Priority Report

Cancer Research

Therapeutic Administration of a Synthetic CpG Oligodeoxynucleotide Triggers Formation of Anti-CpG Antibodies

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Abstract

The synthetic oligodeoxynucleotide CpG 7909, which contains unmethylated cytosine/guanine (CpG) motifs, has potent immunostimulatory effects when coadministered with NY-ESO-1 peptides or recombinant NY-ESO-1 protein, resulting in an enhanced cellular and humoral immune response against the vaccine antigen. In this study, we report the development of anti-CpG-ODN antibodies in 21 of 37 patients who received CpG 7909 either alone or as a vaccine adjuvant. Specific anti-CpG immunoglobulin G (IgG) antibody titers ranged from 1:400 to 1:100,000. The anti-CpG antibodies cross-reacted with other synthetic CpG-ODNs but not with the DNA of mixed bacterial vaccine and were shown to be phosphorothioate backbone specific. Vaccine-related severe side effects observed in some patients were most likely not related to the development of anti-CpG antibodies. In addition, anti-CpG antibodies did not have negative effects on the vaccine immune response. These results show that anti-CpG antibodies develop in humans against short unmethylated CpG dinucleotide sequences after administration of CpG 7909. Our data therefore substantiate the potency of CpG 7909 to directly stimulate human B-cells and suggest that anti-CpG antibody monitoring should be a part of ongoing and planned clinical trials with CpG-ODNs. *Cancer Res; 72(17); 4304–10. ©2012 AACR.*

Introduction

CpG motifs are present in bacterial DNA and many viruses (1). Synthetic oligodeoxynucleotides containing unmethylated CpG motifs can mimic the immunostimulatory effects of bacterial DNA and are recognized by Toll-like receptor 9 (TLR9) expressed in different cell types of the innate immune system (2, 3). Following activation through CpG-ODNs, TLR9 in plasmocytoid dendritic cells and B-cells initiates the secretion of cytokines and the upregulation of costimulatory molecules, which in turn promotes T-helper (T_H)-1–like adaptive immune responses (4). On the basis of their phosphate backbones (phosphodiester or phosphorothioate), location and number of CpG dinucleotides, palindromic sequence, and their effect on human peripheral blood mononuclear cells, CpG-ODNs have been classified into A-, B-, and C-class oligodeoxynucleotides. A-class CpG-ODNs have been found to induce high levels of IFN-α and NK cell activation. B-class CpG-ODNs principally act on B-cell and monocyte activation. They also stimulate the maturation of pDC and IFN-α production but to a lesser extent than A-class oligodeoxynucleotides. C-class oligodeoxynucleotides combine the stimulatory properties of A- and B-type oligodeoxynucleotides (5, 6). Because the natural phosphodiester backbone of native DNA is rapidly digested by serum and cellular nucleases, CpG-ODNs generally use the nuclease-resistant phosphorothioate–modified backbone in which the nonbridging oxygen atoms in the phosphodiester bond have been replaced by sulfur atoms. In recent years, various chemically modified forms of CpG-ODNs have been tested in clinical trials for the treatment of infectious disease, allergies, and cancer (7). The most widely used CpG 7909, a class B oligodeoxynucleotide with the sequence 5′-TCG TCG TTT TGT CGT TTT GTC GTT-3 and a full phosphorothioate backbone, contains four CpG dinucleotides and three times the hexanucleotide sequence 5′-GTCGTT-3′, motifs that are known to be optimal to stimulate human immune cells. Our group and others have shown that CpG 7909, when used as an adjuvant for NY-ESO-1-specific cancer vaccines, has the potency to increase the immunogenicity of peptide- and protein-based vaccines by the induction of antigen-specific T cells and antibodies (8–12). CpG 7909 seems to be generally well tolerated with adverse events including flu-like symptoms and local injection site reactions. However, several safety concerns are raised by the use of CpG-ODNs as a vaccine adjuvant. These include the development of autoimmune disease or the overproduction of cytokines such as TNF-α that can cause life-threatening toxic shock (7). Recently, it has been shown that severe side effects in CpG-ODN–treated mice may be attributed to the phosphorothioate-

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-12-0257

modified backbone that induced anti-CpG-ODN IgM antibodies in a CG-independent manner (13). In the present report, we describe for the first time the occurrence of human anti-CpG immunoglobulin G (IgG) antibodies after treatment with CpG 7909. Anti-CpG antibodies did not appear to be directly related to severe side effects but rather provide important information on the intensity of the immune response triggered by CpG-ODNs.

Materials and Methods

Oligodeoxynucleotides

CpG 7909 was synthesized and provided by Coley Pharmaceutical Group. All other oligodeoxynucleotides were purchased from Invivogen. Oligodeoxynucleotides were resuspended in sterile endotoxin-free water and stored at −20°C.

Measurement of antibody responses by ELISA

CpG-specific antibodies were measured in the serum by standard ELISA assays as previously described (12). Briefly, sera were added to 96-well plates (Nunc Maxisorb) coated with 5 μg/mL (50 μL/well) of oligodeoxynucleotides overnight at 4°C and blocked with 2% bovine serum albumin/PBS. After incubation, plates were washed with PBS and specific antibodies were detected with alkaline phosphatase–conjuncted anti-human IgG (Sigma A9544). Following addition of *p*-nitrophenylphosphate substrate and 3 N NaOH stopping solution, absorbance was measured at 405 nm using an Anthos Laptec Instruments fluorescence reader. Sera were assessed over a range of dilutions from 1:100 to 1:400,000. In addition, sera were measured for serologic responses against the vaccine antigens NY-ESO-1 and MAGE-A3 in standard ELISA at a coating concentration of 2 μg/mL protein. Anti-CpG IgG subtypes were determined by ELISA using biotinylated isotype-specific secondary antibodies B6775, B3998, B3523, and B3648 from Sigma.

Circulating immune complexes

Analysis of circulating immune complexes (CIC) was conducted using standard enzyme immunoassays CIC-C1q and CIC-C3d obtained from Quidel. Serum CICs were measured according to the manufacturers' instructions. Results were graded as positive or negative according to the controls provided with the kit.

Results

Patients

Thirty-seven patients were analyzed for anti-CpG antibodies. Thirty-two of them received CpG 7909 as an adjuvant in combination with NY-ESO-1 peptide and/or NY-ESO-1 protein vaccination. Of these 32 patients, 25 were treated within clinical trials LUD02-007 and LUD03-024 (11, 12) and 7 patients received NY-ESO-1 protein/CpG vaccination according to clinical trial LUD03-024 on single patient protocols. One patient (#34) received MAGE-A3 protein/CpG vaccination as an individual compassionate therapy. Four patients received CpG alone. Three of them (#35, #36, and #37) according to a Coley-sponsored protocol (CO23) and 1 patient (#33) on a compassionate use basis.

Frequency, strength, and duration of anti-CpG 7909 antibodies

Twenty-one of 37 patients developed IgG antibody responses against CpG 7909. Anti-CpG antibodies were predominantly of IgG1 subtype (Supplementary Fig. S1). Antibody titers were variable among the patients. Maximum antibody titer had reached 1:400 to 1:10,000 in 14 patients, 1:10,000 to 1:20,000 in 2 patients, and 1:25,000 to 1:100,000 in 5 patients. In the CpG/peptides group (1 mg CpG s.c.), anti-CpG antibodies developed in 5 of 11 patients (45%). The number of vaccines did not correlate with the level of anti-CpG titer, for example, patient #5 received 37 doses of CpG and maximum anti-CpG titer was 1:3,200, whereas patient #11 received 8 doses of CpG and maximum anti-CpG titer was 1:100,000. In the CpG/protein group (2.5 mg CpG intradermal), anti-CpG antibodies developed in 14 of 23 patients (61%). Also in this group the number of vaccines did not correlate with anti-CpG levels. In the CpG monotherapy group (10–40 mg CpG), anti-CpG antibodies developed in 3 of 4 patients. As summarized in Table 1, there was no significant correlation between dose, application, and vaccine combination with the development of anti-CpG antibodies. Anti-CpG antibodies decreased in all patients without further administration of CpG-ODN but still remained detectable without further vaccination for up to 2 years in patients #5 and #14. Figure 1 shows the course of anti-CpG 7909 antibody in relation to the antibody response against the vaccine antigen in 5 patients. In patients who developed both, anti-CpG and anti-NY-ESO-1/anti-MAGE-A3 antibodies, anti-CpG antibodies generally developed later and not as high in titer.

Severe adverse events and anti-CpG antibodies

After vaccination, all 37 patients developed local erythema and pruritus for 5 to 7 days at the application site. Severe adverse events occurred in 7 of 37 patients (Table 2). All 7 had developed anti-CpG antibodies of different titers ranging from 1:1,600 to 1:100,000. Patients without anti-CpG antibodies had no severe clinical event. Severe events occurred between 20 and 637 days after administration of CpG and between 28 and 553 days after anti-CpG antibody was detected. In patients #36 and #37, anti-CpG measurement could be carried out only before the treatment and 41 and 35 days after the event, respectively. These two time points did not allow for a reliable time frame evaluation of event to CpG antibody. One patient (#11) had no event at all during treatment with NY-ESO-1 peptide/CpG, whereas anti-CpG titers had developed and reached a maximum 1:100,000. The same patient was further vaccinated in a different study with recombinant NY-ESO-1 protein/CpG and had a severe event 637 days after CpG start and 553 days after anti-GpG antibody was measured, whereas anti-CpG titer remained unchanged at 1:100,000. There is no evidence that the anti-CpG antibody itself is actively involved in the occurrence of severe adverse events although severe adverse events were observed more frequently in patients who received CpG in combination with NY-ESO-1 or MAGE-A3 protein (4 of 23) and in patients who received CpG alone (3 of 4), as compared with patients who received CpG in combination with NY-ESO-1 peptide vaccination (0 of 11). Patients who developed

Table 1. Summary of antibody responses in CpG 7909–treated patients

Patient no.	CpG dose, mg	Application	Vaccine combination (NY-ESO-1)	CpG Ab pre	Max. CpG Ab titer	NY-ESO-1 pre	Ab titer post	Severe events
1	2 × 1	Subcutaneous	Peptide p157–165	−	−	+ + +	+ + +	No
2	3 × 1	Subcutaneous	Peptide p157–165	−	−	−	−	No
3	8 × 1	Subcutaneous	Peptide p157–165	−	−	−	−	No
4	8 × 1	Subcutaneous	Peptide p157–165	−	+	+	+ +	No
5	37 × 1	Subcutaneous	Peptide p157–165	−	+	−	−	No
6	4 × 1	Subcutaneous	Peptide p157–165	−	+	−	−	No
7	3 × 1	Subcutaneous	Peptide p157–165	−	−	+ + +	+ + +	No
8	4 × 1	Subcutaneous	Peptide p157–165	−	−	−	−	No
9	8 × 1	Subcutaneous	Peptide p157–165	−	+ +	−	−	No
10	4 × 1	Subcutaneous	Peptide p157–165	−	−	−	+ +	No
11	8 × 1	Subcutaneous	Peptide p157–165	−	+ + +	−	−	No
11	10 × 2.5	Intradermal	Rec. protein	−	+ + +	−	+ + +	Yes
12	8 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
13	2 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
14	14 × 2.5	Intradermal	Rec. protein	−	+ + +	−	+ + +	No
15	5 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
16	8 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
17	20 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
18	4 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
19	7 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	Yes
20	4 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
21	6 × 2.5	Intradermal	Rec. protein	−	+	+ +	+ + +	No
22	6 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
23	6 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
24	4 × 2.5	Intradermal	Rec. protein	−	−	−	+	No
25	8 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
26	4 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
27	4 × 2.5	Intradermal	Rec. protein	−	−	−	+ + +	No
28	2 × 2.5	Intradermal	Rec. protein	−	+	+ +	+ +	No
29	4 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
30	3 × 2.5	Intradermal	Rec. protein	−	+	+	+ + +	No
31	4 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	No
32	8 × 2.5	Intradermal	Rec. protein	−	+	−	+ + +	Yes
33	5 × 2.5	Intralesional	Rec. protein[a]	−	+ + +	−[b]	+ + +[b]	Yes
34	6 × up to 10	Intralesional	CpG only	−	+ +	−	−	Yes
35	8 × 10	Subcutaneous	CpG only	−	−	−	−	No
36	2 × 20 + 2 × 40	Subcutaneous	CpG only	−	+ + +	−	−	Yes
37	4 × 20	Subcutaneous	CpG + DTIC	−	+ + +	−	−	Yes

NOTE: Antibody reciprocal titer: +, <10,000; ++, 10,000–25,000; + + +, >25,000.
[a]Vaccine combination: MAGE-A3 protein.
[b]MAGE-A3 antibody titer.

anti-CpG antibodies and who had severe events were also tested for the presence of antinuclear antibodies that are indicative for autoimmune disease. None of the patients showed elevated levels of antinuclear antibodies. The same sera were also analyzed for increased levels of IgE. Patient #11 had a moderately elevated serum IgE at baseline that decreased from 250 to 72 IU/mL during the treatment with CpG 7909 while anti-CpG antibodies developed. All other patients did not show elevated levels of IgE (data not shown). A subset of 10 patients, including those who had severe events, was further analyzed for the presence of CICs to investigate potential negative immunomodulatory effects of the antibody. As shown in Supplementary Figure S2, CIC levels were within the normal range at any time point and did not change significantly over time.

Specificity of anti-CpG antibodies

We tested anti-CpG 7909 sera against a range of various other CpG motifs that have been modified in backbone and base sequence. As shown in Fig. 2, anti-CpG 7909 antibodies cross-react with other CpG-ODNs in a CG sequence-independent, phosphorothioate backbone–specific manner.

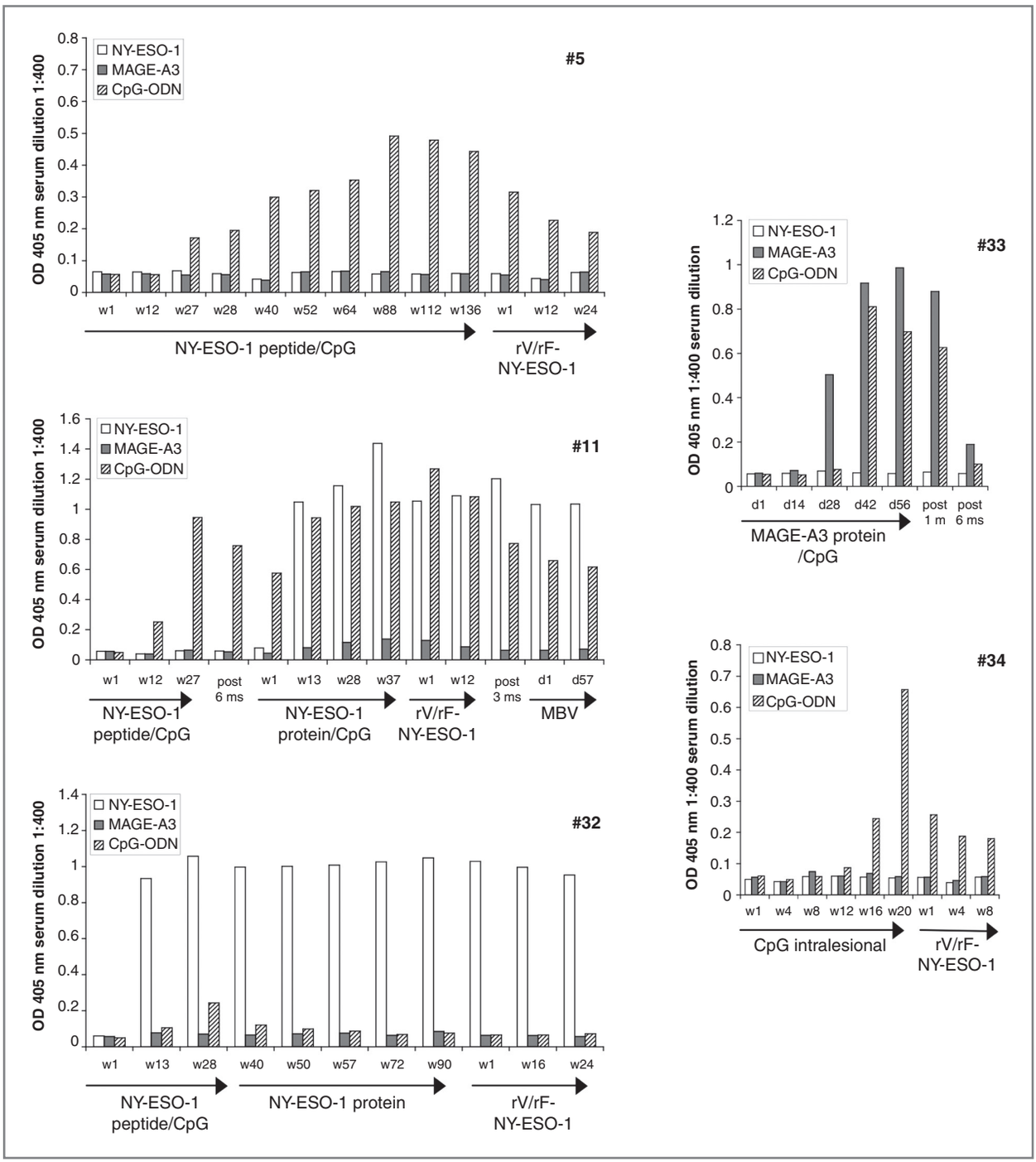


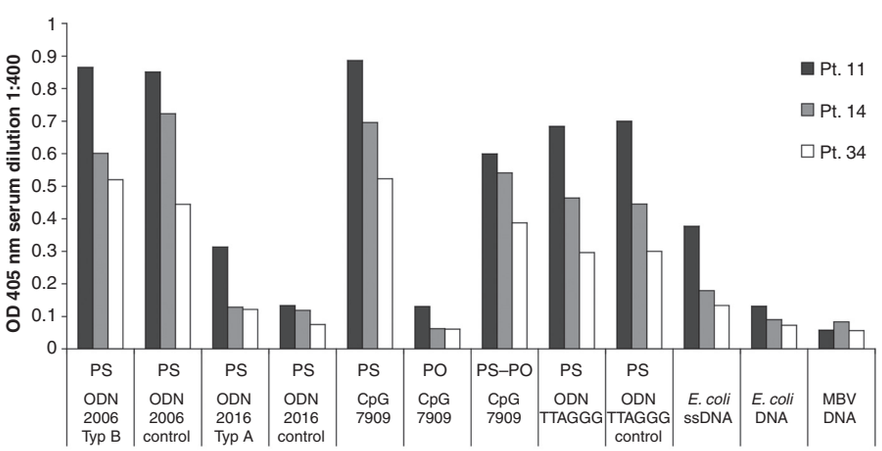
Figure 1. Development and course of anti-CpG antibodies. Patients' received CpG in various vaccine combinations. ELISA antibody results are shown for anti-CpG7909, anti-NY-ESO-1, and anti-MAGE-A3. Patient #5 had developed anti-CpG antibodies at week 27. Antibody titer increased continuously with further CpG administration and decreased after the end of NY-ESO-1 peptide/CpG vaccination. Patient #11 had developed high titer anti-CpG antibodies during vaccination with NY-ESO-1 peptide p157–165. In a different study with CpG and recombinant NY-ESO-1 protein, the patient had a severe event while anti-CpG titer remained unchanged. Anti-CpG titer decreased during subsequent immunotherapies with rV/rF NY-ESO-1 and mixed bacterial vaccine (MBV). Patient #32 developed anti-CpG antibodies during vaccination with NY-ESO-1 protein. On study week (w) 28, an adverse event occurred. Vaccination was continued with NY-ESO-1 protein only. No further severe event occurred and anti-CpG antibody decreased to baseline level. Antibodies against the NY-ESO-1 protein vaccine developed before anti-CpG antibodies became detectable. Patient #33 developed both, anti-CpG antibodies and anti-MAGE-A3 antibodies. Anti-CpG antibodies occurred after the 4th CpG administration and 4 weeks later than anti-MAGE-A3 antibodies. Patient #34 received increasing doses of intratumoral CpG. Anti-CpG antibodies became detectable after the 4th CpG administration and rapidly increased up to the 6th CpG administration. Vaccination was stopped because of dyspnea.

Table 2. Severe events possibly related to the administration of CpG

Patient	Dose of CpG, mg	Application	CpG/vaccine combination	No. of vaccines to event	CpG start to event, d	Event to CpG Ab, d	Severe event	Max. CpG titer
11	8 × 1	Subcutaneous	Peptide	no event	no event	no event	None	100,000
11	10 × 2.5	Intradermal	Rec. protein	10	637	553	Acute chest pain, nausea, skin marbling	100,000
19	7 × 2,5	Intradermal	Rec. protein	4; stop after 7	107	44	Hypersensitivity, intermittent allergic rush	6,400
32	8 × 2.5	Intradermal	Rec. protein	8	189	105	Dyspnea, flush	1,600
33	5 × 2.5	Intralesional	Rec. protein	5	54	28	Dyspnea	25,600
34	Increasing up to 10	Intralesional	CpG only	6	168	101	Dyspnea, flush	12,800
36	2 × 20 + 2 × 40	Intralesional	CpG only	4	20	n.e.	Tachycardia	51,200
37	4 × 20	Intralesional	CpG + DTIC	4	23	n.e.	Generalized urticaria	25,600

Abbreviation: n.e., not evaluable.

Oligodeoxynucleotide 2006, identical to CpG 7909, and the control oligodeoxynucleotide that contains GpC dinucleotides instead of CpGs were both recognized at the same level by anti-CpG 7909 sera. Oligodeoxynucleotide 2216, a type A human TLR9 ligand with a phosphodiester central CpG-containing palindromic motif and a PS 3′poly-G string, was generally not recognized. CpG 7909 oligodeoxynucleotide sequence that was only partially modified (some oxygen atoms replaced by sulfur atoms) or not modified at all was recognized less strongly or not at all, respectively. Inhibitory oligodeoxynucleotide sequence containing 4 times the phosphorothioate base sequence TTAGGG was also recognized. No cross-reactivity was found against *Escherichia coli* K12 ssDNA or mixed bacterial vaccine (Coley's toxin) DNA that contain natural phosphodiester backbone sequences. These results show that unmethylated phosphorothioate CpG 7909 oligodeoxynucleotide leads to the induction of phosphorothioate backbone-specific antibodies.



Oligodeoxynucleotide	Nucleotide Sequence	Modification
ODN 2006 Typ B	5`- tcg tcg ttt tgt cgt ttt gtc gtt - 3	PS
ODN 2006 control	5`- tgc tgc ttt tgt gct ttt gtg ctt - 3	PS
ODN 2216 Typ A	5´- ggG GGA CGA TCG TCg ggg gg - 3	PS-PO
ODN 2216 control	5`- ggG GGA GCA TGC TGg ggg gc - 3	PS-PO
CpG 7909	5`- tcg tcg ttt tgt cgt ttt gtc gtt-3	PS
CpG 7909	5`- TCG TCG TTT TGT CGT TTT GTC GTT-3	PO
CpG 7909	5`- tcG TCg TTT tgt CGT TTT GTC Gtt-3	PS-PO
ODN TTAGGG	5`- ttt agg gtt agg gtt agg gtt agg g-3´	PS
ODN TTAGGG control	5`- gct aga tgt tag cgt-3`	PS

Figure 2. PS backbone-specific cross-reactivity of anti-CpG 7909 antibodies. Anti-CpG 7909 antibodies of patients #11, #14, and #34 react with other phosphorothioate backbone–modified CpG or control oligodeoxynucleotides. CpG-ODNs with the natural phosphodiester backbone or with a partially modified (phosphorothioate–phosphodiester) backbone were not or less strongly recognized, respectively. Nucleotide Sequence, bases shown in lowercase letters are phosphorothioate; those in capital letters are phosphodiester.

Discussion

In the treatment of cancer, various CpG-ODNs have been used in clinical trials to evaluate their potential immunostimulatory properties linking innate and adaptive immune responses (8, 6, 14). We and other groups have shown that CpG 7909 when coadministered with Melan-A- or NY-ESO-1–specific peptide- or protein–based cancer vaccines can enhance both, antigen-specific humoral and cellular immune responses against the vaccine (9–12). However, as CpG-ODNs can also activate suppressive pathways of the immune system, for example, interleukin-10 production and induction of Treg cells (15, 16), chemical modifications of CpG-ODNs are currently being investigated to identify the most effective CpG-ODN for the modulation of different properties of the immune response.

A major focus in the development of more effective cancer vaccines is put on the monitoring of the vaccine-induced immune responses that may allow to determine the impact of immunization on clinical outcomes (17). In this regard, we have investigated patients' immune responses not only against the vaccine antigen but also against the adjuvant and, as a result, identified anti-CpG antibodies in a high proportion of patients. The development of an immune response against the adjuvant itself has been previously described, for example, against keyhole limpet hemocyanin (KLH), and anti-KLH antibodies have generally indicated patients primary *in vivo* antibody responsiveness (18). However, this is the first time that anti-CpG antibodies have been described against short unmethylated CpG dinucleotide sequences in humans. The CpG-directed antibodies did not seem to have neutralizing effects against the vaccine since all patients in our NY-ESO-1 protein trial developed high titer antibody responses against NY-ESO-1 and, in addition, generated NY-ESO-1–specific T-cell responses in the presence of CpG-directed antibodies. The development of anti-CpG antibodies was not accompanied by pathologic CIC levels, increased serum IgE levels, or the occurrence of antinuclear and anti-dsDNA antibodies and thus did not appear to be directly related to immune dysfunctions such as allergy or autoimmune reactions. Therefore, in our patients, anti-CpG antibodies did not seem to be actively involved in the occurrence of severe side effects.

Anti-CpG antibodies were shown to be oligodeoxynucleotide phosphorothioate backbone specific, as they did not cross-react with the unmodified phosphodiester form of CpG 7909. The phosphorothioate backbone modification of CpG-ODNs has raised a number of critical questions. Although it displays higher immunostimulatory effects than partially phosphorothioate-modified or the natural phosphodiester form, phosphorothioate oligodeoxynucleotides are most likely to be responsible for the generation of nonsequence specific toxicity. In primates, administration of high doses of phosphorothioate oligodeoxynucleotides resulted in significant and acute toxicity due to transient complement activation (19).

The biological role and clinical relevance of anti-CpG antibodies is not clear yet and needs to be further investigated. All patients with severe side effects to the vaccine had measurable anti-CpG antibodies and antibodies became detectable already before or after the clinical event was observed. In patients who did not develop anti-CpG antibodies throughout their treatment, severe events were not observed. Thus, anti-CpG antibodies elicited by vaccines may be predictive for the occurrence of more significant immune responses accompanied by more severe side effects. Interestingly, in patients receiving CpG 7909 in combination with a peptide vaccine, no events were observed, whereas events occurred in patients receiving CpG 7909 in combination with a protein vaccine. This observation is even manifested in a single patient (#11) who had a positive anti-CpG antibody and no reaction during treatment with CpG 7909 and NY-ESO-1 peptide, but had a severe adverse event during a subsequent immunotherapy using CpG 7909 together with NY-ESO-1 protein. Twenty-four patients received CpG 7909 in combination with NY-ESO-1 protein vaccine. Anti-NY-ESO-1 antibodies were induced in all NY-ESO-1 baseline seronegative patients (20/20) after protein vaccination, whereas only 1 out of 8 NY-ESO-1 baseline seronegative patients in the NY-ESO-1 peptide vaccine group with CpG 7909 developed NY-ESO-1 antibodies. Although the lack of NY-ESO-1 antibody induction in the peptide group is because of the length of the peptide rather than because of CpG, the combination of CpG 7909 and protein may have a different and probably enhanced effect on the stimulation of the immune system as compared with CpG 7909 combined with peptide vaccines.

Considering that more than 100 active or completed clinical trials with CpG-ODNs are currently listed at http://clinicaltrials.gov, the identification of anti-CpG antibodies as shown here may be of importance. The occurrence of anti-CpG antibodies provide new insights into the adjuvant effect of CpG-ODNs in humans and substantiate the potency of CpG 7909 to activate naive B-cells. The ability of CpG 7909 to promote the induction of anti-CpG 7909 antibodies reflects the capacity of B-cell stimulation to variable extents in vaccinated patients. Thus, anti-CpG antibodies may provide new clues to identify the most immunogenic structure of CpG-ODNs. Furthermore, they may be indicative for the effectiveness of the vaccine and may therefore be used as a marker for the immune response to the cancer vaccine. Anti-CpG monitoring may therefore contribute to an improved understanding of how the induction of CpG antibodies affects the clinical outcome.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J. Karbach, E. Jäger
Development of methodology: J. Karbach, C. Wahle, K. Brand
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Karbach, A. Neumann, E. Jäger
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Karbach, S. Gnjatic, E. Jäger
Writing, review, and/or revision of the manuscript: J. Karbach, S. Gnjatic, E. Jäger
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J. Karbach, C. Wahle, K. Brand
Study supervision: E. Jäger

Grant Support

This study was supported by the Cancer Vaccine Collaborative of the Cancer Research Institute and the Ludwig Institute for Cancer Research as well as by Krebsforschung Rhein-Main e.V.

Received January 27, 2012; revised May 8, 2012; accepted June 4, 2012; published OnlineFirst June 29, 2012.

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