[DESCRIPTION]
[Title of Invention]
CHIRAL NUCLEIC ACID ADJUVANT

[FIELD OF THE INVENTION]
[0001]
This invention is directed to CpG oligonucleotides and a method of using it. In more detail, this invention is directed to cure of diseases that are caused by immune cell which is controlled by activation of dendritic cell using stereoisomer of CpG oligonucleotides and their stereoisomer.
[BACKGROUND OF THE INVENTION]
[0002]
JP 2002-513763 A (Patent Literature 1), JP 2002-154397 A (Patent Literature 2）,JP 2002-521489 A (Patent Literature 3) disclose CpG oligonucleotide and the method of manufacturing them.
[0003]
JP 2010-504750 A (Patent Literature 4) discloses that oligonucleotides, which have lipophilic substituted nucleotide analogues out of CpG motif, cause production of interferon-α (IFN-α).
[0004]
Following Non Patent Literature 1 discloses that the S-form stereoisomer of CpG oligonucleotide trimer promotes MAPK signal.　All of the disclosures of this reference are incorporated herein by reference.
[0005]
Following Non Patent Literature 1 discloses PF-3512676 (Sequence No.128), all parts of the sequence are phosphorothioated and S-form stereoisomer. Natural oligonucleic acid is readily reduced in vivo. Whereas, that are changed phosphoric acid ester bond of oligo nucleic acid (P-O bond) to phosphoric acid thioester bond (P-S bond), P-S modifications are difficult to be reduced in vivo.
[Citation List]
[Patent Literature]
[0006]
[Patent Literature 1] JP 2002-513763 A
[Patent Literature 2] JP 2002-154397 A
[Patent Literature 3] JP 2002-521489 A
[Patent Literature 4] JP 2010-504750 A

[Non Patent Literature]

[0007]

[Non Patent Literature 1] Authur M. Krieg et al. OLIGONUCLROTIDES 13: pp.491-499 (2003)

[Non Patent Literature 2] Clin Cancer Res. 2008 Jul 15;14(14):4532-42.

[0008]

For example, all of the sequence of the CpG oligonucleotide disclosed in Non Patent Literature 2 are phosphorothioated. Therefore CpG oligonucleotides disclosed in Non Patent Literature 2 have problems that they induce inflammation and toxic reaction. When the phosphorothioate backbone modification is removed from the CpG oligonucleotides disclosed in Non Patent Literature 2, the stability of the nucleotide decreases.

[0009]

One object of the present invention is to provide a stable stereoisomer of CpG oligonucleotide.

[0010]

Other object of the present invention is to provide a stereoisomer of CpG oligonucleotides with interferon α (IFN-α) producing ability.

[0011]

Other object of the present invention is to provide a cure for a specific disease by activation of dendritic cells with CpG oligonucleotide.

[0012]

Other object of the present invention is to provide a stereoisomer of CpG oligonucleotides less cytotoxic.

[Means for Achieving the Object]

[0013]

This invention is basically based on the following new finding. It is possible to enhance in-vivo stability of the oligo nucleic acid by controlling the molecular conformation of the oligo nucleic acid. Because of it, it becomes possible to provide a stable oligonucleotide in vivo, without introducing the PS bond to all of the sequences. Because not all of the sequences have PS binding modification, the oligonucleotide of the present invention has excellent biocompatibility.

[0014]

At least one of the above objects is solved by the following invention. That is, the first aspect of the invention relates to an oligonucleotide which contains two to four

sequences each represented by the formula 5'-X_1X_2CpGX_3X_4-3'
(formula (I)) and has a length of 14 to 32 nucleotides.
[0015]
In the formula (I), CpG represents unmethylated CpG without phosphate backbone modifications.
X_1X_2 is either AA, AT, GA or GT which may have phosphate backbone modification. The AA, AT, GA or GT which may have phosphate backbone modification means that it may have 1 or 2 phosphate backbone modifications in either of AA, AT, GA or GT. Same as above.
X_3X_4 is TT, AT, AC, TA, is a TC or CG that may have phosphate backbone modification.
The oligonucleotide may have phosphate backbone modification at a site other than 5'-X_1X_2CpGX_3X_4-3 '. That is, the oligonucleotide may have a phosphate backbone modification on the section other than one of CpG motif consisting of 5'-X_1X_2CpGX_3X_4-3'. However, oligonucleotide having at least one phosphate backbone modification on the section other than one of CpG motifs is preferred.
[0016]
This oligonucleotide is preferred to be that X_1X_2 is AT, GT or GA which may have phosphate backbone modification and X_3X_4, is TT, AT, AC, TA, CG or TC which may have phosphate backbone modification.
[0017]
This oligonucleotide is preferred to be that X_1X_2, is either AA, AT, GA or GT without phosphate backbone modifications and X_3X_4 is, TT, AT, AC, TC or CG without phosphate backbone modifications are preferred.
[0018]
The site having least one of the phosphate backbone modification sites other than 5'-X_1X_2CpGX_3X_4-3 'is preferred to be S-form stereoisomer.
[0019]
The oligonucleotide of this invention is preferred to include any of the following sequences or be an oligonucleotide consisting of any of the following sequences.
[Formula 1]
[0020]

SEQ No.	Sequence
1	T*C*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
2	G*G*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
3	T*C*AACGTT*T*C*AACGTT*T*T
4	T*C*AACGTT*T*C*AACGTT*T*T*GG
5	T*C*AACGTT*T*C*AACGTT*G*G
6	T*C*AACGTT*T*C*AACGTT*G*G*G*G
7	T*C*AACGTT*T*T*AACGTT*T*T*AACGGG
8	T*C*AACGTT*T*A*ACGTT*T*T
9	T*C*AACGT*TAACGTT*T*T
10	T*C*AACGTT*T*A*AACGTT*T*A*AACGGG
11	T*C*AACGTTAACGTTAACGGG
12	T*C*GACGTT*T*T*GACGTT*T*T*GACGGG
15	G*G*GACGT*T*T*TGACGT*T*T*TGACGGGGG
16	T*C*GACGT*T*T*TGACGT*T*T*TGACGT*T*T*TGACGGG
17	T*C*GACGT*T*GACGT*T*GACGGG
20	T*C*GACGT*T*GACGT*T*GACGT*T*GACGGG
21	T*C*GACGTT*T*A*AACGTT*T*A*AACGTT*T*A*AACGGG
22	T*C*GACGTT*T*A*AACGTT*T*A*GACGTT*T*A*AACGGG
23	T*C*GACGTTAACGTTAACGTTAACGGG

[Formula 2]

[0021]

24	GGGACGTT*T*A*AACGTCTAGACGGG
25	T*C*GACGT*ACGT*ACGT*ACGGG
26	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
29	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G
30	T*C*GACGTT*T*T*GACGTT*T*T*GACGT*G*G
31	T*C*GACGTT*T*T*GACGTT*T*T*GACGTG*G*G
32	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
33	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G*G*G
36	T*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G*G*G
37	T*C*GACGT*T*GACGT*T*GACGTG*G*G
38	G*G*T*G*C*ATCGAT*G*C*A*G*G*G*G*G*G
39	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGGG
40	G*G*T*G*C*GACGAT*G*C*A*G*G*G*G*G*G
41	G*G*G*G*GACGATCGTCGGG*G*G*G
42	G*G*GACGATATCGTCG*G*G*G*G*G
43	G*G*GACGACGTCGTCG*G*G*G*G*G
46	G*G*GGGACGATCGTCG*G*G*G*G*G
47	G*G*GACGCGCGTCG*G*G*G*G*G*G*G
48	G*G*G*G*TCGTTCG*G*G*G

[Formula 3]

[0022]

49	T*C*ATCGAT*T*T*ATCGAT*T*T*A*A*C*G*G*G
50	T*C*GACGTTTTGACGTT*T*T*G*A*C*G*G*G
51	T*C*GACGTTTTGACGTTTT*G*A*C*G*G*G
52	T*C*GACGT*T*GACGT*T*GACGG*G
53	T*C*GACGT*T*GACGT*T*GACG*G*G
54	T*C*GACGT*T*GACGT*T*GACGG*G*G
55	T*C*GACGT*T*GACGT*T*GACT*G
56	T*C*GACGT*T*GACGT*T*G*A*C*G*G*G
57	T*C*GACGTTGACGT*T*G*A*C*G*G*G
58	T*C*ATCGATATCGA*T*G*A*C*G*G*G
59	T*C*GACGT*T*GACGT*T*GACG*G*G*G
60	T*C*GACGTT*T*T*GACGTT*T*T*G*G*G*G*G
61	T*C*GACGTT*T*T*GACGTT*T*T*G*A*G*G*G*G
62	T*C*GACGTT*T*T*GACGTT*T*T*G*T*G*G*G*G
63	T*C*G*ACGTT*G*ACGTT*G*A*C*G*G*G
64	C*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G
65	T*C*GACGTT*T*A*GACGTT*T*A*GACG*G*G
66	T*C*GACGTT*T*T*GACGTT*T*T*GACG*A*A
67	T*C*GACGTT*T*T*GACGTT*T*T*GACG*T*T
68	T*C*AACGTT*T*T*AACGTT*T*T*GACG*G*G
69	T*C*GACGTT*T*T*GACGTT*T*T*GGG
70	T*C*GACGTT*T*T*GACGTT*T*T*GACGTTGG
71	T*C*GACGTT*GACGTT*G*G*G
72	T*C*GACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
73	T*C*G*A*CGTT*T*T*G*ACGTTTTGACGGG
74	T*C*G*ACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
75	T*C*GACGTA*GACGTA*GACG*G*G
76	T*A*GACGAT*T*C*GTCGTC*T*A*GACG*G*G
77	T*A*GACGA*C*GTCGT*A*GACG*G*G
78	T*C*G*ACGTTT*T*G*ACGTT*T*T*G*A*C*G*G*G
79	T*C*G*ACGTT*T*T*T*A*ACGAC*T*T*G*A*C*G*G*G

[Formula 4]

[0023]

80	T*C*G*ACGTTT*T*AACGAC*T*T*G*A*C*G*G*G
81	T*C*ATCGAT*T*T*ATCGAT*T*T*GACG*G*G
82	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGA*T*G*G*G
83	T*C*ATCGAT*T*T*ATCGAT*T*T*AT*C*G*G*G
84	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*ATCG*G*G
85	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
86	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*A*T*C*G*G*G
87	T*T*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*G*G
88	T*C*ATCGATATCGAT*T*T*G*A*C*G*G*G
89	TCATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
90	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*A*T
91	T*C*GACGT*T*GACGT*T*GACGT*T*G*G*G
92	T*C*G*ACGT*T*G*ACGT*T*G*A*C*G*G*G
93	T*C*A*TCGAT*T*T*A*TCGAT*T*T*G*A*C*G*G*G
94	T*C*A*TCGAT*A*TCGAT*G*ACGT*T*T*G*G*G
95	T*C*GACGTTTGACGTTT*G*A*C*G*G*G
96	T*C*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
97	G*G*GACGATATCGTCG*G*G*G*G*G
98	G*G*GACGAC*G*TCGTCG*G*G*G*G*G
99	G*G*GACGACGTCGTCG*G*G*G*G
100	T*C*GACGACGTCGTCG*G*G*G*G*G
101	T*C*GACGACGTCGTCT*T*T*G*G*G
102	T*A*GACGACGTCGTCT*T*T*G*G*G
103	T*T*GACGACGTCGTCA*A*A*G*G*G
104	T*C*GACGTAGACGTCT*T*T*G*G*G
105	T*C*GACGTAGACGTTT*A*G*G*G*G
106	T*C*ATCGATATCGATT*T*T*G*G*G
107	T*T*ATCGATATCGATA*A*A*G*G*G
108	T*C*GACGTAGACGATCGA*T*G*G*G
109	T*C*GACGAC*T*T*GACGAC*T*T*G*A*C*G*G*G
110	T*C*GACGAC*T*T*GTCGTC*T*T*G*A*C*G*G*G
111	T*T*ATCGATATCGATA*T*C*G*A*T*G*G*G
112	T*T*ATCGATATCGATT*T*A*A*A*G*G*G
113	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*T*T
114	T*C*ATCGA*T*ATCGA*T*G*A*C*G*G*G*G
115	T*C*ATCGAT*ATCGA*T*G*G*G
116	T*C*GTCGTTGTCGT*T*G*A*C*G*G*G
117	T*C*G*TCGTT*T*T*G*TCGTT*T*T*G*A*C*G*G*G
118	T*C*GTCGTTGTCGTTG*A*C*G*A*C*G*G*G

[0024]

In above formula * indicates the stereoisomer caused by phosphate backbone modification, at least one of the * is S-form stereoisomer. CG of the section corresponding to 5'-X_1X_2CpGX_3X_4-3 in above formula means unmethylated CpG without phosphate backbone modifications.

[0025]

The oligonucleotide of this invention is preferred to be that X_1X_2 is GA, and X_3X_4 is TT or AC.

[0026]

The oligonucleotide of this invention is preferred to be that at least one of the phosphate backbone modifications of sites other than 5'-X_1X_2CpGX_3X_4-3' is an oligonucleotide having phosphorothioate.

[0027]

The sequence consisting of 5'-X_1X_2CpGX_3X_4-3' of formula (I) is defined as CpG motif. The oligonucleotides of this invention is preferred to have a sequence of -$(G)_m$-(m is 2 to 10 integer) by 5'end or 3' end of CpG motif

[0028]

The oligonucleotide of this invention is preferred to have a sequence of -$(G)_m$- (m is 1 to 6 integer) by 5'end or 3' end of CpG motif.

[0029]

The oligonucleotide of this invention is preferred to have a sequence of TC, TA, TG or CC at the point which is close to 5'end from the CpG motif.

[0030]

The oligonucleotide of this invention is preferred to include at least the first CpG motif and the second CpG motif. The first CpG motif and the second CpG motif are bonded directly or the first CpG motif and the second CpG motif include a partial sequence that are represented as - $(T)_n$ - (n is an integer of 1 to 3), TA or TC between the first CpG motif and the second CpG motif.

[0031]

The oligonucleotide consists of either one of following sequences or sequences in which 1, 2, or 3 bases are substituted, inserted, deleted, or added:

$t^{sp}c^{sp}gacgtt^{sp}t^{sp}t^{sp}gacgtt^{sp}t^{sp}t^{sp}gacggg$ (SEQ No.13);

$t^{sp}c^{sp}gacgt^{sp}t^{sp}gacgt^{sp}t^{sp}gacggg$ (SEQ No.18); and

$g^{sp}g^{sp}gacgacgtcgtcg^{sp}g^{sp}g^{sp}g^{sp}g^{sp}g$ (SEQ No.44),

in the sequences “cg” indicates non-methylated CpG with modification of phosphate backbone and “^{sp}” indicates that S form modification of phosphate backbone is added between neighbouring nucleotides.

These oligonucleotide are preferred to show same stability or activity as Sequence Nos.13, 18 or 44. In the sequence, the "cg" indicates unmethylated CpG with a phosphate backbone modification.

[0032]

The oligonucleotide of this invention is preferred to be
$t^{Sp}c^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacggg$ (Sequence No.13),
$t^{Sp}c^{Sp}gacgt^{Sp}t^{Sp}gacgt^{Sp}t^{Sp}gacggg$ (Sequence No.18) or
$g^{Sp}g^{Sp}gacgacgtcgtcg^{Sp}g^{Sp}g^{Sp}g^{Sp}g^{Sp}g$ (Sequence No.44).
In the sequence, "cg" indicates unmethylated CpG with a phosphate backbone modification.

[００３３]

This invention also provides a composition that comprises the oligonucleotides described above.

[００３４]

This invention also provides a vaccine adjuvant that comprises the oligonucleotides described above.

[００３５]

This invention also provides a production inducer of interferon-α (IFN-α) from dendritic cell with any of the oligonucleotides described above.

[００３６]

This invention also provides a medical agent that has an effective amount of oligonucleotide described above as an active ingredient, wherein the medical agent is for cure of infectious diseases, cancer, respiratory diseases, allergic diseases, autoimmune disease, or wound.

[Effect of the Invention]

[００３７]

According to the present invention, it is possible to provide a new CpG oligonucleotide with superior stability.

[００３８]

According to the present invention, it is possible to provide a CpG oligonucleotide with immune adjustment power.

[００３９]

According to the present invention, it is possible to provide a medical agent that comprises immunomodulatory factors, which comprises a CpG oligonucleotide as an active ingredient.

[００４０]

According to the present invention, it is possible to provide CpG oligonucleotide that has less cytotoxicity.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[００４１]

Figure 1 is a gel electrophoresis photograph related to sequence Nos. 26 to 28 for evaluating the serum stability of the S-form and R-form oligonucleotide.
Figure 2 is a gel electrophoresis photograph related to sequence Nos. 43 to 45 for evaluating the serum stability of the S-form and R-form oligonucleotide.
Figure 3 is a gel electrophoresis photograph related to sequence Nos. 33 to 35 for evaluating the serum stability of the S-form and R-form oligonucleotide.
[BEST MODE FOR CARRYING OUT THE INVENTION]
[0042]
The first aspect of the present invention relates to oligonucleotides of 14-32 length nucleotides that comprise 5'-$X_1X_2CpGX_3X_4$-3' (Formula 1) sequences 2 to 4 times.
[0043]
"Oligonucleotide" or "oligo" means sugar (e.g. ribose or deoxyribose) binding multiple nucleotides (i.e. Phosphate groups and substituted organic bases (either of substituted pyrimidines (e.g. cytosine (C), thymine (T) or uracil (U)) or substituted purine (e.g. adenine (A) or guanine (G))). As used in this specification, the term “oligonucleotide” means both of oligoribonucleotide (ORN) and oligodeoxyribonucleotide (ODN). The term “oligonucleotide” includes also oligonucleoside (i.e., the oligonucleotide without phosphate) and any other organic base polymer. Oligonucleotides can be obtained from existing nucleic acid sources (e.g. genome or cDNA), but synthetic one (e.g. produced by oligonucleotide synthesis) is preferred.
[0044]
In formula (I), CpG represents unmethylated CpG without phosphate backbone modification. C is 2'-deoxycytidine. G is 2'-deoxyguanosine. p is a bond between nucleoside with phosphodiester.
[0045]
In formula (I), X_1X_2 is either of AA, AT, GA or GT which may have phosphate backbone modification. In formula (I), X_3X_4 is either of TT, AT, AC, TA, TC or CG which may have phosphate backbone modification.
The oligonucleotide of the present invention may have phosphate backbone modification on the site besides the CpG. The oligonucleotide may have phosphate backbone modification on the part besides the CpG motif consisting of 5'-$X_1X_2CpGX_3X_4$-3'. On the other hand, there is the problem previously said on phosphate backbone with phosphorothioate backbone modification between all of nucleotides, it may be preferable that oxygen atoms are replaced by sulfur atoms by more than 20% less than 95%, it may be more than 30% less than 95%, more than 20% less than 90%, more than 40% less than 95%, more than 40% less than 90%, more than

40% less than 80%, more than 50% less than 95%, more than 50% less than 90%, more than 20% less than 80%.

[0046]

The X_1X_2 of this oligonucleotide is preferred to be either of AT, GA or GT which may have phosphate backbone modification and X_3X_4 is preferred to be either of TT, AT, AC, TA, TC or CG which may have phosphate backbone modification.

[0047]

This oligonucleotide is preferred that X_1X_2 is either of AA, AT, GA or GT without phosphate backbone modification and X_3X_4 is either of TT, AT, AC, TC or CG without backbone modification.

[0048]

In the case that the site besides the CpG motif with phosphate backbone modification is phosphorothioate, the site having at least one phosphate backbone modification portion other than the CpG motif may be S-form stereoisomer.

In the case that at least one of the phosphate backbone modifications other than CpG motif is replaced by atoms or bases other than sulfur atoms, it may be preferred that the site takes s-type conformation when oxygen atoms are replaced by sulfur atoms.

[0049]

The oligonucleotide of the present invention is preferred to have the following, or comprising any sequence, it is preferable that an oligonucleotide has any of the following sequences.

[Formula 5]

[0050]

SEQ No.	Sequence
1	T*C*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
2	G*G*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
3	T*C*AACGTT*T*C*AACGTT*T*T
4	T*C*AACGTT*T*C*AACGTT*T*T*GG
5	T*C*AACGTT*T*C*AACGTT*G*G
6	T*C*AACGTT*T*C*AACGTT*G*G*G*G
7	T*C*AACGTT*T*T*AACGTT*T*T*AACGGG
8	T*C*AACGTT*T*A*ACGTT*T*T
9	T*C*AACGT*TAACGTT*T*T
10	T*C*AACGTT*T*A*AACGTT*T*A*AACGGG
11	T*C*AACGTTAACGTTAACGGG
12	T*C*GACGTT*T*T*GACGTT*T*T*GACGGG
15	G*G*GACGT*T*T*TGACGT*T*T*TGACGGGGG
16	T*C*GACGT*T*T*TGACGT*T*T*TGACGT*T*T*TGACGGG
17	T*C*GACGT*T*GACGT*T*GACGGG
20	T*C*GACGT*T*GACGT*T*GACGT*T*GACGGG
21	T*C*GACGTT*T*A*AACGTT*T*A*AACGTT*T*A*AACGGG
22	T*C*GACGTT*T*A*AACGTT*T*A*GACGTT*T*A*AACGGG
23	T*C*GACGTTAACGTTAACGTTAACGGG

[Formula 6]

[0051]

24	GGGACGTT*T*A*AACGTCTAGACGGG
25	T*C*GACGT*ACGT*ACGT*ACGGG
26	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
29	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G
30	T*C*GACGTT*T*T*GACGTT*T*T*GACGT*G*G
31	T*C*GACGTT*T*T*GACGTT*T*T*GACGTG*G*G
32	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
33	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G*G*G
36	T*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G*G*G
37	T*C*GACGT*T*GACGT*T*GACGTG*G*G
38	G*G*T*G*C*ATCGAT*G*C*A*G*G*G*G*G*G
39	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGGG
40	G*G*T*G*C*GACGAT*G*C*A*G*G*G*G*G*G
41	G*G*G*G*GACGATCGTCGGG*G*G*G
42	G*G*GACGATATCGTCG*G*G*G*G*G
43	G*G*GACGACGTCGTCG*G*G*G*G*G
46	G*G*GGGACGATCGTCG*G*G*G*G*G
47	G*G*GACGCGCGTCG*G*G*G*G*G*G*G
48	G*G*G*G*TCGTTCG*G*G*G

[Formula 7]

[0052]

49	T*C*ATCGAT*T*T*ATCGAT*T*T*A*A*C*G*G*G
50	T*C*GACGTTTTGACGTT*T*T*G*A*C*G*G*G
51	T*C*GACGTTTTGACGTTTT*G*A*C*G*G*G
52	T*C*GACGT*T*GACGT*T*GACGG*G
53	T*C*GACGT*T*GACGT*T*GACG*G*G
54	T*C*GACGT*T*GACGT*T*GACGG*G*G
55	T*C*GACGT*T*GACGT*T*GACT*G
56	T*C*GACGT*T*GACGT*T*G*A*C*G*G*G
57	T*C*GACGTTGACGT*T*G*A*C*G*G*G
58	T*C*ATCGATATCGA*T*G*A*C*G*G*G
59	T*C*GACGT*T*GACGT*T*GACG*G*G*G
60	T*C*GACGTT*T*T*GACGTT*T*T*G*G*G*G*G
61	T*C*GACGTT*T*T*GACGTT*T*T*G*A*G*G*G*G
62	T*C*GACGTT*T*T*GACGTT*T*T*G*T*G*G*G*G
63	T*C*G*ACGTT*G*ACGTT*G*A*C*G*G*G
64	C*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G
65	T*C*GACGTT*T*A*GACGTT*T*A*GACG*G*G
66	T*C*GACGTT*T*T*GACGTT*T*T*GACG*A*A
67	T*C*GACGTT*T*T*GACGTT*T*T*GACG*T*T
68	T*C*AACGTT*T*T*AACGTT*T*T*GACG*G*G
69	T*C*GACGTT*T*T*GACGTT*T*T*GGG
70	T*C*GACGTT*T*T*GACGTT*T*T*GACGTTGG
71	T*C*GACGTT*GACGTT*G*G*G
72	T*C*GACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
73	T*C*G*A*CGTT*T*T*G*ACGTTTTGACGGG
74	T*C*G*ACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
75	T*C*GACGTA*GACGTA*GACG*G*G
76	T*A*GACGAT*T*C*GTCGTC*T*A*GACG*G*G
77	T*A*GACGA*C*GTCGT*A*GACG*G*G
78	T*C*G*ACGTTT*T*G*ACGTT*T*T*G*A*C*G*G*G
79	T*C*G*ACGTT*T*T*T*A*ACGAC*T*T*G*A*C*G*G*G

[Formula 8]

[0053]

80	T*C*G*ACGTTT*T*AACGAC*T*T*G*A*C*G*G*G
81	T*C*ATCGAT*T*T*ATCGAT*T*T*GACG*G*G
82	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGA*T*G*G*G
83	T*C*ATCGAT*T*T*ATCGAT*T*T*AT*C*G*G*G
84	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*ATCG*G*G
85	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
86	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*A*T*C*G*G*G
87	T*T*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*G*G
88	T*C*ATCGATATCGAT*T*T*G*A*C*G*G*G
89	TCATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
90	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*A*T
91	T*C*GACGT*T*GACGT*T*GACGT*T*G*G*G
92	T*C*G*ACGT*T*G*ACGT*T*G*A*C*G*G*G
93	T*C*A*TCGAT*T*T*A*TCGAT*T*T*G*A*C*G*G*G
94	T*C*A*TCGAT*A*TCGAT*G*ACGT*T*T*G*G*G
95	T*C*GACGTTTGACGTTT*G*A*C*G*G*G
96	T*C*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
97	G*G*GACGATATCGTCG*G*G*G*G*G
98	G*G*GACGAC*G*TCGTCG*G*G*G*G*G
99	G*G*GACGACGTCGTCG*G*G*G*G
100	T*C*GACGACGTCGTCG*G*G*G*G*G
101	T*C*GACGACGTCGTCT*T*T*G*G*G
102	T*A*GACGACGTCGTCT*T*T*G*G*G
103	T*T*GACGACGTCGTCA*A*A*G*G*G
104	T*C*GACGTAGACGTCT*T*T*G*G*G
105	T*C*GACGTAGACGTTT*A*G*G*G*G
106	T*C*ATCGATATCGATT*T*T*G*G*G
107	T*T*ATCGATATCGATA*A*A*G*G*G
108	T*C*GACGTAGACGATCGA*T*G*G*G
109	T*C*GACGAC*T*T*GACGAC*T*T*G*A*C*G*G*G
110	T*C*GACGAC*T*T*GTCGTC*T*T*G*A*C*G*G*G
111	T*T*ATCGATATCGATA*T*C*G*A*T*G*G*G
112	T*T*ATCGATATCGATT*T*A*A*A*G*G*G
113	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*T*T
114	T*C*ATCGA*T*ATCGA*T*G*A*C*G*G*G*G
115	T*C*ATCGAT*ATCGA*T*G*G*G
116	T*C*GTCGTTGTCGT*T*G*A*C*G*G*G
117	T*C*G*TCGTT*T*T*G*TCGTT*T*T*G*A*C*G*G*G
118	T*C*GTCGTTGTCGTTG*A*C*G*A*C*G*G*G

[0054]

In above formula * indicates the stereoisomer by phosphate backbone modification. CG of the section corresponding to 5'-$X_1X_2CpGX_3X_4$-3 in above formula means unmethylated CpG without phosphate backbone modifications. The examples of phosphate backbone modifications are phosphothioate backbone modifications,

phosphorodithioate backbone modifications, or phosphoramidate backbone modifications. In these phosphate backbone modifications, phosphothioate backbone modifications are preferred. phosphothioate backbone modifications means that converting one of the two nonbridging oxygen atoms bonding to phosphorus atoms comprising phosphodiester bond of neighbor nucleotides into sulfur atoms. At least one of the * is S-form stereoisomer. Here, S-form stereoisomer means, as described above, stereoisomer that takes S-form when their atoms or bases introduced instead of oxygen atoms are sulfur atoms.

[0055]

The oligonucleotide of the present invention is preferred that the sequence satisfies the article (I) or described sequences above, and X_1X_2 is GA, and X_3X_4 is TT or AC.

[0056]

The oligonucleotide of the present invention is preferred that it is one of the nucleotides described above, and phosphate backbone modifications which exist in at least one of the sites other than CpG motif are oligonucleotides including phosphorothioate. That is, as explained above, it is preferred that the oligonucleotide has phosphorothioate backbone modification also in the sites other than CpG. In this case, as explained above, S-form stereoisomer is preferred. However, in the present invention, it is preferred that no phosphorothioate backbone modification exists between each sequence.

[0057]

The sequence consisting of 5'-X_1X_2CpGX_3X_4-3' of formula (I) define as CpG motif. Then, the oligonucleotide of the present invention is preferred to have a sequence -$(G)_m$- (m is 2 to 10 integer) by 5'end or 3' end of CpG motif.

[0058]

The oligonucleotide of this invention is preferred to have a sequence composed of TC, TA, TG or CC by 5'end of CpG motif.

[0059]

The oligonucleotide of this invention is preferred to include at least first CpG motif and the second one. First CpG motif and second one is bonded directly or including - (T)n- (n is an integer that represents 1 to 3), TA, TC between first CpG motif and second one.

[0060]

The oligonucleotide of this invention is preferred to have the sequences 1, 2 or 3 bases are substituted from, inserted to, deleted from or added to

$t^{Sp}c^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacggg$ (Sequence No.13),

$t^{Sp}c^{Sp}gacgt^{Sp}t^{Sp}gacgt^{Sp}t^{Sp}gacggg$ (Sequence No.18) or

$g^{Sp}g^{Sp}gacgacgtcgtcg^{Sp}g^{Sp}g^{Sp}g^{Sp}g^{Sp}g$ (Sequence No.44)

These oligonucleotide are preferred to exhibit stability or activity same as Sequence No.13, 18 or 44. Here, "cg" indicates unmethylated CpG and "^{Sp}" indicates that S-form phosphate backbone modification is introduced between the neighbor nucleotides.

[0061]

The oligonucleotide of this invention is preferred to include

$t^{Sp}c^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacgtt^{Sp}t^{Sp}t^{Sp}gacggg$ (Sequence No.13),

$t^{Sp}c^{Sp}gacgt^{Sp}t^{Sp}gacgt^{Sp}t^{Sp}gacggg$ (Sequence No.18) or

$g^{Sp}g^{Sp}gacgacgtcgtcg^{Sp}g^{Sp}g^{Sp}g^{Sp}g^{Sp}g$ (Sequence No.44)

Here, "cg" indicates unmethylated CpG with phosphate backbone modification and "^{Sp}" indicates that S-form phosphate backbone modification is introduced between the neighbor nucleotides.

[0062]

Synthetic method of nucleotides

The synthetic method for nucleotides is publicly known. The nucleotides in present invention can be produced by the publicly known method. For example, it can adopt that the methods disclosed in patent No.450870 statement and international application No.2010/064146 pamphlet.

[0063]

The other examples of the method for synthesizing nucleotide are introduced in patent No.4942646 official report and US patent No.5912332. The latter, the use of the solid support attachment linker to parallel synthesis or generic solid support, such as phosphate salt attaching controlled pore glass.

[0064]

Furthermore, nucleotide can be produced by the method e.g. disclosed in patent No.4383534 A. For example, It can be produced by β-cyanoethyl phosphoroamidate method (S.L. Beaucage, M.H. Caruthers, Tetrahedron Lett. 1981, 22, 1859-62) and nucleoside H-phosphonate method (Per J. Garegg et al., Tetrahedron Lett. 1986, 27, 4051-4; Brian C. Froehler et al., Nucl Acid Res 1986, 14, 5399-407; Per J. Garegg et al., Tetrahedron Lett. 1988, 27, 4055-8; Barbara L. Gaffney et al., Tetrahedron Lett., 29, 2619-22). These chemicals can be synthesized by a variety of automated nucleic acid synthesizers available in the market. These nucleic acids are called synthetic nucleic acid. Alternatively, it is possible to generate nucleic acids of the present invention on a large scale in a plasmid. (Sambrook T. et al,. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, 1989) The nucleic acid of this invention can be separated into smaller pieces or administered whole. The nucleic acid is produced from nucleic acid sequence (e.g. genomic sequence and cDNA sequence)

with the use of known techniques (e.g. techniques using restriction enzymes, exonuclease or endonuclease) The nucleic acid that has been prepared in this way is called isolated nucleic acid. An isolated nucleic acid, in general, is a nucleic acid which is separated from components which naturally are normally associated. For example, the isolated nucleic acid is a nucleic acid that is separated from the cells, nucleus, mitochondria and chromatin. The combination motif nucleic acid of the present invention includes both synthesized combination motif nucleic acids and isolated combination motif nucleic acids.

[0065]

The combination motif oligonucleotides, if necessary, have a relatively resistant to degradation (e.g., are stabilized) are preferred in the use of in vivo. A "stabilized nucleic acid molecule" means a nucleic acid molecule that is relatively resistant for in vivo degradation (e.g., exonuclease or endonuclease). The nucleic acid stabilization is achieved through the phosphate backbone modification. The stabilized nucleic acid that is preferred in the present invention has a modified backbone. This modification of the nucleic acid backbone provides increasing the activity of the combination motif oligonucleotide when administered in vivo. In some cases, the combination motif oligonucleotides with phosphorothioate bond provide maximum activity and protect the nucleic acid from degradation by intracellular exonucleases and cellular endonucleases. Other modified nucleic acids, modified phosphodiester nucleic acids, combinations of phosphodiester nucleic acids and phosphorothioate nucleic acids (i.e. chimeric), methylphosphonate, methylphosphorothioate, phosphorodithioate, p-ethoxy, and combinations thereof mentioned are.

[0066]

The modified backbones (e.g., phosphorothioates) can be synthesized by using automated techniques employing either phosphoramidate chemistry or H- phosphonate chemistry. Aryl-phosphonate and alkyl-phosphonates can be generated, for example, as described in US patent No.US4,469,863. And alkylphosphotriester (charged oxygen is alkylated as described in US patent No.US5,023,243 and EP patent No.092,574) can be produced using commercially available reagents by automated solid-phase synthesis. Methods for making modifications and substitutions of other DNA backbone have been described. (e.g., Uhlmann E and Peyman A, Chem. Rev. 1990, 90, 544; Goodchild J., Bioconjugate Chem. 1990, 1, 165)

[0067]

The oligonucleotides obtained by synthesis may be purified by known methods, e.g.,

purified, deprotected, desalted and dialyzed by reversed phase HPLC. In this way, the oligonucleotides of the present invention can be isolated and purified.

[0068]

This invention provides composition with one of the above oligonucleotides. This composition is a medicine composition. The composition contains an effective amount of any of the oligonucleotides described above and it may contain appropriate known carrier. The carrier may be a solvent such as water or alcohol. The carrier can be optional excipients, diluents, fillers, salts, buffers, stabilizers, solubilizers, lipids or other substance which is well known for medicine compositions in the art.

[0069]

This invention also provides a vaccine adjuvant with oligonucleotide described above. The vaccine adjuvant, if necessary, may contain a pharmaceutically acceptable carrier. Patent No.4126252 discloses vaccine adjuvant with oligonucleotide. The vaccine adjuvant with the oligonucleotide of this invention can include the disclosed elements in this publication properly.

[0070]

This invention also provides the interferon-α (IFN-α) production inducing agent from dendritic cells including any of the oligonucleotides above. The present invention also provides a cure for infection, cancer, respiratory diseases, allergic diseases, autoimmune disease or wound healing comprising an effective amount of any of the oligonucleotides described above as an active ingredient.

[0071]

The examples of the infection cure of this invention, are fungal infection, persistent fungal infection, bacterial infection, candidiasis, chronic mucocutaneous candidiasis (CMC), aspergillosis, cryptococcosis disease, viral infection, persistent viral infection, human immunodeficiency virus (HIV) infections, B-type hepatitis virus (HBV) infection, C-type hepatitis virus infection, persistent bacterial infection, mycobacterial infection, M. tuberculosis infection, M. bovis infection, and M. leprae infection. For example, patent No.4688815 official report indicates that interferon-α is effective in the treatment of the infections including hepatitis C virus (HCV) infection, and US4607452 indicates that interferon-α is effective in the treatment of the infections (e.g., mycobacterial disease, malaria, leishmaniasis, toxoplasmosis, schistosomiasis and liver fluke disease). The cure of this invention is also effective in the treatment of infections by producing interferon-α.

[0072]

Cancer in cancer cure of this invention includes known cancer and tumors. For example, patent No.4607452 official report and patent No.2011-503039 official report disclose that interferon-α (IFN-α) is effective in the treatment of cancer and tumors. For this reason, the cancer cure is effective in the treatment of cancer and tumors.

[0073]

The examples of respiratory disease in respiratory disease cure of this invention are blasphemy, asthma, allergic rhinitis, bronchitis, pneumonia, acute respiratory distress syndrome (ARDS), and allergic bronchopulmonary aspergillosis. For example, patent No.2004-505046 official report indicates that interferon-α is effective in the treatment of respiratory diseases.

[0074]

The examples of allergic disease in allergic disease cure of this invention are systemic inflammatory response syndrome (SIRS), anaphylaxis or anaphylactoid reaction, allergic vasculitis, hepatitis, nephritis, nephropathy, pancreatitis, rhinitis, arthritis, inflammatory eye diseases (e.g., conjunctivitis, etc.), inflammatory bowel disease (e.g. , ulcerative colitis, Crohn's disease, eosinophilic gastrointestinal disease, etc.), brain and cardiovascular system diseases (e.g., arteriosclerosis, thrombosis, ischemia / reperfusion injury, restenosis, infarction, etc.), skin disease (e.g., dermatitis (e.g., atopic dermatitis, psoriasis, contact dermatitis, eczema, urticaria, pruritus, etc.), etc.), autoimmune diseases (such as multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus , I type diabetes, glomerulonephritis, Sjogren's syndrome, etc.), transplanted organ rejection. For example, patent No.2004-50546 official report indicates that interferon-α is effective in the treatment of allergic disease. For this reason, the cure for allergic diseases of this invention, by producing interferon-α, is effective in the treatment of allergic diseases.

[0075]

The examples of autoimmune disease in autoimmune disease cure of this invention are acute idiopathic thrombocytopenic purpura, chronic idiopathic thrombocytopenic purpura, Sydenham's chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes, Henoch - Schonlein purpura, post Streptococcus nephritis (post-streptococcalnephritis), erythema nodosum, Takayasu's arteritis, Addison's disease, rheumatoid arthritis, multiple sclerosis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, nodule sex multiple arteritis, ankylosing spondylitis, Goodpasture's syndrome, thromboangiitis obliterans (thromboangitisubiterans), Sjogren's syndrome, primary biliary cirrhosis, Hashimoto's thyroiditis, thyrotoxicosis, scleroderma, chronic active

hepatitis, polymyositis / dermatomyositis, polychondritis, pemphigus vulgaris (parnphigus vulgaris), Wegener's granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, spinal cord wax, giant cell arteritis / polymyalgia pain, pernicious anemia, rapidly progressive glomerulonephritis, psoriasis, and fibrosing alveolitis. For example, JP 2007-528209 A indicates that interferon-α is effective in the treatment of these autoimmune disease. For this reason, The cure for autoimmune diseases of this invention, by producing interferon-α, is effective in the treatment of autoimmune diseases.

[0076]

Wounds in wound cure of this invention include skin disorders, wounds caused by surgery, hypertrophic scars, keloid. For example, patent No.2003-503313 official report disclose that interferon-α (IFN-α) is effective in the treatment of skin disorders. For this reason, the wound cure is also effective in the treatment of wounds by producing interferon-α.

[0077]

The interferon-α (IFN-α) production inducer and these medicine can be produced, for example, by the method disclosed in patent No.4383534.

[0078]

The nucleotide of this invention can be used to inducing type 1 IFN (i.e., IFN-α and IFN-β). This method includes a progress that the cell capable of expressing type 1 IFN is contacted with an effective amount of combination motif oligonucleotides of this invention to induce expression of type 1 IFN with the cell. It has been recognized recently that major IFN-α producer cell type in humans is plasmacytoid dendritic cell (pDC). This cell type exists by very low frequency (0.2~0.4%) and this cell type is characterized by lineage negative (i.e., CD3, CD14, also CD19 are not stained), CD11c negative and phenotypic positive for CD4, CD123 (IL-3Rα) and class II major histocompatibility complex (MHC class II). The methods for measuring type 1 IFN are known by one of ordinary skill in the art, and examples of such methods are enzyme-linked immunosorbent assay (ELISA), bioassay and fluorescence cell analysis (FACS). These types of assay may be performed by readily available reagents and kits.

[0079]

This oligonucleotide is effective to induce systemic immune response and / or mucosal immune response. The combination motif oligonucleotide of this invention can be delivered to a subject exposed to antigens for inducing enhancement of the immune response to antigens. Therefore, for example, the combination motif nucleotide is useful for vaccine adjuvant. Examples of the main agent functioning as adjuvant are a variety

of vaccines. The adjuvant can increase the efficiency of antigen to be incorporated into the immune cells. The adjuvant is preferred to be able to be improved or enhanced or assist the original action with the active ingredient of the main agent.

The examples for the vacctine are vacctine for virus and vacctine for B hepatitis, A hepatitis, Japanese encephalitis, pediatric pneumococcal, diphtheria, cough hundred days, tetanus, measles, rubella, mumps, chicken pox and tuberculosis (BCG vaccine). Examples of the vaccine for virus are influenza vaccine, polio vaccine, human papillomavirus vaccine, rotavirus vaccine, vaccine for non-flops, polio vaccines and AIDS vaccine. The oligonucleotide of this invention functions as adjuvant by a very small amount. Therefore, the oligonucleotide of this invention have low cytotoxic compared with the conventional adjuvant and there are very few side effects. This makes the vaccine which is administrated in many targets very useful.

[００８０]

This oligonucleotide can be administrated with a non-nucleic acid adjuvant. The non-nucleic acid adjuvant is arbitrary molecule or compound, except herein oligonucleotides which can stimulate humoral immune responses and / or cellular immune responses. The examples of the non-nucleic acid are adjuvants causing depot effect, immune stimulating adjuvants, and adjuvants causing depot effect and stimulating immune system. As used herein, the non-nucleic acid mucosal adjuvant is an adjuvant that can induce mucosal immune responses when administered to a mucosal surface with antigen in the subject.

[００８１]

The oligonucleotide of this invention may be formulated as a pharmaceutical composition in a pharmaceutically acceptable carrier. This oligonucleotide may be administrated to a subject directly or with a nucleic acid delivery complex. The nucleic acid delivery complex means a nucleic acid which is associated (e.g., ionic bond or covalent bond, or encapsulated in the way) with a targeting way (e.g., molecules which generate high affinity bond to target cells (e.g., surface of B cell) and / or increase in cellular uptake by target cells.). The examples of the nucleic acid delivery complex are nucleic acid associated with sterols such as cholesterol, lipids (e.g., cationic lipids, virosomes or liposomes) or target cell specific bonding factors (egg, ligands recognized by target cell specific receptor). Preferred complex can be enough stable in vivo to prevent from significant de-coupling before the internalization by the target cell. But the complex can be cleavage under appropriate conditions in the cells so that the nucleic acid is released in a functional form.

[0082]

This oligonucleotide and/or the antigen and/or other therapeutic agents can be administrated separately (e.g. in saline or buffer solution), and may also be administered using any known delivery vehicles.

[0083]

Dose of the compounds described herein for mucosal delivery or topical delivery is typically in the range of about 0.1μg/dose to 10mg/dose. The doses depend on whether it is administered daily, weekly, or monthly, and in any other time. More typically, mucosal doses or local doses are in the range of about 10μg/dose to 5mg/dose. The most typically, it is about 100 μg/dose to 1 mg/dose, and the administrations of 2-4 times are performed apart for a few days or a few weeks. More typically, dose for immune stimulant is in the range of about 1μg/dose to 10mg/dose, most typically, in the range of about 10 μg dose to 1 mg/dose. Then, the administrations are performed daily or weekly. The dose of the compounds (this compound is delivered with an antigen, are not delivered with another therapeutic agent) described herein for parenteral delivery in order to induce an antigen-specific immune response, is typically 5 to 10,000-fold more than effective mucosal dose for vaccine adjuvant or immune stimulating applied. More typically, it is 10 to 1,000-fold greater, and most typically 20 to 100 times greater. In case of that the oligonucleotide is administered in combination with other therapeutic agents or administered using specialized delivery vehicles, the dose of the compounds which is for inducing an innate immune response, increasing ADCC or inducing an antigen-specific immune response, described herein for parenteral delivery is typically in the range of about 0.1 μg/dose to 10mg/dose. The doses depend on whether it is administered daily, weekly, or monthly, and in any other time. More typically parenteral doses for these purposes is in the range of about 10 μg/dose to 5 mg/dose. The most typically, it is about 100 μg/dose to 1 mg/dose, and the administrations of 2-4 times are performed apart for a few days or a few weeks. However, in some embodiments, parenteral doses for these purposes may be used in the 5 to 10,000-fold greater range than the typical doses described above.

[0084]

In the present specification, the term "effective amount" means the required or sufficient amount to achieve the desired biological effect. For example, an effective amount of a nucleic acid for treating an infection means the amount required to treat the infection. Combined with the teachings provided herein, by selecting the various active compounds and weighing factors (For example, potency, relative bioavailability, patient body weight, severity of adverse side-effects and preferred mode of administration), the effective prevention regimen and the effective therapeutic regimen, which are very

effective to treat a particular subject without causing substantial toxicity, can be plan. The effective amount for any particular application may vary depending on factors, such as disease or condition being treated, the particular oligonucleotide being administered, antigen, subject size, and the severity of the disease and conditions. A skilled in the art can be empirically determined the effective amount of a particular oligonucleotide and/or antigen and/or other therapeutic agents without the need for undue experiments.

[0085]

A therapeutically effective amount for any of the compounds described herein can first be determined based on the knowledge obtained in animal experiments. An effective dose for the treatment also can be determined based on the data about the CpG oligonucleotide which has been tested in human (human clinical trials has been started) and the data when the mucosal or local administration of known compounds having similar pharmacological activities [For example, other mucosal adjuvants (for example, LT and other antigens for vaccination)]. For parenteral administration, it is necessary to use higher dose. The applied dose can be adjusted based on the relative bioavailability and potency of the compounds administered. Adjusting the dose to achieve the maximal efficacy using the methods and other methods is well known in the art. In addition, a skilled person can easily adjust the dose.

[0086]

When administered, formulation of the present invention is dissolved in a pharmaceutically demand solutions. The solution conventionally may include salts of pharmaceutically acceptable concentrations, buffering agents, preservatives, compatible carriers, adjuvants, and optionally other therapeutic ingredients.

[0087]

For use in therapy, the oligonucleotide of the effective amount may be administered to a subject using any manner for delivering the nucleic acids to desired surface (for example, a mucosal surface and a systemically surface). Administering the pharmaceutical compositions of this invention may be accomplished by any means known to those skilled in the art. Preferred routes of administration are oral route, parenteral route, intramuscular route, intranasal route, the intratracheal route, inhalation routes, ocular route, sublingual, vaginal routes, rectal route, and the like, but not limited those listed herein.

[0088]

For oral administration, the compounds (i.e., oligonucleotides, antigens, and other therapeutic agents) can be easily prepared by combining the active compound with known pharmaceutically acceptable carriers in the art. Such carriers enable the

compounds of the present invention to be formulated as tablets to be taken orally by a subject to be targeted, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like. The pharmaceutical preparations for oral administration may be obtained as solid excipient by adding suitable auxiliaries if necessary, subsequently grounding the resulting mixture and forming the tablet cores or the dragee cores by processing the mixture of granules. In particular, suitable excipients are fillers [for example, sugar (lactose, sucrose, mannitol and sorbitol); cellulose preparations (for example, corn starch, wheat starch , Rice starch, potato starch, gelatin, tragacanth gum, methyl cellulose, hydroxypropyl methyl-cellulose, sodium carboxymethyl-cellulose) and/or polyvinylpyrrolidone (PVP)]. If necessary, the disintegrating agents [for example, cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof (for example, sodium alginate)] may be added. If necessary, the oral formulations may also be administered in saline or buffer solution to neutralize the acidic internal state. In addition, the oral formulations may be administered without any carriers.

[0089]

The dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions can be used. If necessary, the concentrated sugar solutions may contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol and/or titanium dioxide, lacquer solutions, suitable organic solvents or solvent mixtures. In order to identify or characterize different combinations of active compound doses, dyestuffs or pigments may be added to the tablets or the dragee coatings.

[0090]

Examples of pharmaceutical preparations which can be administered orally are a fabricated push-fit capsule of gelatin, and a soft sealed capsule made of gelatin and a plasticizer (for example, glycerol or sorbitol). The push-fit capsule may contain the active ingredient, if necessary, mixed with fillers (for example, lactose), binders (for example, starch) and/or lubricants (for example, talc or magnesium stearate) and stabilizers. In the soft capsule, the active compounds may be dissolved or suspended in suitable liquids (for example, fatty oils, liquid paraffin, or liquid polyethylene glycol). In addition, the stabilizer may be added. Microspheres formulated for oral administration may also be used. Such microspheres have been well known in the art. All formulations for oral administration may be used in appropriate dosage.

[0091]

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0092]

For inhalation administration, the compounds of the present invention may be administrated by aerosol spray from pressurized packs or a nebulizer using a suitable propellant (for example, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas) as with a conventional usage. When using a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered amount. For use in an inhaler or insufflator, such gelatin capsules and cartridges, which contain a powder mixture of the compound and a suitable powder base, may be provided.

[0093]

If the compound should be delivered systemically, the compound can be provided in a form that can be administered parenterally by injection (for example, bolus injection or continuous infusion). The formulations for injection may be provided in unit dosage form (for example, an ampoule or multi-dose containers) with preservative agent. The compounds may take such forms as solutions, emulsions or suspension in oily or aqueous vehicles. In addition, they may contain the formulations (for example, suspending agents, stabilizing agents and/or dispersing agents).

[0094]

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds which are water soluble. In addition, suspensions of the active compounds may be provided as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils (for example, sesame oil), synthetic fatty acid esters for example, ethyl oleate or triglycerides), or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension (for example, sodium carboxymethyl cellulose, sorbitol, or dextran). In order to prepare highly concentrated solutions, the suspension may also include agents that increase the solubility of the appropriate stabilizers or compounds thereof as necessary.

[0095]

Alternatively, the active compounds may be in powder form which can be configured prior to use with a suitable vehicle (for example, sterile pyrogen-free water).

[0096]

The compounds may be provided in the form for rectal or vaginal administration (for example, suppositories or retention enemas which may contain conventional suppository bases such as cocoa butter or other glycerides).

[0097]

In addition to the above, the compounds may also be provided as a depot preparation.

Such long acting formulations may be provided by using a suitable polymeric or hydrophobic materials (for example, as an emulsion in an acceptable oil), by using an ion exchange resin or by using poorly soluble derivatives (such as salts poorly soluble).

[0098]

The pharmaceutical compositions may also include carriers or excipients which is a suitable solid or gel phase. Examples of such carriers or excipients include calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers (for example, polyethylene glycol), but not limited thereto.

[0099]

Suitable liquid pharmaceutical preparation form or solid pharmaceutical preparation forms are micro-encapsulated, chelated, coated on microscopic gold particles, included in liposomes, contained in the aerosol to be sprayed, included in the pellet for implantation into the skin, dried form in sharp on the object for scratching the skin, aqueous solution for inhalation or saline solution. In addition, the pharmaceutical compositions includes granules, powders, tablets, coated tablets, (micro) capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations, which can release the active compound a long period of time. As described above, the formulations contain the excipients, the additives and/or the adjuvants (for example, disintegrants, binders, coating agents, sweetening agents, lubricants, flavoring agents, sweeteners, or solubilized agents) conventionally. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. Brief review of methods for drug delivery is mentioned in Langer (1990) Science 249: 1527-33 (which is incorporated herein by reference).

[0100]

The oligonucleotide and that containing other therapeutic agent and/or antigen as necessary may be administered without being any processed, or may be administered in the form of a pharmaceutically acceptable salt. When administered in the form of a pharmaceutically acceptable salt, the salt should be pharmaceutically acceptable. However, the pharmaceutically acceptable salt may be used to prepare the pharmaceutically acceptable salts. Examples of such salts are the followings, but not limited thereto: HCl, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, maleic acid, acetic acid, salicylic acid, p- toluene sulfonic acid, salt tartaric acid, citric acid, methane sulfonic acid, formic acid, malonic acid, succinic acid, naphthalene-2-sulfonic acid and benzene sulfonic acid. In addition, such salts may be prepared as alkali metal salts or alkaline earth metal salts (for example, sodium salts of carboxylic acid, potassium salt or calcium salt).

[0101]

Examples of the suitable buffering agents are followings: acetic acid and its salt (1-2% w/v); citric acid and its salt (1-3% w/v); boric acid and its salt (0.5-2.5% w/v); and phosphoric acid and its salt (0.8-2% w/v). Examples of the suitable preservatives are followings: benzalkonium chloride (0.003-0.03% w/v); chlorobutanol (0.3-0.9% w/v); parabens (0.01-0.25% w/v), and thimerosal (0.004-0.02% w/v).

[0102]

The pharmaceutical compositions of the present invention may contain an effective amount of the oligonucleotide, the antigen and/or other agents in a pharmaceutically acceptable carrier as necessary. The term "pharmaceutically acceptable carrier" means one or more compatible filler, diluent, or encapsulating agent which is solid or liquid and is suitable for administration to humans or other vertebrates. The term "carrier" means a natural or synthetic, organic or inorganic component which is added to in order to facilitate the application of the active ingredient. Components of the pharmaceutical compositions can be mixed with the compounds of this invention and each component in a manner that the components do not interact with each other.

[0103]

For the treatment of individual subjects, different capacities of the pharmaceutical compositions of the present invention are required based on activity of the compound, manner of administration, purpose of the immunization (i.e., prophylactic immunization or immunotherapy), the nature and severity of the disorder, the age of the patient and weight of the patient. Administration of a desired dose may be performed by administering an amount corresponding to dosage units at a time or by administering a smaller amount multiple times.

[0104]

Examples of other delivery systems include time-release system, delayed release system, or sustained release system. Such systems may avoid repeated administrations of the compound, and may increase the convenience to the subject and the physician. Many types of release delivery systems are available, and are known to those skilled in the art. Examples of the release delivery systems include a polymer-based system (for example, poly (lactide-glycolide), copoly oxalate, polycaprolactone, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides). For example, microcapsules of the polymer containing the pharmaceutical compositions are described in US Patent No. 5,075,109. The delivery systems also include non-polymeric system. Examples of the non-polymeric system are followings: lipids (sterols (for example, cholesterol, cholesterol ester), and fatty acids or natural fats (for example,

monoglycerides, diglycerides, and triglycerides) and the like); hydrogel release systems; silastic system; peptide-based systems; wax coating; compressed tablets using conventional binders and excipients; partial fused to the implant. In particular, the system includes the followings, but not limited thereto: (a) an erosion-based system which the agent of the present invention is contained in a form located in the matrix (US Patent No. 4,452,775, US Patent No. 4,675,189 and US Patent No. 5,736,152); (b) a diffusion system which the active ingredient penetrate at a controlled rate from the polymer (US Pat. No. 3,854,480, US Pat. No. 5,133,974 and US Pat. No. 5,407,686). In addition, pump-based hardware delivery systems can be used. Some of them are adapted for implantation.

[0105]

The invention is further illustrated by the following examples. The following examples are should not be construed as further limiting. Throughout this specification, all of the contents of the cited documents are incorporated herein.

Working Example 1

[0106]

Synthesis of chiral CpG oligonucleic acid

CpG oligonucleic acid (mixture)

The oligonucleic acid (mixture) which had been synthesized using phosphoramidite method and purified using HPLC were purchased from GeneDesign, Inc.

[0107]

Synthesis of the CpG oligonucleotide of which the three-dimensional structure is modified.

Extension of nucleic acid chain was performed by repeating the following steps (i)-(iv).

(i) 3% DCA (dichloroacetic acid)/CH_2Cl_2 (15 sec),

(ii) Condensation reaction [A mixture of 0.1 M monomer solution in MeCN (See below) and 1 M PhIMT (Trifluoromethanesulfonic acid N-phenylimidazolium) solution in MeCN in ratio 1:1, 5 min],

(iii) Capping reaction [A mixture of 0.5 M CF_3Colm in THF and 1 M DMAN (1,8-bis(dimethylamino)naphthalene)) in THF in ratio 1:1, 30 sec],

(iv) Sulfurization reaction (0.1 M DDTT in MeCN, 90 sec) or oxidization reaction (0.02 M I_2 in H_2O-Pyridine-THF solution, 15 sec).

[0108]

After the chain elongation of nucleic acid, a solid phase carrier was collected in 1.5 ml microtube. The solid phase carrier was treated with high concentrated aqueous

ammonia (1.2 ml, 55 degrees, 48 hours). The solid phase carrier was removed by filtration. A filtrate was dried in reduced pressure, and dissolved in water (1.0 ml). Then, the oligomer was isolated and purified by using a reversed-phase HPLC.

[0109]

A procedure for adjusting 0.1 M monomer solution in MeCN (in case of Rp-Th).

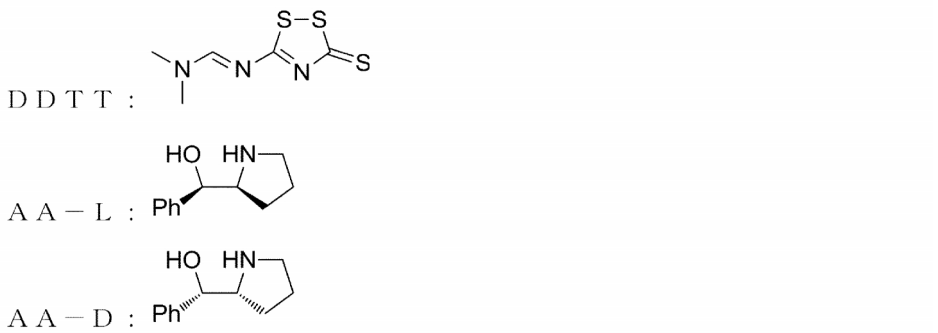
Thymidylic acid H-phosphonate monoester (25μmol) was azeotropic-dried with dehydrated pyridine and dehydrated toluene. It was dissolved in MeCN-CMP (N-Cyanomethylpiperidine) solution (9:1, v/v; 250μL). Subsequently, Ph_3PCl_2 (62.5 μmol) was added, and the solution was stirred for 10 min. Then, AA-L (30 μmol; AA-D was used when Sp form was selected.) was added, and the solution was stirred for 10 min. In this way, the monomer solution was obtained.

[0110]

In the description above, DDTT, AA-L and AA-D mean the abbreviated designation of the following compounds respectively. The obtained oligonucleic acids are shown in Table 1.

[0111]

[Formula 1]



[0112]

[Table 1-1]

Table 1 The oligonucleic acids obtained in Embodiment 1

SEQ No.	Sequence
1	T*C*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
2	G*G*GTCGTT*T*T*GTCGTT*T*T*GTCGGG
3	T*C*AACGTT*T*C*AACGTT*T*T
4	T*C*AACGTT*T*C*AACGTT*T*T*GG
5	T*C*AACGTT*T*C*AACGTT*G*G
6	T*C*AACGTT*T*C*AACGTT*G*G*G*G
7	T*C*AACGTT*T*T*AACGTT*T*T*AACGGG
8	T*C*AACGTT*T*A*ACGTT*T*T
9	T*C*AACGT*TAACGTT*T*T
10	T*C*AACGTT*T*A*AACGTT*T*A*AACGGG
11	T*C*AACGTTAACGTTAACGGG
12	T*C*GACGTT*T*T*GACGTT*T*T*GACGGG
13	TsCsGACGTTsTsTsGACGTTsTsTsGACGGG
14	TrCrGACGTTrTrTrGACGTTrTrTrGACGGG
15	G*G*GACGT*T*T*TGACGT*T*T*TGACGGGGG
16	T*C*GACGT*T*T*TGACGT*T*T*TGACGT*T*T*TGACGGG
17	T*C*GACGT*T*GACGT*T*GACGGG
18	TsCsGACGTsTsGACGTsTsGACGGG
19	TrCrGACGTrTrGACGTrTrGACGGG
20	T*C*GACGT*T*GACGT*T*GACGT*T*GACGGG
21	T*C*GACGTT*T*A*AACGTT*T*A*AACGTT*T*A*AACGGG
22	T*C*GACGTT*T*A*AACGTT*T*A*GACGTT*T*A*AACGGG
23	T*C*GACGTTAACGTTAACGTTAACGGG
24	GGGACGTT*T*A*AACGTCTAGACGGG
25	T*C*GACGT*ACGT*ACGT*ACGGG
26	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
27	TsCsGACGTTsTsTsGACGTTsTsTsGsAsCsGsGsG
28	TrCrGACGTTrTrTrGACGTTrTrTrGrArCrGrGrG
29	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G

[0113]

[Table 1-2]

30	T*C*GACGTT*T*T*GACGTT*T*T*GACGT*G*G
31	T*C*GACGTT*T*T*GACGTT*T*T*GACGTG*G*G
32	T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G
33	T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G*G*G
34	TsCsGACGTTsTsTsGACGTTsTsTsGACGGsGsGsGsG
35	TrCrGACGTTrTrTrGACGTTrTrTrGACGGrGrGrGrG
36	T*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G*G*G
37	T*C*GACGT*T*GACGT*T*GACGTG*G*G
38	G*G*T*G*C*ATCGAT*G*C*A*G*G*G*G*G*G
39	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGGG
40	G*G*T*G*C*GACGAT*G*C*A*G*G*G*G*G*G
41	G*G*G*G*GACGATCGTCGGG*G*G*G
42	G*G*GACGATATCGTCG*G*G*G*G*G
43	G*G*GACGACGTCGTCG*G*G*G*G*G
44	GsGsGACGACGTCGTCGsGsGsGsGsG
45	GrGrGACGACGTCGTCGrGrGrGrGrG
46	G*G*GGGACGATCGTCG*G*G*G*G*G
47	G*G*GACGCGCGTCG*G*G*G*G*G*G*G
48	G*G*G*G*TCGTTCG*G*G*G

[0114]

In above tables, * indicates the position that the S-form phosphorothioate backbone modification and the R-form phosphorothioate modification were induced into the backbone at random. In above table, “s” indicates the S-form phosphorothioate modification. In above table, “r” indicates the R-form phosphorothioate modification.

Working Example 2

[0115]

Induction of production of IFN-α in simian peripheral blood mononuclear cells (PBMC)

The blood derived from Macaca fascicularis which has tested negative for B virus, diluted to 3 times with Hanks’ Balanced Salt Solution. Then, the sample was layered on Ficoll-Paque PLUS centrifugation medium and centrifuged (2,600 rpm, 30 min). A fraction containing the peripheral blood mononuclear cells (PBMC) was obtained. After the PBMC was washed with RPMI medium (1% penicillin and streptomycin), the PBMC was suspended in RPMI medium (10% FBS, 1% penicillin and streptomycin) at a cell density of $3x10^6$ cells/ml. The cells were cultured with various oligonucleic acids (mixture containing oligo DNA and DOTAP in a ratio of 1:3.2) in 96-well round-bottom plate for 17-24 hours using 5% CO_2 incubator. After the cultivation, cell culture supernatant was obtained by centrifuging (500 rpm, 5 min) the culture medium. Then, the concentration of IFN-α in the cell culture supernatant was measured using ELISA kit (PBL Assay Science).

[0116]

The result was shown in Table 2. Table 2 indicates the IFN-α production induced by the oligonucleic acids of SEQ No: 1-48 in simian peripheral blood mononuclear cells.

[0117]

[Table 2]

Table 2 Inductive effect of production of INF-α in simian peripheral blood mononuclear cells

SEQ No.	IFN-α (pg/mL)	
	Average	±SE
1	3583	1634.84
2	1006	1161.33
3	583	299.44
4	2151	1259.46
5	388	144.35
6	1767	1176.08
7	3982	1588.50
8	2887	1080.05
9	2845	1127.16
10	256	173.70
11	1400	508.94
12	4001	607.38
13	2776	1020.29
14	3966	1155.61
15	1453	314.77
16	3719	849.99
17	2694	441.82
18	3207	763.07
19	2583	711.25
20	2457	507.22
21	2287	857.47
22	2492	985.99
23	3082	1379.50
24	1329	302.81
25	2373	1062.95
26	2404	306.42
29	1359	250.85
39	1778	478.20
31	1934	600.05
32	1582	608.10
33	850	417.75
36	581	263.23
37	1880	661.38
38	558	365.34
39	1580	2131.02
40	142	19.37
41	398	33.04
42	1214	532.80
43	3776	1429.12
46	1178	624.04
47	297	132.99
48	929	115.51

Working Example 3

[0118]

Evaluation of stability of the oligonucleic acids in monkey serum

Preparation of samples

A monkey serum was obtained by centrifuging (3000 rpm, 15 min) blood derived from Macaca fascicularis which has tested negative for B virus (The blood was purchased from Shin Nippon Biomedical Laboratories, LTD). The oligonucleic acids (13.4 ng/μl) were incubated in 50% monkey serum at 37 degrees using water bath. The samples were treated at 42 degrees for 1.5 hour using 0.3 mg/ml Proteinase K. Then, an equal volume of phenol/chloroform was added. After centrifugation (1000 rpm, 5 min), the water layer was collected as a sample for analysis by SDS-PAGE.

[0119]

Performing the SDS-PAGE

The sample obtained above (100.5 ng) was loaded onto 20% SDS-polyacrylamide gel. The SDS-PAGE was performed at 20 mA for 120 min. Then, the gel was stained for 40 min using SYBR-Gold solution diluted 1000 times. The oligonucleotides were visualized as fluorescence bands using UV transilluminator. The fluorescence intensity of each band was measured using an image analyzer (IMAGE STATION: Koda).

[0120]

The results are shown in Fig. 1-3 and Table 3. Fig.1 is a photo of the gel electrophoresis as a substitute for a diagram, illustrating the stability of the S-form and R-form oligonucleic acids of SEQ No: 27-28 in the serum. Fig. 2 is a photo of the gel electrophoresis as a substitute for a diagram, illustrating the stability of the S-form and R-form oligonucleic acids of SEQ No: 43-45 in the serum. Fig. 3 is a photo of the gel electrophoresis as a substitute for a diagram, illustrating the stability of the S-form and R-form oligonucleic acids of SEQ No: 33-35 in the serum.

[0121]

[Table 3]

Table 3. Evaluation of stability in serum

			Residual ratio (%)	
SEQ No.	Serum(%)	Time(min)	Sp	Rp
13, 14	30	120	46.1	33.5
27, 28	50	120	41.8	27.5
44, 45	50	180	73.3	17.2
34, 35	50	180	51.7	19.7

Working Example 4

[0122]

Induction test of the production of antigen-specific antibodies in mice

Administration of the test substance

OVA (Wako) and oligonucleic acids which were prepared in a concentration of 0.2 mg/ml respectively using physiological saline were used as the test substance administration solution.

[0123]

Preparation of samples

8-week-old BALB/cAnCrlCrlj mouse were used in the experiment. Using general anesthesia equipment, mouse were anesthetized by isoflurane (2.0%-4.0%, Forane, Abbott Japan Co., LTD.), and maintained anesthesia. After the back of the mouse were shaved, the test substance administration solution was administered subcutaneously at 50μl/body of dose using a disposable syringe and needle. After two weeks of the first administration, the test substance administration solution was administered again. One week later, mice were euthanized, and spleen and whole blood were collected.

[0124]

Measurement of the antibody titer

The plasma obtained from the blood collected from the mouse 5days before and 16 days after administration was used as samples for analysis. Solid-phasing solution was put into holes of the plate for ELISA at 0.1 ml/well. The plate was sealed, and placed overnight in a refrigerator. Then, the solution was removed and wash solution was put into wells of the plate at 0.3 ml/well, and the solution was removed. Same operation was repeated twice. In other word, the well was washed three times in total. Then, the blocking solution was put into wells of the plate at 0.2 ml/well. The plate was sealed, and placed at room temperature for 1-4 hours. Then, the solution was removed, and the well was washed three times in the same manner as described above.

[0125]

The sample (plasma) for measuring was diluted to 1000 times using a solution for dilution. Next, the sample was diluted 7 or more times with common rate 2 (As a result of this process, the sample diluted 100-12800 times were obtained.). The samples were put into wells of the plate for ELISA at 0.1 ml/well. As a blank, the solution for dilution was put into another one well. The plate was sealed, and incubated for 1 hour at 37 degrees in the plate incubator. After the incubation, the solution was removed. The well was washed three times in the same manner as described above. The detection antibody solution was put into wells at 0.1 ml/well.

The plate was sealed, and incubated for 1 hour at 37 degrees in the plate incubator. After the incubation, the solution was removed. The well was washed four times in the same manner as described above.

[0126]

Coloring solution was put into well at 0.1 ml/well. The plate was incubated for 30 min at room temperature. Then, the stop solution was added at 0.1 ml/well in order to stop the reaction. Absorbance of each well was measured using absorption spectrometer for multi-plate (Dominant wavelength was 450 nm, Vice wavelength was 620 nm).

[0127]

The result was shown in Table 4. Table 4 indicates the result of the measurement of anti-OVA-IgG titer of SEQ No: 27-28. Fig. 4 indicates that S-form oligonucleic acids cause higher antibody titers than R-form oligonucleic acids.

[0128]

[Table 4]

Table 4. The effect on the anti-OVA antibody titer of chiral CpG oligonucleic acids

Group	IgG titer
OVA only	100
SEQ No. 27	1000
SEQ No. 28	433

[0129]

Measurement of the weight of spleen

The obtained spleen was washed with cold physiological saline. The weight of the spleen was measured using an electronic balance (HR-200, A&D Company, Limited).

[0130]

The result was shown in Table 5. Table 5 indicates the effect on spleen weight of the oligonucleic acids of SEC ID No. 27-28. Table 5 indicates that the weight of spleen was increased in the mouse treated with R-form oligonucleic acids. This result indicates that R-form oligonucleic acids have toxicity. On the other hand, the weight of the spleen was not increased in the mouse treated with S-form oligonucleic acids. This indicates that S-form oligonucleic acids have no toxicity or have low toxicity.

[0131]

[Table 5]

Table 5. Effect on spleen weight of chiral CpG oligonucleic acids

Group	Spleen mg/100gBW	SE
OVA only	570.4	9.6
SEQ No. 27	566.7	8.2
SEQ No. 28	607.2	7.6

Working Example 5

[0132]

The CpG oligonucleic acids of which the three-dimensional structure is modified were synthesized in the same manner as described in embodiment 1. The sequences of the synthesized oligonucleic acids were shown in Table 6. The notation in the table is the same as one described in Embodiment 1. In addition, for reference, a known nucleic acid sequence is shown in SEQ No. 119.

[0133]

[Table 6]

Table 6. The oligonucleic acids obtained in Embodiment 5

49	T*C*ATCGAT*T*T*ATCGAT*T*T*A*A*C*G*G*G
50	T*C*GACGTTTTGACGTT*T*T*G*A*C*G*G*G
51	T*C*GACGTTTTGACGTTTT*G*A*C*G*G*G
52	T*C*GACGT*T*GACGT*T*GACGG*G
53	T*C*GACGT*T*GACGT*T*GACG*G*G
54	T*C*GACGT*T*GACGT*T*GACGG*G*G
55	T*C*GACGT*T*GACGT*T*GACT*G
56	T*C*GACGT*T*GACGT*T*G*A*C*G*G*G
57	T*C*GACGTTGACGT*T*G*A*C*G*G*G
58	T*C*ATCGATATCGA*T*G*A*C*G*G*G
59	T*C*GACGT*T*GACGT*T*GACG*G*G*G
60	T*C*GACGTT*T*T*GACGTT*T*T*G*G*G*G*G
61	T*C*GACGTT*T*T*GACGTT*T*T*G*A*G*G*G*G
62	T*C*GACGTT*T*T*GACGTT*T*T*G*T*G*G*G*G
63	T*C*G*ACGTT*G*ACGTT*G*A*C*G*G*G
64	C*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G
65	T*C*GACGTT*T*A*GACGTT*T*A*GACG*G*G
66	T*C*GACGTT*T*T*GACGTT*T*T*GACG*A*A
67	T*C*GACGTT*T*T*GACGTT*T*T*GACG*T*T
68	T*C*AACGTT*T*T*AACGTT*T*T*GACG*G*G
69	T*C*GACGTT*T*T*GACGTT*T*T*GGG
70	T*C*GACGTT*T*T*GACGTT*T*T*GACGTTGG
71	T*C*GACGTT*GACGTT*G*G*G
72	T*C*GACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
73	T*C*G*A*CGTT*T*T*G*ACGTTTTGACGGG
74	T*C*G*ACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G
75	T*C*GACGTA*GACGTA*GACG*G*G
76	T*A*GACGAT*T*C*GTCGTC*T*A*GACG*G*G
77	T*A*GACGA*C*GTCGT*A*GACG*G*G
78	T*C*G*ACGTTT*T*G*ACGTT*T*T*G*A*C*G*G*G
79	T*C*G*ACGTT*T*T*T*A*ACGAC*T*T*G*A*C*G*G*G

[0134]

[Table 6-2]

80	T*C*G*ACGTTT*T*AACGAC*T*T*G*A*C*G*G*G
81	T*C*ATCGAT*T*T*ATCGAT*T*T*GACG*G*G
82	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGA*T*G*G*G
83	T*C*ATCGAT*T*T*ATCGAT*T*T*AT*C*G*G*G
84	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*ATCG*G*G
85	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
86	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*A*T*C*G*G*G
87	T*T*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*G*G
88	T*C*ATCGATATCGAT*T*T*G*A*C*G*G*G
89	TCATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
90	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*A*T
91	T*C*GACGT*T*GACGT*T*GACGT*T*G*G*G
92	T*C*G*ACGT*T*G*ACGT*T*G*A*C*G*G*G
93	T*C*A*TCGAT*T*T*A*TCGAT*T*T*G*A*C*G*G*G
94	T*C*A*TCGAT*A*TCGAT*G*ACGT*T*T*G*G*G
95	T*C*GACGTTTGACGTTT*G*A*C*G*G*G
96	T*C*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G
97	G*G*GACGATATCGTCG*G*G*G*G*G
98	G*G*GACGAC*G*TCGTCG*G*G*G*G*G
99	G*G*GACGACGTCGTCG*G*G*G*G
100	T*C*GACGACGTCGTCG*G*G*G*G*G
101	T*C*GACGACGTCGTCT*T*T*G*G*G
102	T*A*GACGACGTCGTCT*T*T*G*G*G
103	T*T*GACGACGTCGTCA*A*A*G*G*G
104	T*C*GACGTAGACGTCT*T*T*G*G*G
105	T*C*GACGTAGACGTTT*A*G*G*G*G
106	T*C*ATCGATATCGATT*T*T*G*G*G
107	T*T*ATCGATATCGATA*A*A*G*G*G
108	T*C*GACGTAGACGATCGA*T*G*G*G
109	T*C*GACGAC*T*T*GACGAC*T*T*G*A*C*G*G*G
110	T*C*GACGAC*T*T*GTCGTC*T*T*G*A*C*G*G*G
111	T*T*ATCGATATCGATA*T*C*G*A*T*G*G*G
112	T*T*ATCGATATCGATT*T*A*A*A*G*G*G
113	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*T*T
114	T*C*ATCGA*T*ATCGA*T*G*A*C*G*G*G*G
115	T*C*ATCGAT*ATCGA*T*G*G*G
116	T*C*GTCGTTGTCGT*T*G*A*C*G*G*G
117	T*C*G*TCGTT*T*T*G*TCGTT*T*T*G*A*C*G*G*G
118	T*C*GTCGTTGTCGTTG*A*C*G*A*C*G*G*G
119	T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*G*T*C*G*T*T

Working Example 6

[0135]

Using the same method as described in Embodiment 2, the induction of the production of IFN-α of the oligonucleic acids in the peripheral blood mononuclear cells (PBMC) was investigated. The values in the table show the relative values compared to the value of the nucleic acid of SEQ No. 128. The result was indicated in Table 7.

[0136]

[Table 7-1]

Table 7. Inductive effect of production of INF-α in simian peripheral blood mononuclear cells

No.	Sequence (* indicates the S-form modification.)	Ratio	±SE	Number of individuals
49	T*C*ATCGAT*T*T*ATCGAT*T*T*A*A*C*G*G*G	195	40	4
50	T*C*GACGTTTTGACGTT*T*T*G*A*C*G*G*G	241	32	4
51	T*C*GACGTTTTGACGTTTT*G*A*C*G*G*G	265	52	3
52	T*C*GACGT*T*GACGT*T*GACGG*G	297	71	3
53	T*C*GACGT*T*GACGT*T*GACG*G*G	361	60	5
54	T*C*GACGT*T*GACGT*T*GACGG*G*G	225	101	3
55	T*C*GACGT*T*GACGT*T*GACT*G	124	39	4
56	T*C*GACGT*T*GACGT*T*G*A*C*G*G*G	210	59	6
57	T*C*GACGTTGACGT*T*G*A*C*G*G*G	269	71	3
58	T*C*ATCGATATCGA*T*G*A*C*G*G*G	255	42	4
59	T*C*GACGT*T*GACGT*T*GACG*G*G*G	139	23	4
60	T*C*GACGTT*T*T*GACGTT*T*T*G*G*G*G*G	123	38	5
61	T*C*GACGTT*T*T*GACGTT*T*T*G*A*G*G*G*G	123	30	5
62	T*C*GACGTT*T*T*GACGTT*T*T*G*T*G*G*G*G	117	17	5
63	T*C*G*ACGTT*G*ACGTT*G*A*C*G*G*G	155	29	3
64	C*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G	84	36	4
65	T*C*GACGTT*T*A*GACGTT*T*A*GACG*G*G	246	20	4
66	T*C*GACGTT*T*T*GACGTT*T*T*GACG*A*A	188	56	5
67	T*C*GACGTT*T*T*GACGTT*T*T*GACG*T*T	244	93	5
68	T*C*AACGTT*T*T*AACGTT*T*T*GACG*G*G	220	97	5
69	T*C*GACGTT*T*T*GACGTT*T*T*GGG	98	44	5
70	T*C*GACGTT*T*T*GACGTT*T*T*GACGTTGG	242	80	4
71	T*C*GACGTT*GACGTT*G*G*G	238	48	3
72	T*C*GACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G	225	76	3
73	T*C*G*A*CGTT*T*T*G*ACGTTTTGACGGG	102	22	3
74	T*C*G*ACGTT*T*T*G*ACGTT*T*T*G*ACG*G*G	247	120	5
75	T*C*GACGTA*GACGTA*GACG*G*G	188	54	4
76	T*A*GACGAT*T*C*GTCGTC*T*A*GACG*G*G	260	49	3
77	T*A*GACGA*C*GTCGT*A*GACG*G*G	86	17	4
78	T*C*G*ACGTTT*T*G*ACGTT*T*T*G*A*C*G*G*G	98	6	3
79	T*C*G*ACGTT*T*T*T*A*ACGAC*T*T*G*A*C*G*G*G	142	26	3

[0137]

[Table 7-2]

80	T*C*G*ACGTTT*T*AACGAC*T*T*G*A*C*G*G*G	223	22	3
81	T*C*ATCGAT*T*T*ATCGAT*T*T*GACG*G*G	389	163	3
82	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGA*T*G*G*G	263	38	4
83	T*C*ATCGAT*T*T*ATCGAT*T*T*AT*C*G*G*G	308	53	4
84	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*ATCG*G*G	334	40	3
85	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G	170	21	4
86	T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*A*T*C*G*G*G	170	25	4
87	T*T*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*G*G	208	86	3
88	T*C*ATCGATATCGAT*T*T*G*A*C*G*G*G	288	135	3
89	TCATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G	279	45	3
90	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*A*T	374	205	3
91	T*C*GACGT*T*GACGT*T*GACGT*T*G*G*G	135	41	5
92	T*C*G*ACGT*T*G*ACGT*T*G*A*C*G*G*G	97	25	3
93	T*C*A*TCGAT*T*T*A*TCGAT*T*T*G*A*C*G*G*G	109	34	3
94	T*C*A*TCGAT*A*TCGAT*G*ACGT*T*T*G*G*G	103	7	2
95	T*C*GACGTTTGACGTTT*G*A*C*G*G*G	222	37	4
96	T*C*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G	129	35	3
97	G*G*GACGATATCGTCG*G*G*G*G*G	167	11	5
98	G*G*GACGAC*G*TCGTCG*G*G*G*G*G	138	107	3
99	G*G*GACGACGTCGTCG*G*G*G*G	230	87	4
100	T*C*GACGACGTCGTCG*G*G*G*G*G	125	53	3
101	T*C*GACGACGTCGTCT*T*T*G*G*G	404	52	3
102	T*A*GACGACGTCGTCT*T*T*G*G*G	387	126	3
103	T*T*GACGACGTCGTCA*A*A*G*G*G	302	64	5
104	T*C*GACGTAGACGTCT*T*T*G*G*G	354	75	4
105	T*C*GACGTAGACGTTT*A*G*G*G*G	297	65	4
106	T*C*ATCGATATCGATT*T*T*G*G*G	224	26	4
107	T*T*ATCGATATCGATA*A*A*G*G*G	154	61	6
108	T*C*GACGTAGACGATCGA*T*G*G*G	370	70	4
109	T*C*GACGAC*T*T*GACGAC*T*T*G*A*C*G*G*G	235	76	3
110	T*C*GACGAC*T*T*GTCGTC*T*T*G*A*C*G*G*G	133	42	4
111	T*T*ATCGATATCGATA*T*C*G*A*T*G*G*G	200	8	4
112	T*T*ATCGATATCGATT*T*A*A*A*G*G*G	257	40	4
113	T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*T*T	209	27	4
114	T*C*ATCGA*T*ATCGA*T*G*A*C*G*G*G*G	155	37	3
115	T*C*ATCGAT*ATCGA*T*G*G*G	95	12	2
116	T*C*GTCGTTGTCGT*T*G*A*C*G*G*G	235	23	4
117	T*C*G*TCGTT*T*T*G*TCGTT*T*T*G*A*C*G*G*G	177	61	4
118	T*C*GTCGTTGTCGTTG*A*C*G*A*C*G*G*G	88	14	4
119	T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*G*T*C*G*T*T	100	0	41

[0138]

Using the same method as described in Embodiment 2, the concentration of IFN-α in case of administering the oligonucleic acids of SEQ No. 119 was measured. The result was shown in Table 8.

[0139]

[Table 8]

Table 8. Concentration of IFN-α

IFN-alfa (ng/mL)		
52.2	6.2	Measured value

[0140]

The present invention can be utilized in the field of pharmaceutical industry.

Claims

1. An oligonucleotide which comprises two to four sequences each represented by 5'-$X_1X_2CpGX_3X_4$-3' and has a length of 14 to 32 nucleotides,

wherein the CpG is non-methylated CpG without modified phosphate backbones,

wherein the oligonucleotide comprises at least one modified phosphate backbone at a site other than the parts represented by 5'-$X_1X_2CpGX_3X_4$-3',

wherein the X_1X_2 is either one of AA, AT, GA, or GT without modified phosphate backbones,

wherein the X_3X_4 is TT, AT, AC, TC or CG without modified phosphate backbones, and

wherein the site of the oligonucleotide which includes at least one modified phosphate backbones at the site other than the parts represented by 5'-$X_1X_2CpGX_3X_4$-3' is a Sp type stereoisomer.

2. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide comprises either one of the sequences represented by Sequence Nos. 1-7, 10-12, 21-24, 26, 29-33, 36, 39, 42, 43, 49-51, 60-62, 64-71, 75, 76, 81-90,95-97, 99-103, 106, 107, 109-113, and 118.

Seq. No.	Sequence
1	**T*C*GTCGTT*T*T*GTCGTT*T*T*GTCGGG**
2	**G*G*GTCGTT*T*T*GTCGTT*T*T*GTCGGG**
3	**T*C*AACGTT*T*C*AACGTT*T*T**
4	**T*C*AACGTT*T*C*AACGTT*T*T*GG**
5	**T*C*AACGTT*T*C*AACGTT*G*G**
6	**T*C*AACGTT*T*C*AACGTT*G*G*G*G**
7	**T*C*AACGTT*T*T*AACGTT*T*T*AACGGG**
10	**T*C*AACGTT*T*A*AACGTT*T*A*AACGGG**
11	**T*C*AACGTTAACGTTAACGGG**
12	**T*C*GACGTT*T*T*GACGTT*T*T*GACGGG**
21	**T*C*GACGTT*T*A*AACGTT*T*A*AACGTT*T*A*AACGGG**
22	**T*C*GACGTT*T*A*AACGTT*T*A*GACGTT*T*A*AACGGG**

23	**T*C*GACGTTAACGTTAACGTTAACGGG**

24	**GGGACGTT*T*A*AACGTCTAGACGGG**
26	**T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G**
29	**T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G**
30	**T*C*GACGTT*T*T*GACGTT*T*T*GACGT*G*G**
31	**T*C*GACGTT*T*T*GACGTT*T*T*GACGTG*G*G**
32	**T*C*GACGTT*T*T*GACGTT*T*T*G*A*C*G*G*G**
33	**T*C*GACGTT*T*T*GACGTT*T*T*GACGG*G*G*G*G**
36	**T*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G*G*G**
39	**T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGGG**
42	**G*G*GACGATATCGTCG*G*G*G*G*G**
43	**G*G*GACGACGTCGTCG*G*G*G*G*G**

49	**T*C*ATCGAT*T*T*ATCGAT*T*T*A*A*C*G*G*G**
50	**T*C*GACGTTTTGACGTT*T*T*G*A*C*G*G*G**
51	**T*C*GACGTTTTGACGTTTT*G*A*C*G*G*G**
60	**T*C*GACGTT*T*T*GACGTT*T*T*G*G*G*G*G**
61	**T*C*GACGTT*T*T*GACGTT*T*T*G*A*G*G*G*G**
62	**T*C*GACGTT*T*T*GACGTT*T*T*G*T*G*G*G*G**
64	**C*C*GACGTT*T*T*GACGTT*T*T*GACG*G*G**
65	**T*C*GACGTT*T*A*GACGTT*T*A*GACG*G*G**
66	**T*C*GACGTT*T*T*GACGTT*T*T*GACG*A*A**
67	**T*C*GACGTT*T*T*GACGTT*T*T*GACG*T*T**
68	**T*C*AACGTT*T*T*AACGTT*T*T*GACG*G*G**
69	**T*C*GACGTT*T*T*GACGTT*T*T*GGG**
70	**T*C*GACGTT*T*T*GACGTT*T*T*GACGTTGG**
71	**T*C*GACGTT*GACGTT*G*G*G**
75	**T*C*GACGTA*GACGTA*GACG*G*G**
76	**T*A*GACGAT*T*C*GTCGTC*T*A*GACG*G*G**
81	**T*C*ATCGAT*T*T*ATCGAT*T*T*GACG*G*G**
82	**T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGA*T*G*G*G**
83	**T*C*ATCGAT*T*T*ATCGAT*T*T*AT*C*G*G*G**
84	**T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*ATCG*G*G**
85	**T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G**
86	**T*C*ATCGAT*T*T*ATCGAT*T*T*ATCGAT*A*T*C*G*G*G**
87	**T*T*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*G*G**
88	**T*C*ATCGATATCGAT*T*T*G*A*C*G*G*G**
89	**TCATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G**
90	**T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*A*T**
95	**T*C*GACGTTTGACGTTT*G*A*C*G*G*G**

96	**T*C*ATCGAT*T*T*ATCGAT*T*T*A*T*C*G*G*G**
97	**G*G*GACGATATCGTCG*G*G*G*G*G**
99	**G*G*GACGACGTCGTCG*G*G*G*G**
100	**T*C*GACGACGTCGTCG*G*G*G*G*G**
101	**T*C*GACGACGTCGTCT*T*T*G*G*G**
102	**T*A*GACGACGTCGTCT*T*T*G*G*G**
103	**T*T*GACGACGTCGTCA*A*A*G*G*G**
106	**T*C*ATCGATATCGATT*T*T*G*G*G**
107	**T*T*ATCGATATCGATA*A*A*G*G*G**
109	**T*C*GACGAC*T*T*GACGAC*T*T*G*A*C*G*G*G**
110	**T*C*GACGAC*T*T*GTCGTC*T*T*G*A*C*G*G*G**
111	**T*T*ATCGATATCGATA*T*C*G*A*T*G*G*G**
112	**T*T*ATCGATATCGATT*T*A*A*A*G*G*G**
113	**T*C*ATCGAT*T*T*ATCGAT*T*T*G*A*C*G*T*T**
118	**T*C*GTCGTTGTCGTTG*A*C*G*A*C*G*G*G**

in the above formula, * refers to a stereoisomer which has modified phosphate backbone and at least one of * in each formula is the Sp stereoisomer,

in the above formula, CG at a site corresponding to 5'-X_1X_2CpGX_3X_4-3' means non-methylated CpG without modified phosphate backbones.

3. The oligonucleotide as claimed in claim 1,

wherein the X_1X_2 is GA, and

wherein the X_3X_4 is TT or AC.

4. The oligonucleotide as claimed in claim 1,

wherein the at least one modified phosphate backbone at the site other than the part represented by 5'-X_1X_2CpGX_3X_4-3' comprises phosphorothioate.

5. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide comprisess a sequence represented as - $(G)_m$ - (m is an integer of 2 to 10) at either 5' end side or 3' end side of CpG motif, the CpG motif being the sequence of 5'-X_1X_2CpGX_3X_4-3'.

6. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide comprise a sequence represented as - $(G)_m$ - (m is an integer of 1 to 6) at 3' end side of CpG motif, the CpG motif being the sequence of 5'-$X_1X_2CpGX_3X_4$-3'.

7. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide comprises a sequence represented as TC, TA, TG, or CC at 5' end side of CpG motif, the CpG motif being the sequence of 5'-$X_1X_2CpGX_3X_4$-3'.

8. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide comprises at least a first CpG motif and a second CpG motif, the first and the second CpG motifs being the sequence of 5'-$X_1X_2CpGX_3X_4$-3',

the oligonucleotide comprises no sequence between the first CpG motif and the second CpG motif, or comprise a sequence represented as - $(T)_n$ - (n is an integer of 1 to 3), TA or TC between the first CpG motif and the second CpG motif.

9. The oligonucleotide as claimed in claim 1,

wherein the oligonucleotide consists of either one of following sequences or following sequences in which 1, 2, or 3 bases are substituted, inserted, deleted, or added:

$t^{sp}c^{sp}gacgtt^{sp}t^{sp}t^{sp}gacgtt^{sp}t^{sp}t^{sp}gacggg$ (SEQ No.13);

$t^{sp}c^{sp}gacgt^{sp}t^{sp}gacgt^{sp}t^{sp}gacggg$ (SEQ No.18); and

$g^{sp}g^{sp}gacgacgtcgtcg^{sp}g^{sp}g^{sp}g^{sp}g^{sp}g$ (SEQ No.44),

in the sequences “cg” indicates non-methylated CpG without modified phosphate backbone and “^{sp}” indicates that S type modified phosphate backbone is added between neighbouring nucleotides.

10. The oligonucleotide as claimed in claim 1, wherein the oligonucleotide consists of either one of following sequences:

$t^{sp}c^{sp}gacgtt^{sp}t^{sp}t^{sp}gacgtt^{sp}t^{sp}t^{sp}gacggg$ (SEQ No.13);

$t^{sp}c^{sp}gacgt^{sp}t^{sp}gacgt^{sp}t^{sp}gacggg$ (SEQ No.18); and

$g^{sp}g^{sp}gacgacgtcgtcg^{sp}g^{sp}g^{sp}g^{sp}g^{sp}g$ (SEQ No.44),

in the sequences “cg” indicates non-methylated CpG without modified phosphate backbone and “sp” indicates that S type modified phosphate backbone is added between neighbouring nucleotides.

11. A compound which comprises the oligonucleotide as claimed in claim 1.

12. A vaccine adjuvant which comprises the oligonucleotide as claimed in claim 1.

13. An agent for inducing generation of interferon-α (IFN-α) in dendritic cells, wherein the agent comprises the oligonucleotide in accordance with claim 1.

14. The therapeutic agent for infectious diseases, cancers, respiratory diseases, allergic diseases, autoimmune diseases, or wound healing,

wherein the agent comprises an effective amount of the oligonucleotides in accordance with claim 1 as an effective ingredient.