amounts of TNF [38]. Peripheral blood mononuclear cells from allergic patients have been made to express IL-12 and IL-18 [108]. In addition, nonhuman primates respond to CpG motifs in experimental vaccination [81]. Thus, although the optimal CpG motif for human use still seems to be elusive, the data so far indicate that human cells respond in a similar way to murine cells. The ongoing phase I trial on CpG ODNs as adjuvant will further contribute to a definite clarification of this issue.

CpG Motifs and Genetic Vaccination

The immunization of mice with naked DNA plasmids encoding an antigen has been found to cause strong and persistent humoral, as well as cellular, immune responses [116]. Surprisingly, plasmid immunization has been shown to preferentially induce Th1-type responses [98]. The strength of immunization was dependent on nonantigen-coding regions of the plasmid. It turned out that these regions contained CpG motifs and critically influenced the efficacy of DNA vaccines [26, 27, 117]. However, the overall role of CpG motifs in DNA vaccines is still controversial. This may be due to conflicting results obtained with the coinjection of plasmids and CpG ODNs [118], and to the different amounts of plasmid used for immunization. It has been suggested that low amounts of plasmids (e.g. in gene gun experiments) may preferentially induce Th2 responses [119], while higher doses of DNA vaccines (e.g. after i.m. vaccination) may induce Th1 responses [98, 119]. DNA vaccines could therefore be seen as a two-component entity. The first component of the plasmic DNA codes for the antigen to be expressed, while the second component consists of CpG motifs within the plasmid. These CpG motifs activate APCs and thus deliver a 'danger' signal necessary to express the full complement of costimulatory molecules. Accordingly, injection protocols using higher amounts of plasmid DNA and thus delivering more CpG motifs might therefore preferentially induce Th1 responses. These rules might also apply to immunization with plasmid DNA encoding allergens. Initial reports have confirmed the Th1-inducing capacity of allergen vaccines and the principle feasibility of this approach [107, 120–123]. It should be mentioned, however, that some plasmids might still contain inhibitory DNA sequences and thus

might possess a reduced potency to induce Th1 responses. On the other hand, the construction of plasmids with inhibitory sequences might prevent immunization against the antigen expressed. This effect could be of importance in gene therapy, in which, after plasmid delivery, immunization is not desired.

Concluding Remarks

Although the biology of CpG DNA has been analyzed only recently, the information gained from murine models has been prodigious. This information may not only lead to new approaches for vaccine formulas and for directed immune modulation, but also contributes to our knowledge of host-parasite interactions and the development of diseases. Intriguing is the fact that bacterial and CpG DNA facilitate Th1-type immune reactions. Recent reports have indicated an inverse correlation between bacterial infections and the predisposition to develop allergic disorders [124, 125]. Accordingly, our Western lifestyle may have selected against severe bacterial infection, which in turn could contribute to the prevalence of Th2 diseases [126]. Although this concept has not been proved at all, some murine experiments support this hypothesis. Infection with mycobacteria has been shown to efficiently prevent asthmatic inflammation in mice [127, 128]. This raises the possibility that CpG DNA could be used to imitate bacterial infection in order to induce a Th1-promoting milieu [123]. Thus, unraveling the immunobiology of bacterial and CpG DNA has led to the discovery of new rules and mechanisms governing the interplay between innate and adaptive immune cells, with the promise of new therapeutic applications in vaccinology and allergy.

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