Clin Exp Immunol 1997; **109**:27–31

Specificity and immunochemical properties of antibodies to bacterial DNA in sera of normal human subjects and patients with systemic lupus erythematosus (SLE)

Z.-Q. WU, D. DRAYTON & D. S. PISETSKY *Medical Research Service, Durham VA Medical Centre, Division of Rheumatology, Allergy and Clinical Immunology, Department of Medicine, Duke University Medical Centre, Durham, NC, USA*

(Accepted for publication 25 March 1997)

SUMMARY

To elucidate the mechanisms of anti-DNA production, we assessed the binding of sera of normal human subjects (NHS) and patients with SLE to a panel of bacterial and mammalian DNA. Using single-stranded DNA as antigens in an ELISA, NHS showed significant binding to some but not all bacterial DNA, while lacking reactivity to calf thymus DNA. Among bacterial DNA, the highest levels of binding were observed with DNA from *Micrococcus lysodeikticus* and *Staphylococcus aureus*. In contrast, SLE sera showed high levels of binding to all DNA tested. To evaluate further immunochemical properties of the anti-DNA antibodies, the subclass distribution of these responses was evaluated by subclass-specific reagents. While NHS showed a predominance of IgG2 antibodies to bacterial DNA, SLE sera had a predominance of IgG1 antibodies to these antigens. Together, these results provide further evidence for the antigenicity of bacterial DNA and suggest that NHS and SLE anti-DNA differ in the patterns of epitope recognition as well as mechanisms of induction.

Keywords bacterial DNA anti-DNA antibodies antigenicity systemic lupus erythematosus

INTRODUCTION

DNA is a complex macromolecule whose immunological properties reflect structural microheterogeneity. Although mammalian DNA is immunologically inert, bacterial DNA displays potent immunostimulatory properties both *in vitro* and *in vivo*. These properties include the induction of cytokines as well as polyclonal activation of B cells [1,2]. As shown using synthetic oligonucleotides, immune activation by bacterial DNA results from sequences that consist of two 5′ purines, an unmethylated CpG core and two 3′ pyrimidines. These sequences are much more common in bacterial DNA than mammalian DNA, and suggest that bacterial DNA can function like endotoxin and trigger innate immunity during infection [3–7].

While the role of bacterial DNA in non-specific immune activation is speculative at this time, its ability to induce specific antibody responses appears well established. Thus, sera from normal human subjects (NHS) show significant binding to DNA from two bacterial species, *Micrococcus lysodeikticus* (MC) and *Staphylococcus epidermidis* (SE). These antibodies bind with high selectivity to their respective DNA antigens and do not cross-react with other DNA. NHS antibodies to MC DNA are predominately IgG2κ, suggesting induction by a T cell-independent mechanism that resembles the response to a bacterial carbohydrate [8,9]. Immunization experiments in mice fully confirm the ability of bacterial DNA to elicit specific antibody production [10,11].

As demonstrated with MC DNA, the immunochemical properties of anti-DNA antibodies in NHS differ markedly from those in patients with SLE. This prototypic autoimmune disease is characterized by anti-DNA that cross-react widely with DNA from various species and bind conserved backbone determinants. Furthermore, SLE anti-DNA are predominantly IgG1 and IgG3, with a more equivalent distribution of κ and λ light chains. The isotype distribution of these antibodies points to a T-dependent mechanism for anti-DNA autoantibody induction in disease [9].

In the initial studies on the immune response to DNA in NHS, only four bacterial DNA antigens were tested. To elucidate further the antigenicity of bacterial DNA and mechanisms of antibody induction, we assessed a larger panel of bacterial DNA antigens for binding by NHS and SLE sera. Single stranded (ss) preparations were tested as antigens by ELISA, using subclass-specific reagents to determine the isotype distribution of these responses. As shown here, NHS express antibodies to DNA from a variety of bacterial species. These responses all show an IgG2 predominance, in contrast to an IgG1 predominance in SLE sera. These findings provide further evidence for the immunogenicity of bacterial DNA and suggest that the mechanisms for anti-DNA production differ in normal immunity and SLE.

Correspondence: David S. Pisetsky MD, PhD, Durham VA Medical Centre, 508 Fulton Street, Box 151G, Durham, NC 27705, USA.

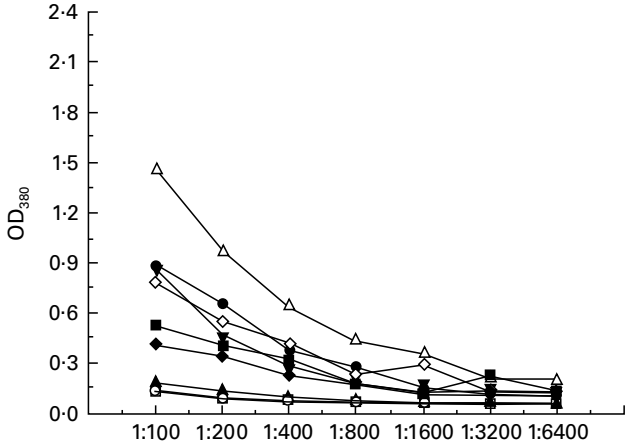

Fig. 1. Representative data on titrations of antibodies to bacterial DNA in a normal human serum. Eight bacterial species were tested. Calf thymus DNA was used for comparison. Results are presented as optical density (OD) 380. □, *Streptococcus pyogenes*; ○, Corynebacterium species; ●, *Micrococcus lysodeikticus*; ◇, *Clostridium perfringens*; ◆, *Salmonella typhimurium*; △, *Klebsiella pneumoniae*; ▲, calf thymus; ▼, *Proteus vulgaris*; ■, *Serratia marcescens*.

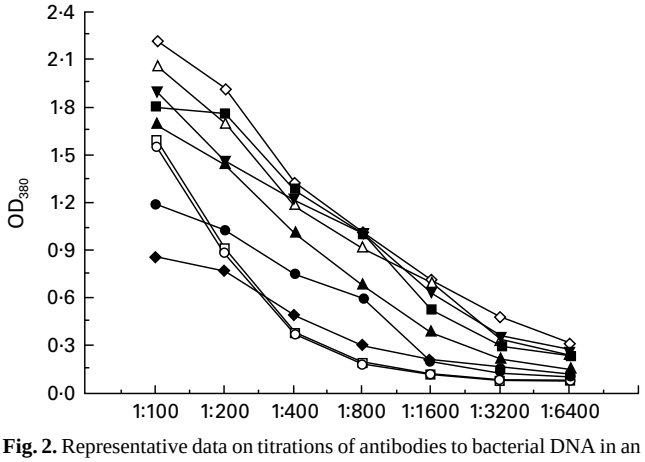

Fig. 2. Representative data on titrations of antibodies to bacterial DNA in an SLE serum. Eight bacterial species were tested. Calf thymus DNA was used for comparison. Results are presented as optical density (OD) 380. □, *Streptococcus pyogenes*; ○, Corynebacterium species; ●, *Micrococcus lysodeikticus*; ◇, *Clostridium perfringens*; ◆, *Salmonella typhimurium*; △, *Klebsiella pneumoniae*; ▲, calf thymus; ▼, *Proteus vulgaris*; ■, *Serratia marcescens*.

Table 1. Anti-DNA responses of SLE and normal human subject (NHS) sera to bacterial DNA*

	SLE	NHS
Bacterial DNA		
Streptococcus pyogenes	1·019 ± 0·443 ($n = 5$)	0·133 ± 0·012 ($n = 5$)
Corynebacterium sp.	1·041 ± 0·558 ($n = 5$)	0·146 ± 0·019 ($n = 6$)
Proteus vulgaris	1·846 ± 0·015 ($n = 5$)	0·641 ± 0·201 ($n = 10$)
Serratia marcescens	1·849 ± 0·156 ($n = 5$)	0·573 ± 0·198 ($n = 10$)
Haemophilus influenzae	2·108 ± 0·231 ($n = 10$)	0·858 ± 0·278 ($n = 10$)
Staphylococcus aureus	1·772 ± 0·103 ($n = 3$)	1·282 ± 0·121 ($n = 10$)
Enterobacter cloacae	1·432 ± 0·533 ($n = 6$)	1·073 ± 0·488 ($n = 6$)
Micrococcus lysodeikticus	2·031 ± 0·541 ($n = 6$)	1·310 ± 0·345 ($n = 12$)
Escherichia coli	1·561 ± 0·130 ($n = 6$)	0·730 ± 0·171 ($n = 6$)
Clostridium perfringens	1·833 ± 0·247 ($n = 4$)	0·687 ± 0·219 ($n = 10$)
Salmonella typhimurium	1·512 ± 0·160 ($n = 4$)	0·508 ± 0·327 ($n = 10$)
Mammalian DNA		
Calf thymus	1·652 ± 0·074 ($n = 4$)	0·264 ± 0·040 ($n = 10$)

* Results presented are the means ± s.d. of ELISA derterminations of anti-DNA activity. Values in parentheses indicate the number of sera tested.

MATERIALS AND METHODS

Sera

Twenty-three sera identified as NHS were obtained from normal healthy volunteers without known autoimmune diseases. Twelve sera from patients with the diagnosis of SLE by ACR Revised Criteria for SLE were obtained from the Duke University Medical Centre Clinical Immunology Laboratory. These sera were selected on the basis of an elevated activity to calf thymus (CT) dsDNA by ELISA. Because of availability, some sera were tested for activity with only some DNA. Of these sera, 12 NHS and eight SLE were chosen for IgG subclass assays because of high reactivity to individual DNA.

Antigens

Highly purified DNA from MC and CT were purchased from Sigma Chemical Co. (St Louis, MO). The following bacteria were grown in our laboratory and harvested for the production of DNA: *Salmonella typhimurium* (ST), *Streptococcus pyogenes* (SP), Corynebacterium species (CS), *Proteus vulgaris* (PV), *Serratia marcescens* (SM), *Haemophilus influenzae* (HI), *Clostridium perfringens* (CP), *Klebsiella pneumoniae* (KP), *Enterobacter cloacae* (E clo), and *Staphylococcus aureus* (SA). DNA were obtained using Qiagen Bacterial DNA Isolation Protocol (Qiagen Inc., Chatsworth, CA). These preparations were further purified by extraction with phenol, followed by isoamyl alcohol and chloro-

Table 2. Subclass distribution of anti-DNA in normal human subject (NHS) sera*

	IgG1	IgG2	IgG3	IgG4
Haemophilus influenzae	29·4 ± 4·1	33·0 ± 6·0	16·0 ± 4·6	17·8 ± 2·7
Micrococcus lysodeikticus	10·8 ± 2·1	80·4 ± 4·6	4·6 ± 4·6	4·1 ± 2·4
Clostridium perfringens	28·7 ± 6·0	57·6 ± 9·0	4·1 ± 3·4	9·0 ± 2·1
Klebsiella pneumoniae	11·9 ± 6·1	79·2 ± 7·3	4·3 ± 1·0	4·0 ± 2·2
Staphylococcus aureus	17·4 ± 8·0	63·7 ± 9·8	8·4 ± 0·4	10·6 ± 1·4

* Results presented are the means ± s.d. of percentage of the total IgG response as determined by isotype-specific assays. Twelve sera were tested. Because of low antigenicity, some of the bacterial DNA were not tested by this assay.

form. The DNA were precipitated and resuspended in Tris-EDTA buffer. ssDNA was obtained by boiling the native DNA for 10 min followed by immediate immersion in ice. The concentration of DNA was determined by absorbance measurement at OD_{260}, and purity was tested by OD_{260}/OD_{280} ratios. Digestion with pancreatic DNase (Sigma) was performed to assure that the antigenic component of all preparations was DNA. Synthetic oligonucleotides were purchased from Midland Certified Reagents (Midland, TX).

ELISA

For assay of anti-DNA, 96-well Immulon polystyrene plates (Dynatech Labs, Alexandria, VA) were coated with ssDNA in SSC (0·15 M NaCl, 0·015 M sodium citrate pH 8·0) at 5 μg/ml and incubated for 2 h at 37°C. The plates were then washed three times with PBS pH 7·4. Serial dilutions of sera in PBS–1% bovine serum albumin (BSA)–0·05% Tween 20 (PBS–T) were added to the plates and incubated for 1 h. After washing, peroxidase-conjugated goat anti-human IgG (γ-chain-specific; Sigma) diluted 1:1000 in PBS–T was added and the plates were incubated for 1 h at room temperature. Following further washing, a substrate solution of 0·015% 3,3′,5,5′-tetramethylbenzidine diluted 1:50 with 0·015% H_2O_2 in 0·1 M citrate pH 4·0 was added and the plates were incubated for 35 min at room temperature. Absorbance values were measured at 380 nm using a Titertek Multiskan Plus plate reader (Flow Labs, McLean, VA).

IgG subclass assay

Mouse monoclonal anti-IgG1 (HP-6001, 1:500), anti-IgG2 (HP-6014, 1:500), anti-IgG3 (HP-6050, 1:5000), anti-IgG4 (HP-6025, 1:2500) were used to detect the subclasses of anti-DNA antibodies in NHS and SLE sera. These antibodies were purchased from Sigma. These MoAbs were extensively pre-tested with standards derived from purified human myeloma proteins to produce dilutions of equivalent sensitivity. Briefly, plates were coated with different bacterial ssDNA as described above. After 2 h incubation, plates were washed and SLE or NHS sera were added at 1:50 and 1:100. After incubation for 1 h, plates were washed with PBS, followed by the addition of mouse monoclonal anti-human reagents. After incubation and washing, goat anti-mouse IgG peroxidase conjugate was added, followed by substrate and reading at optical density (OD) 380 as previously described. The percentage of each IgG subclass was calculated by dividing each individual absorbance value by the sum total of OD values of all four subclasses and multiplying by 100. Results are represented as the mean ± s.d. Statistical significance was assessed by the Microsoft Excel program.

RESULTS

To evaluate the antigenicity of bacterial DNA, a panel of highly purified ssDNA antigens was tested for activity in ELISA with sera from NHS as well as patients with SLE. Figure 1 presents representative data for a single NHS serum tested with nine of the DNA. As Fig. 1 shows, NHS showed minimal reactivity with SP and CS DNA and greater reactivity with the other DNA.

To demonstrate that all the DNA in the panel were antigenically active, the binding of an SLE serum was assessed under the same conditions (Fig. 2). As this figure shows, an SLE serum bound all DNA, including those that were inactive with NHS serum. Some differences in the magnitude of binding were observed, in accord with previous observations that SLE sera vary in the strength of interaction with ssDNA antigens [12]. In all instances, digestion of the DNA preparation by DNase eliminated activity in the ELISA.

Table 1 presents a comparison of responses of NHS and SLE sera. As these data indicate, SLE sera bound well to all DNA tested, while NHS showed variable levels of binding. For some DNA, levels of binding of NHS were similar to those of SLE. These findings are consistent with previous results showing that NHS sera do not react with mammalian DNA while nevertheless able to react with some but not all bacterial DNA [8].

In previous studies, antibodies to MC DNA in NHS sera were shown to display primarily the IgG2 isotype, suggesting induction by a mechanism similar to that of bacterial carbohydrates. This pattern contrasts SLE anti-DNA, which display an IgG1 predominance with both bacterial and mammalian [9]. To determine whether an IgG2 predominance is characteristic of the response to all bacterial DNA, anti-DNA assays were performed with subclass-specific reagents in the ELISA. These reagents were all used at concentrations that produced similar sensitivity and quantitative detection of antibodies. Tables 2 and 3 summarize these results and indicate that an IgG2 predominance characterizes the response to all bacterial DNA in NHS, whereas SLE anti-DNA directed to these same antigens are predominantly IgG1.

To determine whether antibodies in NHS or SLE sera are directed to immunostimulatory CpG motifs, sera were tested for binding to 30 mer oligonucleotides containing the sequences AACGTT or CGATCG in the context of dT residues. These sequences can induce antibody production *in vitro* [7,13]. As a control, oligo (dT) 20 was tested to assess the influence of flanking residues. As data in Fig. 3 indicate, NHS showed minimal binding to the synthetic oligonucleotides even with a CpG motif. In contrast, SLE sera bound at equivalent levels to the oligonucleotides,

Table 3. Subclass distribution of anti-DNA in SLE sera*

	IgG1	IgG2	IgG3	IgG4
Haemophilus influenzae	46·6 ± 18·5	31·4 ± 16·4	10·0 ± 11·6	4·6 ± 1·4
Micrococcus lysodeikticus	61.4 ± 16·6	21·0 ± 10·3	7·1 ± 7·2	10·5 ± 5·8
Clostridium perfringens	64·0 ± 15·2	20·2 ± 8·4	7·0 ± 6·5	8·9 ± 4·3
Klebsiella pneumoniae	60·7 ± 12·5	26·4 ± 16·5	9·0 ± 7·4	3·9 ± 2·6
Staphylococcus aureus	68·4 ± 3·1	23·2 ± 2·1	5·3 ± 0·4	3·2 ± 0·7

* Results are presented as described in Table 2. Eight sera were tested.

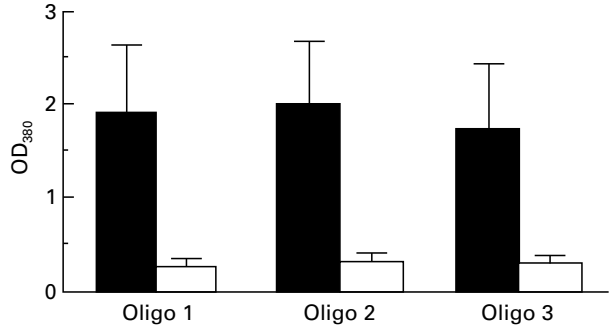

Fig. 3. The reactivities of normal human subject (NHS, □; $n = 5$) and SLE (■; $n = 5$) sera to 30 mer oligonucleotides containing immunostimulatory CpG motifs in the context of dT residues. Oligo 1, CGATCG; oligo 2, AACGTT; oligo 3, dT 20. Data are based on a 1:200 dilution.

suggesting interaction with a backbone determinant. These results suggest that bacterial DNA epitopes recognized by NHS differ from immunostimulatory motifs, at least as presented in the context of small oligonucleotides.

DISCUSSION

Results presented here demonstrate that sera of normal human subjects contain antibodies to DNA from a variety of bacterial species. These antibodies display the IgG2 subclass and differ in specificity and immunochemical properties from both anti-DNA autoantibodies found in SLE sera as well as naturally occurring autoantibodies that have also been described in NHS. Natural autoantibodies are IgM and bind DNA, among other antigens, at low avidity [14]. In contrast, antibodies to bacterial DNA are highly selective in their binding and have high avidity [15]. Since NHS bind DNA from both Gram-positive and Gram-negative organisms as well as pathogens and non-pathogens, immune responses to DNA appear to be a common feature of encounters with bacteria.

The conclusion of these studies contrasts with previous concepts on the immunology of DNA. Since conventional anti-DNA assays are rarely positive with sera of NHS or patients with other inflammatory diseases, anti-DNA have been considered virtually pathognomonic of SLE and a criterion in the classification of patients with SLE. These studies, however, were based on assays with very few DNA (including human, calf thymus, *Escherichia coli* and *Crithidia luciliae*), on the assumption that DNA are antigenically uniform because of the presence of conserved backbone determinants [14]. In our studies, NHS had low binding to *E. coli* DNA as well as other bacterial DNA and calf thymus DNA; in some instances, however, NHS reactivity to bacterial DNA approached that of SLE sera. The existence of a robust immune response to bacterial DNA was previously missed because of the failure to test appropriate DNA as antigens.

The predominant expression of the IgG2 isotype in NHS sera is consistent with a T cell-independent antibody induction that is similar to that for bacterial polysaccharides. According to the current model for these responses, B cell activation occurs when surface immunoglobulin receptors are cross-linked by antigen of repeating structure in the presence of cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) or interferon-gamma (IFN-γ) [16]. The source of these cytokines is natural killer (NK) cells or T cells which have been stimulated by bacterial antigens. Bacterial DNA is capable of inducing a T-independent mechanism, since the immunostimulatory motifs can cause the induction of IFN-γ among other cytokines; furthermore, like polysaccharides, DNA is a large molecule with multiple repeating determinants.

The response to bacterial DNA can be understood in terms of the role of two types of sequences. Immunostimulatory sequences with CpG motifs stimulate IL-12 and tumour necrosis factor-alpha (TNF-α) by macrophages; these cytokines can in turn cause NK cell production of IFN-γ, with consequent effects on B cells [5,6,17,18]. Other sequences on the bacterial DNA will bind surface receptors on B cells and trigger activation and antibody production. These determinants represent linear epitopes recognized by antibodies.

The relationship between immunostimulatory sequences and antigenic determinants has not yet been determined, although these sequences appear to be distinct. As shown previously, the binding of antibodies to MC DNA cannot be inhibited by SE DNA and *vice versa*, suggesting non-conserved sequences variably expressed on DNA depending on species origin [8]. This selective binding is consistent with a high-avidity interaction. Furthermore, we have shown in preliminary experiments that absorption of NHS on affinity columns of one bacterial DNA does not affect reactivity to other DNA (Drayton and Pisetsky, unpublished observations). Since the CpG motifs appear to be ubiquitous among bacterial DNA, the antigenic epitopes must have other sequences whose presence varies among bacterial DNA. The failure of NHS to bind to oligonucleotides bearing immunostimulatory motifs is consistent with this notion, although we cannot exclude the possibility that an immunostimulatory motif in the context of other bases or intact DNA may be antigenic.

While these considerations can account for the induction of anti-DNA by a T-independent mechanism, they do not explain the varying antigenicity of bacterial DNA that we observed. The

differences may reflect the extent of exposure to various bacteria; site of contact (e.g. skin *versus* gut); tolerance to DNA from certain organisms; content of antigenic determinants; and content of immunostimulatory motifs. In this regard, while CpG motifs have been proposed as a general trigger for innate immunity, bacterial DNA differ in the frequency of these motifs as calculated from available DNA sequences. Importantly, DNA from various bacterial species differ in their *in vitro* induction of IFN-γ, with levels consistent with the content of immunostimulatory motifs [5]. These considerations raise the possibility that the ability of a bacterial DNA to induce cytokines may determine the magnitude of induced response by T cell-independent mechanism [16].

Together, these studies suggest that the specificity and immunochemical properties of anti-DNA differ in NHS and patients with SLE. Whereas NHS produce antibodies to non-conserved linear sequences on bacterial DNA, SLE patients produce antibodies to conserved conformational determinants on all DNA. In this regard, we have noted variability in the activity of various DNA antigens in the ELISA. Such differences have been previously demonstrated and suggest that SLE anti-DNA may differ in avidity for conformational sites that are common among DNA [12]. The binding to a common conformational determinant is supported by studies indicating that various DNA can all inhibit binding of SLE anti-DNA to a bacterial DNA antigen [8,19].

A similar dichotomy in the binding specificity of normal and autoimmune anti-DNA has been observed in mice immunized with preparations of *E. coli* dsDNA as protein complexes in adjuvant. Under conditions in which normal mice produce antibodies that bind selectively to the immunizing bacterial antigen, autoimmune mice produce cross-reactive antibodies that bind both mammalian and bacterial DNA. These antibodies can also be distinguished by the pattern of reactivity with synthetic dsDNA antigens [10]. Studies on the sequences of induced antibodies suggest that abnormalities in the composition of the B cell repertoire (i.e. content of autoreactive precursors with certain variable region) may underlie the generation of cross-reactive anti-DNA in autoimmune mice [20].

These findings further define the antigenicity of bacterial DNA and the relationship between anti-DNA in the setting of normal and aberrant immunity. In conjunction with our previous studies as well as observations on antibody response to BK polyomavirus [21,22], they refute the notion that anti-DNA responses are exclusive to SLE. Indeed, they suggest that anti-DNA production accompanies many encounters with bacteria and viruses, and may be analogous to the response to bacterial carbohydrates in the mechanism of induction. Studies are in progress to identify the antigenic determinants on bacterial DNA and their range of immunomodulatory effects.

REFERENCES

1 Pisetsky DS. The immunological properties of DNA. J Immunol 1996; **156**:421–3.

2 Pisetsky DS, Reich C, Crowley SD *et al.* Immunological properties of bacterial DNA. Ann NY Acad Sci 1995; **772**:152–63.

3 Messina JP, Gilkeson GS, Pisetsky DS. Stimulation of *in vitro* murine lymphocyte proliferation by bacterial DNA. J Immunol 1991; **147**:1759–64.

4 Tokunaga T, Yano O, Kuramoto E *et al.* Synthetic oligonucleotides with particular base sequences from the cDNA encoding protein of *Mycobacterium bovis* induce interferons and activate natural killer cells. Microbiol Immunol 1992; **36**:55–66.

5 Yamamoto S, Yamamoto T, Shimada S *et al.* DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. Microbiol Immunol 1992; **36**:983–97.

6 Yamamoto S, Yamamoto T, Katoaka T *et al.* Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN and augment IFN-mediated natural killer activity. J Immunol 1992; **148**:4072–6.

7 Krieg AM, Yi A-K, Matson S *et al.* CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 1995; **374**:546–9.

8 Karounos DG, Grudier JP, Pisetsky DS. Spontaneous expression of antibodies to DNA of various species origin in sera of normal subjects and patients with systemic lupus erythematosus. J Immunol 1988; **140**:451–5.

9 Robertson CR, Gilkeson GS, Ward MM *et al.* Patterns of heavy and light chain utilization in the antibody response to single-stranded bacterial DNA in normal human subjects and patients with systemic lupus erythematosus. Clin Immunol Immunopathol 1992; **62**:25–32.

10 Gilkeson GS, Grudier JP, Karounos DG. Induction of anti-dsDNA antibodies in normal mice by immunization with bacterial DNA. J Immunol 1989; **142**:1482–6.

11 Gilkeson GS, Pritchard AJ, Pisetsky DS. Specificity of anti-DNA antibodies induced in normal mice by immunization with bacterial DNA. Clin Immunol Immunopathol 1991; **59**:288–300.

12 Yu C-L, Huang M-H, Tsai C-Y *et al.* The reactivity of sera from patients with systemic lupus erythematosus to seven different species of single and double stranded deoxyribonucleic acids. Clin Exp Rheumatol 1996; **14**:137–44.

13 Yi A-K, Chace JH, Cowdery JS *et al.* IFN-γ promotes IL-6 and IgM secretion in response to CpG motifs in bacterial DNA and oligonucleotides. J Immunol 1996; **156**:558–64.

14 Emlen W, Pisetsky DS, Taylor RP. Antibodies to DNA: a perspective. Arthritis Rheum 1986; **29**:1417.

15 Robertson CR Pisetsky DS. Specificity analysis of antibodies to single-stranded micrococcal DNA in the sera of normal human subjects and patients with systemic lupus erythematosus. Clin Exp Rheumatol 1992; **10**:589–94.

16 Snapper CM, Mond JJ. Towards a comprehensive view of immunoglobulin class switching. Immunol Today 1993; **14**:15–17.

17 Halpern MD, Kurlander RJ, Pisetsky DS. Bacterial DNA induces murine interferon-γ production by stimulation of interleukin-12 and tumor necrosis factor-α. Cell Immunol 1996; **167**:72–78.

18 Cowdery J, Chase JH, Yi A-K *et al.* Bacterial DNA induces NK cells to produce IFN-gamma *in vivo* and increases the toxicity of lipopolysaccharides. J Immunol 1996; **156**:4570–5.

19 Bunyard MP, Pisetsky DS. Characterization of antibodies to bacterial double-stranded DNA in the sera of normal human subjects. Int Arch Allergy Immunol 1994; **105**:122–7.

20 Gilkeson GS, Bloom DD, Pisetsky DS *et al.* Molecular characterization of anti-DNA antibodies induced in normal mice by immunization with bacterial DNA. Differences from spontaneous anti-DNA in the content and location of V CDR3 arginines. J Immunol 1993; **151**:1353–64.

21 Fredriksen K, Skogsholm A, Flaegstad T *et al.* Antibodies to dsDNA are produced during primary BK virus infection in man, indicating that anti-dsDNA antibodies may be related to virus replication *in vivo*. Scand J Immunol 1993; **38**:401–5.

22 Fredriksen K, Osei A, Sundsfjord A *et al.* On the biological origin of anti-double-stranded (ds) DNA antibodies: systemic lupus erythematosus-related anti-dsDNA antibodies are induced by polyomavirus BK in lupus-prone (NZB$\times$NZW) F_1 hybrids, but not in normal mice. Eur J Immunol 1994; **24**:66–70.