Synergy between CpG- or non-CpG DNA and specific antigen for B cell activation

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Abstract

DNA or oligodeoxynucleotides (ODN) containing unmethylated CpG motifs (CpG DNA) activate antigen-presenting cells and switch on T_h1 immunity to antigen. B cells are synergistically activated by CpG DNA in combination with non-physiologic B cell stimulators such as polyclonal mitogen and surface Ig cross-linkers. This study shows the unexpected finding that not only CpG ODN, but also non-CpG and methylated ODN synergize with specific antigen, hen egg lysozyme (HEL), in stimulating HEL-specific B cells to proliferate, to express the early activation marker CD69 and to activate the NF-κB pathway. In vivo, non-CpG and methylated CpG ODN also enhanced anti-HEL antibody production in HEL-immunized mice, with a bias towards the production of T_h1-associated isotypes. The synergy with all ODN to enhance B cell immune function was epitope-specific since neither denatured HEL nor other antigens enhanced the ODN effect on HEL-specific B cells. Furthermore, the synergy was independent of whether the ODN backbone was phosphorothioate or phosphodiester, or whether natural vertebrate genomic DNA was used. In all functional analyses, non-CpG and methylated CpG ODN showed lower activity than CpG ODN. These studies demonstrate that the presence of specific physiologic antigen might broaden the spectrum of DNA/ODN that stimulate B cells, with potential implications for the initiation and regulation of normal and pathologic immune responses.

Introduction

The immunostimulatory activity of DNA prepared from bacteria was first reported in 1984 (1). It was over a decade later that we reported the immunostimulatory elements as unmethylated CpG motifs in DNA or synthetic oligodeoxynucleotides (ODN) (2). Since then, CpG ODN have been studied intensively in different areas of immunology. Briefly, CpG ODN exert their immune effects mainly through modifying the function of antigen-presenting cells, including dendritic cells (DC), macrophages and B cells, and, as a consequence, activating innate immunity as well as adaptive antigen-specific cellular and humoral responses [reviewed in (3–5)]. CpG ODN also directly or indirectly act on other immune cells including mast cells (6), myocytes (7), neutrophils (8), and brain microglial cells and astrocytes (9). The immune responses triggered by CpG ODN can have positive or negative effects. On one hand, the immunostimulatory activity of CpG ODN has therapeutic applications in disease. For example, CpG ODN have been successful either as monotherapies or vaccine adjuvants in preventing or curing infectious diseases caused by Leishmania major (10,11), Francisella (12), Listeria monocytogenes (13), Trypanosoma Cruzi (14) or HIV (15–17). CpG ODN may also be useful in preventing or treating asthma (18–22). Accumulating evidence shows that CpG ODN can be used as therapeutics to induce specific immunity to subsequent tumor challenge (23–26) or to eliminate existing tumors in animals (1,27–30). In addition to affecting host immune system components, CpG ODN also modulate biological properties of tumor cells per se. For example, CpG ODN increase cytokine production by, and cytokine receptor or co-stimulatory molecules expression on, tumor cells, hence improving tumorigenicity of tumor cells and inducing stronger immune reactions against them (31–33). On the other hand, activation of immune cells caused by CpG DNA/ODN in unwanted places or at unwanted times and improper levels...
may lead to diseases such as arthritis (34–36) or shock (37). In DNA vaccines CpG motifs are necessary to achieve the desired immunization effect (38,39,40). CpG motifs also are present in gene therapy vectors, in which case the resulting immune system activation generally is unfavorable and can lead to decreased expression of the target gene (41–43) and/or unwanted and toxic immune activation [see review (44,45)].

We have reported that B cells are synergistically activated to proliferate and secrete Ig by the combination of CpG ODN and anti-IgM, but we did not observe synergy in this model with non-CpG ODN (2). However, Goeckeritz (46) reported using the Tris±NH₄Cl method. After adhering to flasks for 1 h at 37°C, non-adherent cells were collected and referred to as splenic lymphocytes. To purify Ig-Tg B cells, B cells expressing endogenous IgMβ as well as T cells were removed from Ig-Tg mice splenic lymphocytes using the MACS system (Miltenyi Biotec, Auburn, CA) as recommended by the manufacturer. Briefly, splenic lymphocytes were treated with a cocktail of 1 μg/ml biotinylated rat anti-mouse CD4, biotinylated rat anti-mouse CD8 and biotinylated rat anti-mouse IgMβ (PharMingen, San Diego, CA) at 4°C for 15 min and then incubated with MACS streptavidin-labeled microbeads at 6°C for 15 min. After running through the Type BS depletion columns, effluent cells were collected and washed. Resulting B cells were confirmed by FACS to be >95% pure by IgMβ staining.

Proliferation assay
Ig-Tg splenic cells were incubated in the presence of different concentrations of HEL and ODN for 40 h. Then 1 μCi [³H]thymidine (Amersham Pharmacia Biotech, Piscataway, NJ) in 50 μl medium was added to each well, and incubation continued for 8 h before harvesting cells on glass fiber filters and counting by a 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland).

Western blot
B cells were treated with ODN in the presence of HEL for 1 h at 37°C. Then cells were put on ice for 10 min and then washed twice with cold PBS buffer. Cells were suspended in cold lysis buffer for 5 min and then microcentrifuged at 6000 r.p.m. for 5 min. Supernatants were removed and mixed with protein loading buffer. The samples were separated on SDS–PAGE gels and transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) using a semi-dry transfer cell (Bio-Rad, Hercules, CA). Western blotting was performed following the procedure supplied by with the ECL Western blotting detec-

### Methods

#### Mice

Inbred C57BL/6 mice were obtained from the National Cancer Institute (Frederick, MD). Anti-HEL Ig transgenic (Ig-Tg) mouse line MD4, originally generated by Goodnow (47), was a generous gift from Dr F. Martin (UAB, Birmingham, AL) and maintained hemizygous on the B6 background. All mice were kept under specific pathogen-free conditions in the University of Iowa Animal Care Unit during the experiments following the institutional regulations on animal usage.

#### DNA and antigens

Two sets of ODN (Coley Pharmaceutical Group, Wellesley, MA) with similar base compositions were used (Table 1). The immunostimulatory potency of each ODN has been well defined in this laboratory and by others. *Escherichia coli* (EC) DNA and calf thymus (CT) DNA (Sigma, St Louis, MO) were extracted with phenol–chloroform at least 5 times to remove any residual endotoxin. All ODN and DNA used in cell culture were confirmed to be free of detectable endotoxin by Limulus assay. HEL and BSA (Sigma) were dissolved in PBS and filtered. Denatured HEL (dHEL) was prepared using the described protocol (48).

#### Cell preparation

HL-1 medium (BioWhittaker, Walkersville, MD) was used for cell preparation and culture in this study. Spleens were removed from mice and pressed through 70-μm nylon mesh to obtain single-cell suspensions. Red blood cells were lysed using the Tris–NH₄Cl method. After adhering to flasks for 1 h at 37°C, non-adherent cells were collected and referred to as splenic lymphocytes.

### Table 1. Sequences and backbones of ODN used in this study

<table>
<thead>
<tr>
<th>ODN no.</th>
<th>Sequence</th>
<th>Backbone</th>
<th>Classification</th>
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<tbody>
<tr>
<td>1826</td>
<td>TCCATGACGTTCTGACGTT</td>
<td>PS</td>
<td>CpG, immunostimulatory</td>
</tr>
<tr>
<td>1982</td>
<td>TCCAGAATTCCTTACAGTT</td>
<td>PS</td>
<td>non-CpG, not stimulatory</td>
</tr>
<tr>
<td>1911</td>
<td>TCCAGAATTCCTTACAGTT</td>
<td>PS</td>
<td>non-CpG, not stimulatory</td>
</tr>
<tr>
<td>1845</td>
<td>TCCATGAGTTCTTACAGTT</td>
<td>PS</td>
<td>mCpG, not stimulatory</td>
</tr>
<tr>
<td>2006</td>
<td>TCGTCGTTTTGTCGTTTTGTCGTT</td>
<td>PO</td>
<td>CpG, immunostimulatory</td>
</tr>
<tr>
<td>2059</td>
<td>TCGTCGTTTTGTCGTTTTGTCGTT</td>
<td>PO</td>
<td>CpG, immunostimulatory</td>
</tr>
<tr>
<td>2063</td>
<td>TCGTCGTTTTGTCGTTTTGTCGTT</td>
<td>PO</td>
<td>mCpG, not stimulatory</td>
</tr>
<tr>
<td>2077</td>
<td>GCTAGCTTATAGCGTTAGAGCTT</td>
<td>PO</td>
<td>non-CpG, not stimulatory</td>
</tr>
</tbody>
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Z = 5-methyl-C.

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tion reagent kit using rabbit anti-IkBα (Santa Cruz Biotechnology, Santa Cruz, CA) as primary antibodies and goat anti-rabbit IgG–horseradish peroxidase conjugate (Bio-Rad) as secondary antibody.

**Early activation antigen (CD69) assay**

After treatment with ODN and antigens for 1 h at 37°C, Ig-Tg splenic cells were stained on ice with anti-IgM–FITC and anti-CD69–phycoerythrin (PE; PharMingen), both at 1 μg/ml. After two washes, samples were run on FACScan using CellQuest software (Becton Dickson Immunocytometry System, San Jose, CA) and collected data were analyzed using FlowJo software (Tree Star, San Jose, CA). The FACScan was calibrated using beads as per the manufacturer's instructions. FSC versus SSC was used for gating on viable lymphocytes.

**Immunization of mice and antibody detection**

C57BL/6 mice were immunized (i.p.) with different doses of HEL in 100 μl PBS plus ODN 1826, ODN 1845 or ODN 1982. Three weeks later tail vein blood was collected and anti-HEL IgG in sera was measured by conventional ELISA. Briefly, Corning 96-well Easy-wash ELISA plates (Corning Costar, Corning, NY) were coated with 20 μg/ml HEL in 0.1 M NaHCO3 (pH 9.5) overnight at 4°C and blocked with 5% milk in PBS for 2 h at room temperature. Serial dilutions of serum were added to plates and the plates were incubated at 4°C overnight. Horseradish peroxidase-labeled goat anti-mouse IgG, anti-mouse IgG1 or anti-mouse IgG2a (Santa Cruz Biotechnology) at 1 μg/ml was used as secondary antibody for 1 h at room temperature. Between each step plates were washed 5 times using PBS/Tween buffer. Then 100 μl TMB liquid substrate (Sigma) was added to each well. After 10 min, 50 μl of 2 N H2SO4 was added to stop the reaction, and the plates were read at wavelengths of 450 and 570 nm. OD450/570 was used to calculate the titers of antibody.

**Results**

**Synergy of ODN with HEL to stimulate specific B cell proliferation**

We have previously reported that CpG DNA/ODN activates B cell proliferation in synergy with anti-IgM-mediated stimulation through the BCR (2). In order to determine whether this synergy would also exist in the activation of B cells induced by specific antigen, we performed **in vitro** assays with splenic cells from mice transgenic for anti-HEL Ig. We also used this B cell proliferation model to assay the ability of control non-CpG ODN 1982 to activate B cells in the presence or absence of HEL, for which the Ig-Tg B cells are specific. As shown in Fig. 1, while CpG ODN 1826 alone at concentrations of at least 0.3 μg/ml stimulated Ig-Tg B cells to proliferate, non-CpG ODN 1982 showed no notable stimulation for B cells even at 10 times this concentration. When 0.3 μg/ml of HEL was added to the culture, the threshold for ODN 1826 to cause B cell proliferation was lowered by at least a factor of 3 and the peak level of B cell proliferation was increased (Fig. 1A). HEL alone failed to induce substantial B cell proliferation. Surprisingly, a similar synergy was also observed between HEL and non-CpG ODN 1982, indicating that this synergy was not unique to ODN 1826 (data not shown).

**Synergy of ODN with HEL to induce CD69 expression and NF-κB activation**

B cell proliferation in response to HEL plus CpG or non-CpG ODN could result from the activation of shared or distinct signaling pathways. To investigate this mechanism, we studied the expression of the very early activation antigen CD69...
and NF-κB activation in IgM\(^{+}\) B cells in response to stimulation with ODN and HEL, and the percentage of CD69\(^{+}\) IgM\(^{+}\) cells and mean fluorescence intensity (MFI) were calculated. In the absence of HEL, 1 h of incubation with 3 or 0.3 mg/ml CpG ODN 1826, but not with the same concentrations of non-CpG ODN 1982, drove Ig-Tg B cells to express CD69 in a dose-dependent fashion (Fig. 2, bottom row). While HEL alone induced CD69 expression, there was no significant difference between 0.3 and 3 mg/ml HEL-treated cells as to the percentage of positive cells or MFI (Fig. 2, right column). CD69 expression induced by HEL could be further up-regulated either by addition of CpG ODN 1826 or by the addition of the non-CpG ODN 1982 (Fig. 2), the non-CpG ODN 1911 or the mCpG ODN 1845 (data not shown). Increasing HEL concentration from 0.3 to 3 μg/ml could further increase CD69 expression in ODN 1982-treated, but not in ODN 1826-treated, B cells. This may reflect the saturation of CD69 expression in CpG ODN 1826 plus HEL-treated B cells and also showed that ODN 1982 was weaker than ODN 1826 in stimulating cell activation in synergy with HEL.

Previous studies have demonstrated that either antigen (49) or CpG ODN (50) can activate the NF-κB signal transduction pathway in B cells, but non-CpG ODN do not. When added alone to culture at 3 μg/ml, either HEL or CpG ODN 1826, but not non-CpG ODN 1982, led to NF-κB activation in Ig-Mg B cells as indicated by the degradation of IκBα. When HEL and ODN 1982 were present together, however, B cell IκBα degradation was substantially higher than that induced by HEL alone, and reached approximately the same level as seen in B cells cultured with ODN 1826 plus HEL (Fig. 3). These studies again demonstrate the synergy between HEL and ODN 1982 to activate B cells, and that this synergy involves the NF-κB pathway.

**Backbone independence of synergy between ODN and HEL**

Phosphorothioate (PS) CpG ODN are reportedly ~200 times as potent at activating B cells compared to native phosphodiester (PO) CpG DNA (2). To look into whether the PS backbone is responsible for the synergy between non-CpG ODN and HEL, we tested the possibility of synergy between HEL and DNA/ODN with a PO backbone, such as genomic DNA prepared from cells or another set of ODN having a PO backbone. At 1 μg/ml, only ODN 2006 with the PS backbone and EC DNA with the PO backbone, such as genomic DNA prepared from cells or another set of ODN having a PO backbone, showed stimulatory activity as single agents (bottom row in Fig. 4). However, all of these DNA or ODN showed different potencies to synergize with HEL to induce CD69 expression by B cells, with the CpG motif-containing ODN 2006 and EC DNA being strongest, while non-CpG ODN 2077 and CT DNA were weakest among all those with the PO backbone (Fig. 4).
Antigen specificity of ODN synergy

To check whether only specific antigen or any protein can synergize to lower the threshold of ODN to activate B cells, we substituted native HEL with BSA or dHEL at the same concentration in the 1-h culture and measured CD69 expression on IgMa B cells. We found that neither BSA nor dHEL affected the response of Ig-Tg B cells to ODN (data not shown). The anti-HEL Ig transgene in this system was derived from the clone HyHEL10 and the product of this gene (surface IgMa on B cells) is specific for a discontinuous epitope distributed in four different regions of the linear sequence of HEL (47). This epitope was disrupted in the denaturing treatment. The inability of dHEL to synergize with non-CpG ODN demonstrated that the synergy with ODN is not only antigen-specific, but also epitope-specific.

Synergy of ODN with HEL to induce antibody production in vivo

To further detect the potential influence of non-CpG ODN on the intact immune system, the adjuvant effects of ODN 1826, ODN 1845 and ODN 1982 were measured in C57BL/6 mice. We observed that both CpG ODN 1826 and mCpG ODN 1845 enhanced antibody production in a dose-dependent manner, while ODN 1982 did not show a clear dose-dependent effect. We attributed this to the saturation of the ODN 1982 effect. At the same dosage, ODN 1845 and ODN 1982 were always weaker than ODN 1826 in promoting antibody production (Fig. 5), indicating that non-CpG ODN are less potent than CpG ODN in synergizing with antigen to induce antibody production.

The CpG motif is noted for its preferential effects on enhancing T h1 responses. To assess the similarity to non-CpG ODN, we compared the effect of CpG ODN and non-CpG ODN on the T h1/Th2 balance as indicated by antibody isotype in immunized mice. Figure 6 shows that HEL alone induced antibody production that is predominantly the Th2-type antibody such as IgG1 (IgG2a:IgG1 = 0.003). Addition of ODN 1826 greatly increased the T h1-type antibody (IgG2a) percentage (IgG2a:IgG1 = 0.088). ODN 1982 also increased the IgG2a:IgG1 ratio (to 0.037) significantly, although not to the same degree as the CpG ODN. Thus, the synergy between non-CpG ODN and antigen drives a relatively Th1-type antibody response.

Discussion

In the studies reported in this manuscript, we investigated the interactions between the signaling pathways induced by physiologic specific antigen, by CpG, non-CpG and mCpG ODN, and by unmethylated and predominantly methylated genomic DNAs. Synergy between CpG DNA with other immunomodulators such as BCR ligands, mitogens, etc., has been known since the discovery of the CpG motif (2) [reviewed in (51)], but non-CpG ODN or mCpG ODN or...
highest dilution factor that gave an OD 450/570 reading that was 2-fold determined using ELISA as described in Methods. The titer is the of IgG1 and IgG2a classes at day 21 after immunization were m or with 100 

Fig. 6. C57BL/6 mice were immunized (i.p.) with 150 µg HEL alone or with 100 µg of the indicated ODN in PBS. Anti-HEL antibodies of IgG1 and IgG2a classes at day 21 after immunization were determined using ELISA as described in Methods. The titer is the highest dilution factor that gave an OD_450/570 reading that was 2-fold that of blank wells. No specific anti-HEL antibody could be detected in the sera from naive mice. This experiment was repeated twice with similar results.

genomic DNAs are usually used as negative controls for CpG ODN and are thought to have little or no activity at low concentrations. Unexpectedly, in the present studies we found that non-CpG ODN and vertebrate genomic DNA synergize with physiologic antigen to activate specific B lymphocytes.

Although the concept of the immunostimulatory CpG motif has become widely accepted, we have observed that this is not an ‘all or none’ phenomenon. Not all DNA/ODN containing CpG motifs are equally stimulatory; neither are all DNA/ODN without CpG motifs equally non-stimulatory. While some non-CpG DNA never show any stimulatory activity in model systems, some others manifest significant stimulatory ability when used at high, but not at lower doses (A. Krieg, unpublished data). Non-CpG effects have been especially prominent in experiments using ODN with a nuclease-resistant PS backbone and are much less pronounced with native PO ODN. DNA is a polyanion and has been reported to have a powerful multivalent cross-linking signal, to activate polyclonal B cell proliferation (52,53). Goeckeritz et al. reported that mCpG and non-CpG ODN can synergize with dextran-conjugated anti-IgD, a non-physiologic and extremely powerful multivalent cross-linking signal, to activate polyclonal B cell proliferation (46). Davis et al. reported in a mucosal vaccine system that non-CpG ODN showed a T_{H2}-biased immunostimulatory effect which can be shifted to the T_{H1} direction by CpG motifs (54). However, to our knowledge, the present studies are the first to demonstrate a direct synergy at the level of the isolated B cell between a physiologic BCR ligand and non-CpG DNA, and thus extend those prior studies.

A problem in past studies of the interaction between the BCR and CpG DNA has been the use of polyclonal B cell populations, which require non-physiologic stimuli for their activation, or B cell lines, which may not mimic the responses of primary B cells. In the present studies, we used the Ig-Tg mouse model which gives a highly enriched B cell population that is specific to a single epitope of HEL. This made it possible for us to directly examine the effect of CpG ODN or DNA on the response of a primary B cell to its physiologic soluble native antigen. Our data demonstrate that DNA or ODN without regular CpG motifs have immunostimulatory priming effects on normal B cells, but that these are too weak or incomplete to be detected by conventional assays. The ability of non-CpG DNA/ODN to synergize with antigen made it possible for us to show the stimulatory activity of these ODN, first in a transgenic B cell model and then in normal mice. This report is consistent with, but extends, our previous work in several aspects. First, specific antigen, or the exact B cell epitope, is needed to help mCpG and non-CpG to achieve detectable stimulatory activity at ODN concentrations where CpG DNA alone shows notable activity. Second, when the same level of specific antigen is present, the concentration of mCpG or non-CpG ODN needed to show effective synergy with antigen is higher than that of CpG ODN and, unless B cell activation is saturated, non-CpG or mCpG DNA/ODN is always weaker than CpG ODN.

Our studies were limited in scope to murine cells and it remains unclear whether the same effects would be observed with human cells or in humans. In fact, there are substantial differences between the biologic effects of CpG ODN injection into humans or monkeys and those observed in mice. First, mice injected s.c. with CpG have marked increases in serum tumor necrosis factor-α, IL-12 and IFN-γ (4), but humans and non-human primates showed no detectable change in these parameters until very high doses (Krieg et al., unpublished data). The same differences in cytokine responses are seen in vitro, despite a strong response of human and NHP blood cells to endotoxins. This may be due to a species-specific difference in the cells expressing the Toll-like receptor (TLR) 9 receptor. In humans, TLR9 expression appears to be restricted to B cells and CD123+ DC (‘plasmacytoid DC’), but in mice, TLR9 is also expressed in monocytes and macrophages (4). Humans injected with a CpG ODN that is optimized for interacting with the human TLR9 molecule s.c. do show high serum levels of certain T_{H1}-like chemokines, despite their lack of the ‘classic’ T_{H1} cytokine response that is seen in mice. Further studies will be required to determine whether human B cells will show the same pattern of non-specific co-stimulation with antigen that we have now described in mouse B cells.

Based on the fact that CpG motifs are more common in bacterial genomes than in those of vertebrates, we suggested that CpG motifs in bacteria might act as a ‘danger signal’ for mammalian immune systems (4). In spite of the fact in this report that non-CpG and mCpG ODN, which are abundant in mammalian genome DNA, also manifest immunostimulatory effects under certain conditions, the immune system of animals like humans should be able to distinguish bacterial DNA from self DNA, because only the former has direct immune B cell stimulatory activity in the absence of a BCR signal and only the former activates DC directly. If the immune system were exposed to large amounts of self DNA as a result of tissue damage and cell death secondary to infection or trauma, then it is possible that the self DNA would act as an adjuvant for enhancing immune responses to foreign antigens.
and further help to protect the host. It seems unlikely that self DNA would overcome self tolerance and act as an adjuvant for self antigens, inducing an autoimmune response, but further studies would be required to formally exclude this possibility. DC and macrophages have the ability to engulf apoptotic or dead cells, become activated and present the antigens to T cells efficiently [reviewed in (55)]. Recently, Ishii et al. reported that genomic DNA released from dying cells induces maturation of DC and proposed that double-stranded genomic DNA may also serve as a danger signal to the immune system (56). However, these effects required transfection of the DNA into the DC and thus are of unclear relevance to our experimental system, in which transfection or lipofection were not required. At present it is unclear whether self-DNA that is not transfected would modulate antigen presentation by DC in a synergistic manner when these APC encounter dead cells.

TLR9 has been reported to act as the receptor for CpG DNA/ODN (57,58), although it has not yet been shown to bind the DNA directly. TLR9 does not appear to transduce any stimulatory signal in response to non-CpG DNA, at least when administered as a single agent. It remains unclear whether perhaps there is some weak interaction of TLR9 with non-CpG or mCpG DNA, leading to the transduction of a qualitatively or quantitatively distinct signal, or whether some other pathway may mediate the effects we have observed. The present data indicate that regardless of whether a particular DNA molecule has a CpG motif or not, it delivers a signal to B cells that interacts with that derived from the BCR. Thus when the BCR signal is absent, DNA containing CpG motif(s) induces a signal that is sufficient to cause significant cell activation, while mCpG or non-CpG DNA fail to do so. When the non-CpG or mCpG motif and specific antigen act simultaneously on these cells, they synergize and the B cells are activated.

In summary, we showed that although DNA or ODN that do not contain CpG motifs may not be stimulatory alone, they synergize with specific antigen to activate efficiently B lymphocyte proliferation, expression of early activation molecules and the NF-κB pathway. This synergy does not depend on the type of DNA backbone, but is strictly epitope-specific. The detailed molecular mechanisms for this synergy are under investigation, as well as the possible roles in normal health and disease.

Note in proof

While this manuscript was in revision, Leadbetter et al. reported that the signals initiated through the TLR9 pathway and surface IgM engagement synergize to activate autoimmune B cells (59). In that report, autologous chromatin and self-IgG appeared to act as non-CpG DNA and antigen respectively to stimulate rheumatoid factor-specific B cells. While our work utilized synthetic CpG, non-CpG or mCpG ODN as sources of DNA, our findings show similar response patterns of B cells to combinations of specific antigen and DNA. Taken together, these observations imply a possible significance of non-CpG or CpG DNA in the pathogenesis of autoimmune diseases regardless of the original source of DNA.

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Abbreviations

CpG DNA DNA containing CpG motifs
CT calf thymus
DC dendritic cell
dHEL denatured hen egg lysozyme
EC Escherichia coli
HEL hen egg lysozyme
Ig-Tg Ig transgenic
mCpG methylated CpG
MFI mean fluorescence intensity
ODN oligodeoxynucleotide
PS phosphorothioate
PO phosphodiester
TLR9 Toll-like receptor 9

References


