Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2879–2883, April 1996
Immunology

CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ

DENNIS M. KLINMAN*, AE-KYUNG YI†, SERGE L. BEAUCAGE‡, JACQUELINE CONOVER*, AND ARTHUR M. KRIEG†

*Section of Retroviral Immunology, Division of Viral Products, and ‡Division of Hematologic Products, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892-4555; and †Department of Internal Medicine, Division of Rheumatology, University of Iowa College of Medicine and Veterans Affairs Medical Center, Iowa City, IA 52242

Communicated by Marvin H. Caruthers, University of Colorado, Boulder, CO, December 15, 1995 (received for review October 12, 1995)

ABSTRACT **Bacterial infection stimulates the host to mount a rapid inflammatory response. A 6-base DNA motif consisting of an unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′ pyrimidines was shown to contribute to this response by inducing polyclonal B-cell activation. This stimulatory motif is 20 times more common in the DNA of bacteria than higher vertebrates. The current work shows that the same motif induces the rapid and coordinated secretion of interleukin (IL) 6, IL-12, and interferon γ (but not IL-2, IL-3, IL-4, IL-5, or IL-10) *in vivo* and *in vitro*. Stimulatory CpG DNA motifs induced B, T, and natural killer cells to secrete cytokine more effectively than did lipopolysaccharide. Thus, immune recognition of bacterial DNA may contribute to the cytokine as well as the antibody production characteristic of an innate inflammatory response.**

The mammalian immune system responds to bacterial infection by rapidly initiating an inflammatory reaction that limits the early spread of pathogens and facilitates the emergence of antigen-specific immunity (for review, see ref. 1). Microorganisms have evolved to avoid such recognition by altering their expression protein and lipid products (1). Yet DNA is an indispensable and highly conserved component of all bacteria. Indeed, the genomes of otherwise diverse bacteria share DNA motifs that are rarely found in higher vertebrates (2–4). Recent studies suggest that immune recognition of these motifs may contribute to the host's innate inflammatory response.

Yamamoto and others (5–9) showed that bacterial but not mammalian DNA boosted the lytic activity of natural killer (NK) cells and induced interferon γ (IFN-γ) production, effects attributed to palindromic sequences present in bacterial DNA (5, 10). Other investigators showed that bacterial DNA, especially when complexed to DNA-binding proteins, induced B-cell activation (3, 4, 7–9, 11). Recent work from our laboratory suggested that this B-cell stimulation was mediated by a 6-base nt motif consisting of an unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′ pyrimidines (12). This motif is expressed nearly 20 times more frequently in bacterial than vertebrate DNA (12–14).

In addition to B-cell activation, the innate immune response to bacterial infection is characterized by the production of immunomodulatory cytokines (15, 16). These include interleukin (IL) 6, which contributes to T- and B-cell activation (17–20), IFN-γ, which enhances the capacity of macrophages to eliminate a broad array of intracellular and extracellular pathogens (21), and IL-12, which regulates IFN-γ production and contributes to NK cell activation (22–24).

We examined whether bacterial DNA was involved in the release of immunomodulatory cytokines. Sensitive and specific ELIspot assays were used to monitor the secretory state of lymphocytes stimulated *in vitro* and *in vivo* with bacterial DNA or synthetic oligodeoxyribonucleotides (ODNs). Results indicate that ODNs containing unmethylated CpG dinucleotides induced the rapid and coordinated production of IL-6, IFN-γ, and IL-12 by NK, B, and $CD4^+$ T lymphocytes.

EXPERIMENTAL PROCEDURES

Animals. Eight- to 15-week-old female BALB/c and C57BL/6 mice were killed by cervical dislocation, and their organs were removed aseptically. Single spleen cell suspensions were prepared in complete medium (RPMI 1640 medium/10% fetal calf serum/1.5 mM L-glutamine/penicillin/streptomycin at 100 units/ml) as described (12).

Treatments. Cell suspensions were incubated at 37°C with DNA, ODN, or with lipopolysaccharides extracted from *Escherichia coli* (Difco) (12). Inhibition experiments were conducted by adding neutralizing anti-cytokine antibodies at a final concentration of 10 μg/ml to ODN-stimulated spleen cells. The monoclonal antibodies used were as follows: anti-IL-6 (clone MPS-20F3, Endogen, Cambridge, MA), anti-IFN-γ (clone R4-6A2, Lee Biomolecular Laboratories, San Diego), anti-granulocyte/macrophage colony-stimulating factor (clone MP1-22E9, Endogen), and anti-IL-12 (clone 17.8, from Georgio Trinchieri).

Cell Culture. Cells (2×10^5 per ml) were cultured in complete medium at 37°C in a 5% CO_2/air humidified incubator. Culture duration varied depending upon the experiment and nature of the assay.

Cytokine and IgM ELISA and ELIspot Assays. Nitrocellulose-backed microtiter plates (96-well Milititer HA plate, Millipore) were coated with primary anti-cytokine at 10 μg/ml or anti-IgM antibody as described (25, 26). Plates were blocked with phosphate-buffered saline (PBS)/1% bovine serum albumin and washed with PBS/0.025% Tween 20. Serial dilutions of a single-cell suspension were incubated on these plates for 4–6 hr at 37°C in a humidified 5% CO_2 incubator (25, 26).

Plates were overlaid with secondary biotinylated anti-cytokine antibody at 1 μg/ml (25, 26), washed, and treated with a 1:2000 dilution of avidin-conjugated alkaline phosphatase (Vector Laboratories) or phosphatase-conjugated anti-IgM antibody (Southern Biotechnology Associates). Individual cytokine or IgM-secreting cells were visualized and quantitated as described (25–27).

The concentration of cytokine in culture supernatants was determined by using a modification of the assay system described above. Supernatants were serially diluted in PBS/1% bovine serum albumin and incubated on anti-cytokine-coated microtiter plates for 2 hr. Biotinylated anti-cytokine antibody followed by avidin-conjugated alkaline phosphatase were used in a colorimetric assay to detect the presence of bound cytokine (27).

Cell Purification. Mononuclear cells were incubated with biotinylated anti-CD4, anti-CD8, anti-CD11, anti-CD14, or

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IFN, interferon; IL, interleukin; ODN, oligodeoxyribonucleotide; NK, natural killer; pODN, phosphorothioate.

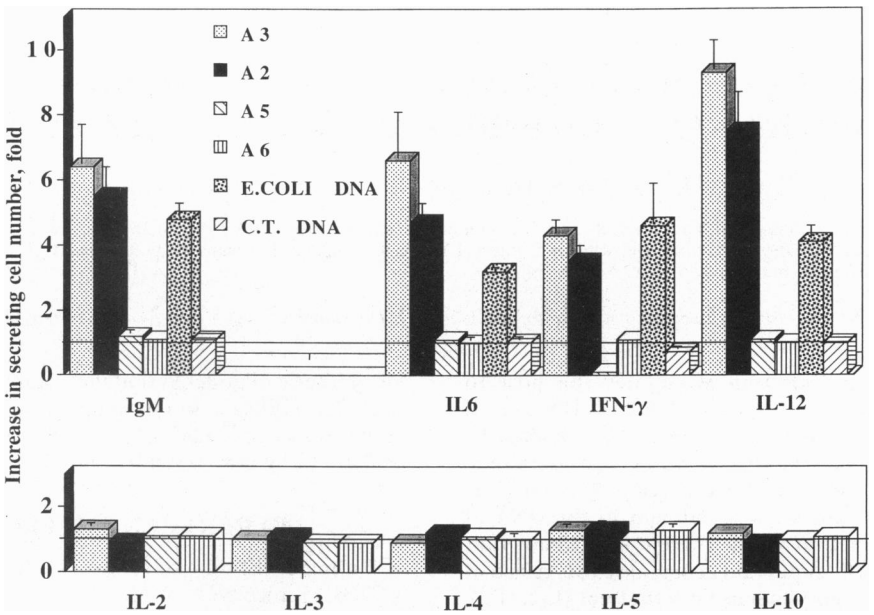

FIG. 1. Activation of cytokine-secreting cells by CpG-containing pODNs and *E. coli* DNA. BALB/c spleen cells were incubated with 1 μM of stimulatory (A1, A2) or control (A5, A6) pODNs, or *E. coli* at 100 μg/ml or calf thymus (C.T.) DNA. Optimal cytokine production was detected by ELIspot assay after 10–12 hr. The stimulation index is the fold increase in number of cytokine-secreting cells over background. See Table 3 for spontaneous cytokine production.

anti-NK1.1 antibodies (Becton Dickinson) for 15°C at 4°C. Cells were washed and incubated with avidin-conjugated MACS (magnetic cell separator) microbeads (Miltenyi Biotec, Sunnyvale, CA) at 4°C for 15 min. Phenotype-positive cells were then isolated using the MACS magnetic purification system (28). The purity of the isolated cell populations as determined by staining-sorted populations with fluorescein isothiocyanate labeled phenotype-specific monoclonal antibodies (PharMingen) followed by cell cytometry analysis was 87–94%.

Nucleic Acid Reagents. ODN and phosphorothioate ODN (pODN) were synthesized as described (12). *E. coli* DNA and calf thymus DNA were purchased from Sigma. All DNA and ODN preparations were purified by extraction with phenol/chloroform/isoamyl alcohol, 25:24:1 and/or ethanol precipitation. *E. coli* and calf thymus DNA were rendered single-stranded before use by boiling for 10 min and then cooled on ice for 5 min.

Data Analysis. All results represent the average of two to four separate experiments involving triplicate or serial-dilution assays. Statistical significance was established by using Student's *t* test.

RESULTS

Bacterial DNA and Synthetic ODNs Elicit the Production of IL-6, IL-12, and IFN-γ. Spleen cells from BALB/c mice were treated with bacterial DNA, mammalian DNA, or synthetic ODNs. Immune activation was monitored using ELIspot assays to detect and quantitate cells secreting specific inflammatory and immunoregulatory cytokines. As seen in Fig. 1, bacterial DNA stimulated normal spleen cells to produce IL-6, IFN-γ, and IL-12 (*Upper*), but not IL-2, IL-3, IL-4, IL-5, or IL-10 (*Lower*).

The capacity of LPS-free synthetic ODNs and nuclease-resistant pODNs to induce cytokine secretion was also examined. ODNs and pODNs with the same nucleotide sequence had similar properties. ODNs and pODNs that activated resting B cells to release IgM also stimulated whole spleen cells to produce IL-6, IFN-γ, and IL-12. In contrast, mammalian DNA and ODNs that did not stimulate IgM production did not induce cytokine secretion (Fig. 1 and Table 1).

DNA Motifs Containing a CpG Dinucleotide Trigger Cytokine Production. A panel of synthetic ODNs was used to examine the sequence motif(s) responsible for inducing the production of IL-6, IL-12, and IFN-γ by bacterial DNA.

Table 1. Stimulation of cytokine-secreting cells by CpG ODNs: Effect of multiple CpGs on cytokine production

pODN	Sequence	Stimulation index		
		IL-6	IL-12	IFN-γ
	E. coli DNA	3.2 ± 0.2	3.8 ± 0.4	4.7 ± 2.3
	Calf thymus DNA	0.8 ± 0.2	1.1 ± 0.2	0.8 ± 0.3
A1	TCCATGACGTTCCTGATGCT	3.3 ± 0.6	5.7 ± 1.3	3.0 ± 0.7
A2	GCTAGACGTTAGCGT	4.4 ± 0.5	7.7 ± 1.8	3.7 ± 0.4
A3	ATCGACTCTCGAGCGTTCTC	6.0 ± 2.7	9.9 ± 1.2	4.0 ± 0.6
A4	GAACCTTCCATGCTGTTCCG	1.3 ± 0.2	ND	ND
A5	GCTAGATGTTAGCGT	1.3 ± 0.3	1.1 ± 0.2	1.0 ± 0.1
A6	TCCATGAGCTTCCTGATGCT	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.2

BALB/c spleen cells were incubated with 1 μM of pODN, 30 μM of ODN (see Table 2), or DNA at 100 μg/ml for 10 hr. Stimulation index is calculated as fold increase in number of cytokine-secreting cells over background. The pODNs in Table 1 are representative of a set of 26 studied, whereas the ODNs in Table 2 are representative of >125 studied. ND, not done.

pODNs containing the dinucleotide CpG consistently triggered cytokine release (see ODNs A1, A2, A3, Table 1), whereas pODNs lacking this motif (A6) did not. Multiple CpGs generally resulted in greater stimulatory capacity (Table 1, compare pODN A1 with A3), although CpGs located at the terminus of an ODN were ineffective (A4, A5).

The latter finding suggested that sequences flanking the CpG dinucleotide contributed to ODN stimulatory capacity. To define the size and composition of this stimulatory motif, a series of >150 synthetic ODNs and pODNs was examined. Optimal stimulation of IL-6-, IL-12-, and IFN-γ-secreting cells was induced when a central CpG was flanked by two 5′ purines (GpA or ApA) and two 3′ pyrimidines (TpC or TpT) (Table 2, B1, B2, B8). Immune stimulation persisted despite purine/purine or pyrimidine/pyrimidine replacements (ODN B8), even if these substitutions eliminated a palindromic sequence (ODN B9). In contrast, stimulation was significantly reduced when a purine was substituted for pyrimidine, or vice versa, even if a palindromic sequence was maintained or created (B10, B11).

If either base pair of the CpG was eliminated, stimulatory activity was lost (B3, B4), whereas optimizing the flanking region (B5) or incorporating two CpGs into a single ODN increased stimulation. The minimal length of a stimulatory ODN was 8 bp (compare B1 to B6 and B7, Table 2).

Kinetics and Magnitude of the Cytokine Response Induced by CpG-Containing ODNs. pODNs induced cytokine release within 4 hr, with peak production at 12 hr (Fig. 2). Maximal cytokine production was observed using pODNs at a concentration 0.10–0.33 μg/ml (Table 2 and data not shown). By comparison, lipopolysaccharide-dependent cytokine production was observed over a broad concentration range, peaking at 10–30 μg/ml. The maximal amount of IL-6, IL-12, and IFN-γ released into culture after 48 hr of pODN treatment exceeded that induced by LPS 4–14 fold (Table 3).

Phenotype of Cells Triggered to Secrete Cytokine. Phenotype-specific monoclonal antibodies were used to identify $CD4^+$ and $CD8^+$ T cells, $CD14^+$ monocytes/macrophages, $CD11^+$ B cells and $NK1.1^+$ NK cells in normal mice. Each cell population was enriched by MACS sorting to 87–94% purity. Cell populations were then treated *in vitro* with stimulatory (A2, A3) or control (A5, A6) pODNs. CpG-containing pODNs stimulated purified B cells to release IL-6 and IL-12 (as well as IgM), $CD4^+$ T cells to release IL-6 and IFN-γ, and NK cells to release IFN-γ (Fig. 3). No significant effect on $CD8^+$ T cells or macrophages was observed.

The phenotype of cells activated to secrete cytokine *in vivo* was also examined. pODNs A1 and A2 injected i.p. into normal mice induced a significant increase in the number of spleen cells producing IL-6, IL-12, and IFN-γ within 4 hr. Phenotype-positive spleen cells from these animals were purified by MACS and analyzed for cytokine production. Consistent with results from *in vitro* experiments, B cells and $CD4^+$ T lymphocytes were the primary sources of pODN-induced IL-6 *in vivo*, B cells were the dominant source of IL-12, and NK and $CD4^+$ lymphocytes were major sources of IFN-γ (Fig. 3, *Lower*).

Table 2. Stimulation of cytokine-secreting cells by CpG ODNs: Identification of stimulatory motif

ODN	Sequence	Stimulation index IL-6	IL-12	IFN-γ
B1	TCAACGTT	3.5 ± 0.4	5.7 ± 1.7	4.3 ± 1.1
B2	G . . . _ _ . .	3.4 ± 0.6	5.0 ± 1.2	5.2 ± 0.9
B3	. . . . GC . .	1.1 ± 0.2	0.9 ± 0.1	1.0 ± 0.2
B4	. . . . . C . .	1.2 ± 0.2	1.0 ± 0.2	1.1 ± 0.2
B5	T_G . _ . C	7.1 ± 1.3	9.5 ± 2.8	8.1 ± 1.7
B6	. . . _ _ . .	1.2 ± 0.2	ND	ND
B7	. . _ _ . .	1.1 ± 0.1	1.1 ± 0.2	0.8 ± 0.1
B8	. . . G _ C .	4.2 ± 0.4	5.4 ± 1.0	5.9 ± 1.5
B9	. . . . _ C .	3.5 ± 0.5	4.6 ± 0.8	4.5 ± 1.1
B10	. . . T _ A .	1.5 ± 0.3	1.6 ± 0.4	1.3 ± 0.3
B11	. . TT _ AA	1.3 ± 0.4	1.4 ± 0.4	1.2 ± 0.2

See legend for Table 1. Dots indicate identity with ODN B1.

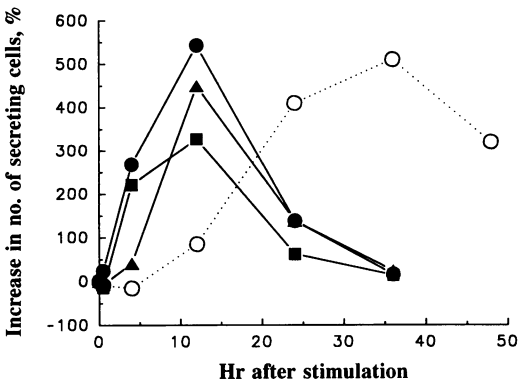

FIG. 2. Kinetics of activation of cytokine-producing cells by pODN. BALB/c spleen cells were incubated with pODN A1 at 1 μg/ml. The number of cells induced to secrete IL-6 (■), IL-12 (▲), IFN-γ (●), and IgM (○) was monitored by ELIspot assay. Results represent the percentage increase in number of antibody- or cytokine-secreting cells over background at each time point (see Fig. 1 legend for details). Similar results were obtained by using pODN A2.

Relationship Between ODN-Mediated Cytokine and Antibody Production. Kinetic studies indicated that there was a 24-hr lag between the peak activation of cytokine- versus IgM-secreting cells in ODN-treated cultures (Fig. 2). This difference in kinetics suggested that cytokines might contribute to the activation of IgM-producing lymphocytes. To investigate this possibility, anti-cytokine antibodies were added to cultures of pODN-treated spleen cells.

As seen in Table 4, neutralizing anti-IL-6 monoclonal antibody at 10 μg/ml significantly reduced the number of B cells secreting IgM after 36 hr of culture but had no effect on the production of IL-12 or IFN-γ. Antibodies reactive with granulocyte/macrophage colony-stimulating factor (another cytokine implicated in the regulation of immunoglobulin production), IL-12, or IFN-γ had no effect on IgM production. However, neutralizing anti-IL-12 antibodies significantly reduced the number of spleen cells stimulated by ODNs to secrete IFN-γ.

DISCUSSION

This study shows that a DNA motif consisting of an unmethylated CpG dinucleotide flanked by two 5′ purines and two 3′

Table 3. Concentration of cytokine in stimulated cultures

Treatment	Fold increase in cytokine concentration IL-6	IL-12	IFN-γ
pODN			
3.0 μg/ml	11.6	69.0	14.5
0.3 μg/ml	14.4	74.1	23.6
0.03 μg/ml	4.7	62.2	16.1
Lipopolysaccharide			
30.0 μg/ml	3.8	5.2	7.6
3.0 μg/ml	2.9	4.4	6.2
0.3 μg/ml	1.3	3.8	3.9
PBS	1.0	1.0	1.0

BALB/c spleen cells (2×10^5/ml) were incubated with pODN A1, lipopolysaccharide, or PBS for 48 hr. The relative concentration of IL-6, IL-12, and IFN-γ in these cultures was determined by limiting-dilution analysis of culture supernatants by ELISA assay.

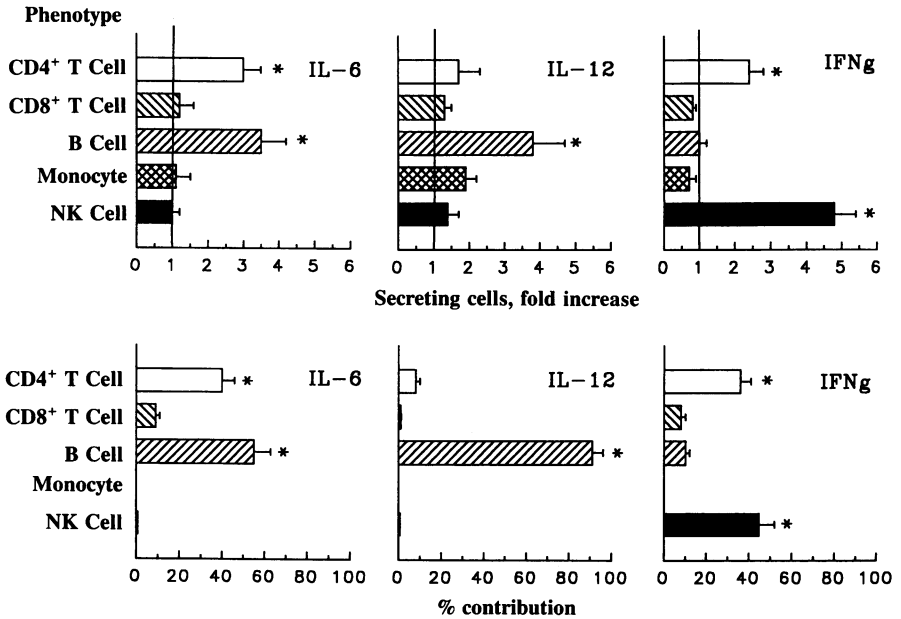

FIG. 3. Phenotype of cells induced by CpG ODNs to secrete cytokine. (*Upper*) Phenotype-positive cells were isolated by MACS technology. Each population was incubated *in vitro* with medium alone or 1 μM pODN (A1, A2). Control pODNs (A5, A6) did not induce cytokine production in any cell population. Phenotype-specific antibodies did not alter ODN-induced cytokine production by unseparated spleen cells. BALB/c mice were used to analyze the contribution of CD4-, CD8-, CD11-, and CD14-expressing cells, while C57BL/6 mice were used to analyze CD11-, CD14-, and NK 1.1-expressing cells. (*Lower*) Phenotype-positive cell populations were MACS purified from BALB/c or C57BL/6 spleens 8 hr after i.p. injection of 600 μg of pODNs A1 or A2. Percentage contribution of cells secreting cytokine *in vivo* is shown. No increase in cytokine production over control was seen in mice treated with the nonstimulatory pODN A6. Results represent the average of four experiments. *, Statistically significant increase ($P < 0.05$).

pyrimidines rapidly stimulates B cells to produce IL-6 and IL-12, $CD4^+$ T cells to produce IL-6 and IFN-γ, and NK cells to produce IFN-γ both *in vivo* and *in vitro*. Although lymphocyte stimulation was polyclonal and antigen-nonspecific in nature, specificity was retained with respect to the phenotype of cells activated and the type of cytokine they produced. The stimulatory motif is commonly expressed in bacterial but not mammalian DNA and elicited greater cytokine production at lower optimal concentrations than lipopolysaccharide.

Yamamoto *et al.* (5) showed that bacterial DNA could induce murine NK cells to produce IFN-γ and attributed this effect to palindromic sequences present in bacterial DNA (5, 10, 29). We find that immune stimulation is due to a 6-base pair motif containing a central CpG dinucleotide. Palindromes are not required because nucleotide substitutions eliminating palindromes but maintaining the CpG motif were immunostimulatory. Of note, the stimulatory palindromes identified by Yamamoto *et al.* (5) all contained CpG dinucleotides.

Table 4. Effect of anti-cytokine antibodies on ODN-induced cytokine production

Treatment	Inhibition of ODN-dependent activation, %			
	IL-6	IL-12	IFN-γ	IgM
ODN + control Ab	0	0	0	0
ODN + anti-IL-6	100*	0	6 ± 3	92 ± 4
ODN + anti-IL-12	0	100*	78 ± 4	0
ODN + anti-IFN-γ	0	0	100*	0
ODN + anti-GMCSF	0	0	0	0

Anti-cytokine antibody at 10 μg/ml was added to C57BL/6 spleen cells treated with 1 μM of stimulatory pODN (A1 or A2). The number of cells induced to secrete cytokine (after 10 hr) or IgM (after 36 hr) was monitored by ELIspot assay. The mean ± SD from three independent experiments is shown. The spontaneous (background) number of cytokine-secreting spleen cells was ≈900 per 10^6 for IL-6, 100 per 10^6 for IL-12, 500 per 10^6 for IFN-γ, and 1300 per 10^6 for IgM. GMCSF, granulocyte/macrophage colony-stimulating factor; Ab, antibody.

*The presence of anti-cytokine antibodies interferes with ELIspot detection of cells producing homologous cytokine.

The sequence motif identified in our studies is present at a much higher frequency in bacterial than mammalian DNA. This reflects the common use of methylated cytosines by mammalian but not microbial DNA, differences in the frequency with which 5′ purines and 3′ pyrimidines flank CpGs in these organisms, and the poorly understood phenomenon of "CpG suppression" in vertebrate DNA (3, 4, 12, 14). The finding that NK and T cells as well as B cells are triggered by CpG-containing ODNs suggests that immune recognition of this motif is evolutionarily conserved among multiple types of immunologically active cells.

The cytokines induced by CpG motifs perform critical immunomodulatory functions. IL-12 and IFN-γ promote type 1 cytokine production (22–24) and play important roles in the elimination of human pathogens (16, 23, 24, 30). IL-6 is a type 2 cytokine that facilitates the growth/differentiation of T and B lymphocytes (17–20) and stimulates immunoglobulin production (31). IL-6 knockout mice are extremely susceptible to infection (15, 32). Thus, bacterial DNA induces the production of cytokines involved in both cell-mediated and humoral immune responses.

Neutralizing anti-IL-6 antibodies significantly reduced ODN-dependent IgM production. Thus, B-cell-derived IL-6 may be acting in an autocrine or paracrine capacity to promote IgM secretion. Neutralization experiments also showed that anti-IL-12 antibodies significantly reduced the number of cells activated to secrete IFN-γ.

The ELIspot technique used in these studies detects cells secreting immunoglobulin and cytokine *in vivo* (26, 33). Indeed, the background levels of IL-6, IL-12, and IFN-γ production in untreated mice has been attributed to spontaneous immune activation caused by environmental and self-antigens in our specific pathogen-free colony (26, 27, 33). The 3- to 7-fold increase in spleen cells secreting cytokine after pODN treatment thus represents a profound increase over physiologic

rates of B, T and NK cell activation. The stimulatory effect of pODNs was not limited to BALB/c mice; similar results were observed when spleen cells from C57Bl/6 and the lipopolysaccharide nonresponsive C3H/HeJ mice were examined. It should be noted that all ODNs were synthesized and studied under identical conditions, eliminating the trivial possibility that bacterial infection and/or lipopolysaccharide contamination might account for differences in activity between ODNs with and without CpG dinucleotides.

Taken together, findings in this report support the view that a complex series of cytokine and cell-mediated interactions contribute to the host's innate response to microbial pathogens. Bacterial DNA, alone or in combination with other bacterial products, can trigger the release of IL-6, IL-12, IFN-γ, and IgM. The stimulatory capacity of CpG-containing DNA motifs may also confound studies involving antisense, gene therapy, and plasmid DNA vaccines produced in bacteria. Studying the interactions between bacterial products and the innate immune system should improve our understanding of the interface between antigen-specific and non-specific immune stimulation as it impacts on host protection.

We thank Dr. Basil Golding for his critical review of this manuscript and Drs. Georgio Trinchieri and Victor VanCleve for providing IL-12 specific reagents. A.M.K. is a Pfizer Scholar and was supported in part by grants from the Carver Trust, Arthritis Foundation, Lupus Foundation of America, the Department of Veterans Affairs and National Institutes of Health R29-AR42556-01.

1. Marrack, P. & Kappler, J. (1994) *Cell* **76,** 323–332.
2. Bird, A. P. (1987) *Trends Genet.* **3,** 342–347.
3. Gilkeson, G. S., Grudier, J. P., Karounos, D. G. & Pisetsky, D. S. (1989) *J. Immunol.* **142,** 1482–1486.
4. Messina, J. P., Gilkeson, G. S. & Pisetsky, D. S. (1991) *J. Immunol.* **147,** 1759–1764.
5. Yamamoto, S., Yamamoto, T., Shimada, S., Kuramoto, E., Yano, O., Kataoka, T. & Tokunaga, T. (1992) *Microbiol. Immunol.* **36,** 983–997.
6. Yamamoto, S., Katoaka, T., Yano, O., Kuramoto, E., Shimada, S. & Tokunaga, T. (1989) *Proc. Jpn. Soc. Immunol.* **48,** 272–281.
7. Field, A. K., Tytell, A. A., Lampson, G. P. & Hilleman, M. R. (1967) *Proc. Natl. Acad. Sci. USA* **58,** 1004–1010.
8. Oehler, J. R. & Herverman, R. B. (1978) *Int. J. Cancer* **21,** 221–229.
9. Vilcek, J., Ny, M. H., Friedmann-Kien, A. E. & Krawciw, T. (1968) *J. Virol.* **2,** 648–650.
10. Yamamoto, S., Yamamoto, T., Katoaka, T., Kuramoto, E., Yano, O. & Tokunaga, T. (1992) *J. Immunol.* **148,** 4072–4076.
11. Gilkeson, G. S., Pippen, A. M. & Pisetsky, D. S. (1995) *J. Clin. Invest.* **95,** 1398–1402.
12. Krieg, A. M., Yi, A., Matson, S., Waldschmidt, T. J., Bishop, G. A., Teasdale, R., Koretzky, G. A. & Klinman, D. M. (1995) *Nature (London)* **374,** 546–548.
13. Cardon, L. R., Burge, C., Clayton, D. A. & Karlin, S. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3799–3803.
14. Razin, A. & Friedman, J. (1981) *Prog. Nucleic Acid Res. Mol. Biol.* **25,** 33–52.
15. Van Damme, J., Schaafama, M. R., Fibbe, W. E., Falkenburg, J. H. F., Opdenakker, G. & Billiau, A. (1989) *Eur. J. Immunol.* **19,** 163–177.
16. Paul, W. E., Seder, R. A. & Plaut, M. (1993) *Adv. Immunol.* **53,** 1–29.
17. Uyttenhove, C., Couli, P. G. & Van Snick, J. (1988) *J. Exp. Med.* **167,** 1417–1427.
18. Muraguchi, A., Hirano, T., Tang, B., Matsuda, T., Horii, Y., Nakajima, K. & Kishimoto, T. (1988) *J. Exp. Med.* **167,** 332–340.
19. Le, J. M. & Vilcek, J. (1989) *Lab. Invest.* **61,** 588–602.
20. Hirano, T., Akira, S., Taga, T. & Kishimoto, T. (1990) *Immunol. Today* **11,** 443–449.
21. Murray, H. W. (1990) *Diagn. Microbiol. Infect. Dis.* **13,** 411–421.
22. Trinchieri, G. (1994) *Blood* **84,** 4008–4027.
23. Zhan, Y. & Cheers, C. (1995) *Infect. Immun.* **63,** 1387–1390.
24. Bohn, E., Heesemann, J., Ehlers, S. & Autenrieth, I. B. (1994) *Infect. Immun.* **62,** 3027–3032.
25. Klinman, D. M. & Nutman, T. B. (1994) in *Current Protocols in Immunology*, eds. Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M. & Strober, W. (Greene, Brooklyn, NY), pp. 6.19.1–6.19.8.
26. Shirai, A., Holmes, K. & Klinman, D. M. (1993) *J. Immunol.* **150,** 793–799.
27. Shirai, A., Sierra, V., Kelly, C. I. & Klinman, D. M. (1994) *Cytokine* **6,** 329–336.
28. Semple, J., Allen, D., Chang, W., Castaldi, P. & Freedman, J. (1993) *Cytometry* **14,** 955–960.
29. Yamamoto, T., Yamamoto, S., Katoaka, T. & Tokunaga, T. (1994) *Microbiol. Immunol.* **38,** 831–836.
30. Heinzel, F. P., Sadick, M. D., Mutha, S. S. & Locksley, R. M. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7011–7015.
31. Hirano, T., Yasukawa, K., Harada, H., Taga, T., Watanabe, Y., Matsuda, T., Kashiwamura, S., Nakajima, K., Koyama, K., Iwamatsu, A., Tsunasawa, S., Sakiyama, F., Matsui, H., Takahara, Y., Taniguchi, T. & Kishimoto, T. (1986) *Nature (London)* **324,** 73–76.
32. Kopf, M., Baumann, H., Freer, G. & Freundenberg, M. (1994) *Nature (London)* **368,** 339–341.
33. Klinman, D. M., Haynes, B. F. & Conover, J. (1995) *AIDS Res. Hum. Retroviruses* **11,** 97–105.