CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Jan. 1998, p. 1–6 Vol. 5, No. 1
1071-412X/98/$04.00+0
Copyright © 1998, American Society for Microbiology

MINIREVIEW

Antibody Responses to DNA in Normal Immunity and Aberrant Immunity

DAVID S. PISETSKY*

Rheumatology Section, Medical Research Service, Durham Veterans Affairs Medical Center, and Division of Rheumatology, Allergy and Clinical Immunology, Duke University Medical Center, Durham, North Carolina 27705

Among antibodies directed to biological macromolecules, antibodies to DNA (anti-DNA) are unique in their association with the pathological state. These antibodies are the serologic hallmark of systemic lupus erythematosus (SLE) and serve as markers for diagnosis and prognosis. Furthermore, as indicated by the correlation of antibody levels with disease activity, anti-DNA play a major role in the pathogenesis of lupus nephritis. The close association of anti-DNA with SLE has implied that immune responses to DNA are an exclusive feature of autoimmune disease (5).

Although assessment of anti-DNA remains clinically valuable, recent data suggest that the current conceptualization of anti-DNA needs revision. These data provide a new perspective on lupus serology and show clearly that production of anti-DNA occurs in hosts with normal immunity as well as those with aberrant immunity. The salient feature of the production of normal anti-DNA, however, is its specificity for bacterial DNA. Furthermore, as shown in studies with both mice and humans, antibody induction is just one facet of bacterial DNA's far-reaching immunological properties. This review considers current information on antibody responses to DNA, focusing on their role in SLE as well as normal host defense.

IMMUNOLOGY OF SLE

As a systemic autoimmune disorder, SLE is associated with protean manifestations that can involve essentially every organ in the body. These manifestations occur unpredictably and vary in frequency and intensity among patients (30). Despite the marked heterogeneity in clinical features, patients with SLE almost invariably express antibodies to components of the cell nucleus (antinuclear antibodies). These antibodies are highly diverse and target a host of nuclear macromolecules. Of these antibodies, however, only two, anti-DNA and anti-Sm antibodies, represent criteria for disease classification. By conventional assays, these antibodies are found essentially only in the sera of patients with SLE. Whereas the levels of anti-DNA vary during the course of disease, anti-Sm antibody levels remain more static, limiting their utility in patient monitoring (25, 41).

Of the manifestations of SLE, anti-DNA are most closely linked with glomerulonephritis. The capacity of anti-DNA to cause renal damage has been confusing since DNA, like other nuclear antigens, is ubiquitous among cells and is sequestered intracellularly. Following injury or death, however, cells may release DNA, providing a source of extracellular antigen that can form phlogistic complexes. Since DNA exists in nucleosomes inside the cell, any released antigens are likely to exist as complexes whose protein components may also influence pathogenicity (5, 6).

While the mechanisms of lupus nephritis are not well understood, anti-DNA may provoke renal injury by one of four mechanisms: formation of circulating immune complexes, in situ immune complex formation in the kidney with DNA trapped in the glomerulus, cross-reactive binding to a non-DNA glomerular antigen, and penetration of antibodies into glomerular cells. Evidence of the pathogenicity of anti-DNA comes from both the correlation of the levels of anti-DNA with renal activity as well the provocation of nephritis in animals by infusion of preparations of anti-DNA (6, 34, 42, 46, 47). Other manifestations of SLE are less clearly related to anti-DNA, although they may result from other pathogenic autoantibodies.

ANTIGENICITY OF DNA

Because of the role of anti-DNA in the pathogenesis of disease, assays for antibody measurement have been directed to two major goals: (i) providing specific markers for patient diagnosis and (ii) providing sensitive markers for disease activity. Underlying the use of these assays has been the notion that antibodies that bind with high affinities to double-stranded DNA (dsDNA) are the most specific and reliable for diagnosis. Assays that detect antibodies to single-stranded DNA (ssDNA), however, generally yield a higher frequency of positive responses among patient sera, most likely because of the detection of a broader array of specificities, including low-affinity antibodies (1, 19, 25, 41).

The distinction between antibodies to ssDNA and antibodies to dsDNA, while often emphasized in studies on serology, is somewhat artificial because many antibodies can bind to both antigenic DNA forms. Indeed, only a minority of antibodies have exclusive specificity for either ssDNA or dsDNA. The ability to bind to both DNA forms suggests reactivity with determinants on the phosphodiester backbone that can be present on either the ssDNA or the dsDNA antigen. In its antigenic properties, ssDNA may be more active than dsDNA since it is structurally flexible and can interact more readily with antibody in solution than the more rigid and rod-like dsDNA (3, 4, 39).

Assays for anti-DNA have used DNA from only a limited number of species on the assumption that all DNAs are antigenically alike by virtue of their backbone and, in the case of dsDNA, their display of the classic Watson-Crick double helix, also called B DNA. This assumption was never investigated in

* Mailing address: VA Medical Center, Box 151G, 508 Fulton St., Durham, NC 27705. Phone: (919) 286-6835. Fax: (919) 286-6891. E-mail: dpiset@acpub.duke.edu.

any detail, in part, because most assays for anti-DNA perform well in the clinic and produce comparable results for diagnosis and disease assessment.

INDUCTION OF ANTI-DNA

The strong association of anti-DNA with autoimmunity has been confirmed in experiments replicating lupus by immunizing normal mice with DNA. Even when coupled to a protein carrier and presented in adjuvant, mammalian DNA fails to elicit significant antibody production (22). This failure is in contrast to animal models of disease induced by immunization with protein autoantigens (e.g., collagen-induced arthritis or experimental allergic encephalomyelitis), suggesting that DNA differs from other biological macromolecules in its immunological capacity.

The weak activity of DNA in immunization models is in contrast to its apparent strong activity in spontaneous disease. Thus, as shown by molecular analysis of monoclonal antibodies from patients as well as mice with lupus, anti-DNA bear the features expected for an antigen-specific response to DNA. These features include clonal expression, V-region somatic mutations, and a high content of heavy-chain CDR3 arginines. Since arginine can bind to DNA by both electrostatic interactions and hydrogen bonds, these findings have suggested that anti-DNA in SLE are selected by a receptor mechanism, with DNA being the relevant antigen in vivo (31). These observations have further suggested that SLE represents a unique setting for the expression of anti-DNA, with flagrant immunoregulatory disturbances allowing for immune recognition of an otherwise inert molecule.

IMMUNOLOGICAL PROPERTIES OF BACTERIAL DNA

While this conceptualization of DNA's immunological properties has long dominated investigations of SLE, it is nevertheless flawed. As recent data show, DNA, rather than being uniform and bland, is immunologically complex, with sequence microheterogeneity contributing to a variety of immunological properties. Indeed, bacterial DNA, by virtue of characteristic sequence motifs, can activate the immune system and drive the production of antibodies to sequential as opposed to backbone DNA determinants (26, 27). In its antigenic properties, foreign DNA resembles foreign proteins in that it has an epitope structure based on nonconserved sequences that are absent from the host and that are therefore not subject to tolerance.

The existence of antibody responses to bacterial DNA was long missed because of a failure to survey an adequate number of DNAs for their activities with sera from normal hosts as well as sera from hosts with SLE. As many studies showed, sera from hosts with SLE recognize predominantly backbone determinants which can be presented by any DNA. Among the commonly used assays, various DNA sources were in fact used to measure antibodies to these backbone determinants. Since these assays effectively distinguished sera from normal subjects from sera from patients with SLE, there was little reason to suspect that differences in the behavior of DNA from other sources.

The first clear evidence for the antigenic heterogeneity of DNA came in an analysis of binding of sera to a panel of DNAs from various mammalian and bacterial species (15). The goal of these investigations was to determine whether the source of DNA antigen could influence the quantitative detection of antibodies in an enzyme-linked immunosorbent assay. These effects could reflect either the influence of a base sequence on the backbone orientation or the presence in the sera of patients with SLE antibodies that bound to the sequence as well as to the backbone. A study by Stollar et al. (40) raised these possibilities, although these observations were never pursued in detail.

As shown by Karonous et al. (15), sera from patients with SLE generally bind to all DNAs equivalently, consistent with the importance of the backbone to antigenicity. In marked contrast to previous studies, however, those investigators found that normal human serum (NHS) shows highly significant binding to DNA from certain bacteria, including *Staphylococcus epidermidis* and *Micrococcus lysodeikticus*. These antibodies were of the immunoglobulin G (IgG) isotype and were present at levels comparable to those in the sera of patients with SLE. Despite binding to these bacterial DNAs, NHS did not bind to mammalian DNA and therefore differed from natural autoantibodies which are IgM and bind to DNA broadly (15).

Subsequent studies on the specificities of these responses have demonstrated that NHS can bind to DNA from many bacterial species, although, interestingly, they do not bind to DNA from *Escherichia coli*. This binding is very species specific. Thus, antibodies that bind to the DNA of one bacterial species do not bind cross-reactively to the DNA of another bacterial species (48). Furthermore, antibody reactivity extends to viral DNA, since NHS binds to DNA from BK polyomavirus (7). While not anticipated from previous work, anti-DNA in NHS can easily be explained as a specific response, induced during ordinary encounters with bacteria or viruses, to sites on foreign DNA that differ in sequence from the host DNA.

As shown in an analysis of antibodies to *Micrococcus* DNA, anti-DNA in NHS differ from anti-DNA in sera from patients with SLE in important immunochemical properties. Thus, anti-DNA in NHS are primarily IgG2 and show restriction to the κ light chain; in contrast, anti-DNA in sera from patients with SLE are primarily IgG1 and have more equivalent levels of expression of κ and λ. The predominance of IgG2 is reminescent of the response to bacterial polysaccharide antigens and a T-cell-independent response. Other differences between anti-DNA in NHS and sera from patients with SLE include the high degrees of specificity and avidity of anti-DNA in NHS and the role of nonionic interactions in antibody binding (32, 33). Like anti-DNA in sera from patients with SLE, however, anti-DNA in NHS can bind both ssDNA and dsDNA (2). Together, these properties indicate that anti-DNA in NHS bind selectively to nonconserved sequences on foreign DNA. These antibodies are probably not pathogenic because of their isotype, which does not fix complement well, and the limited availability of their target antigen, which should disappear as the infection resolves.

To test the possibility that bacterial DNA can drive antibody production, normal mice were immunized with bacterial DNA as complexes with methylated bovine serum albumin in complete Freund's adjuvant. Although mammalian DNA elicits a limited response to ssDNA but not to dsDNA under these conditions, bacterial DNA induces abundant antibody production. By using bacterial dsDNA as the immunogen, the induced antibodies bind only to bacterial dsDNA without cross-reactivity to mammalian dsDNA. In contrast, immunization with bacterial ssDNA leads to a cross-reactive response to both mammalian and bacterial ssDNAs (10, 11).

IMMUNOSTIMULATION BY BACTERIAL DNA

Although the immunogenicity of bacterial DNA could reflect its content of nonconserved sequences, subsequent stud-

ies have shown that bacterial DNA has immunostimulatory properties that enhance responsiveness. These properties were initially discovered in studies of the antitumor effects of extracts of mycobacteria. These extracts promoted tumor resistance by stimulation of natural killer cell activity through the action of alpha/beta interferon (IFN-α/β) and IFN-γ. As shown by biochemical fractionation studies, mycobacterial DNA is the active component of these extracts, with further studies demonstrating that DNAs from many different bacteria produce the same stimulatory effects (43, 49, 50).

In a series of elegant experiments with cloned DNA, cytokine induction was shown to result from sequence motifs characteristic of bacterial DNA (16, 51). These sequences, called CpG motifs or immunostimulatory sequences (ISSs), have the general structure of two 5′ purines, an unmethylated CpG dinucleotide, and two 3′ purines. ISSs occur in bacterial DNA much more frequently than in mammalian DNA for at least two reasons. In mammalian DNA, cytosine is commonly methylated. Furthermore, cytosine and guanosine occur in tandem much less frequently than predicted by base composition, a phenomenon known as CpG suppression (13, 20). While the biological advantages of cytosine methylation and CpG suppression are speculative, the difference in the occurrence of mammalian and bacterial DNAs creates a system for immune recognition.

The immunostimulatory activities of bacterial DNA are varied and encompass the mitogenicity of B cells and the induction of cytokines including IFN-α/β, IFN-γ, tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-12 (17, 21, 23). Together, these activities resemble those of endotoxin and suggest that bacterial DNA may have a similar role in innate immunity. In current terminology, bacterial DNA may function as a danger signal. Since these activities poise the immune system for responsiveness, they may explain the effectiveness of bacterial DNA as an immunogen. The CpG motifs are common to all bacterial DNAs, however, and must differ from the target sequences of anti-DNA in NHS which are variably present in bacterial DNAs, depending on the species.

ROLE OF BACTERIAL DNA IN SLE

The observations described above suggest a plausible mechanism by which bacterial DNA can drive antibody responses in normal immunity and aberrant immunity. In normal individuals, bacterial DNA can elicit antibodies that are highly specific for nonconserved sequential determinants on the DNA from infecting or colonizing organisms. These antibodies may arise from a T-cell-independent mechanism, reflecting the ability of bacterial DNA to both cross-link B-cell surface receptors and induce cytokine production. In their induction, these antibodies would resemble antibodies to bacterial carbohydrate. While T-cell-dependent induction of antibodies to bacterial DNA is possible, it does not appear to be a major mechanism. The preference for T-cell independence may result from the pattern of cytokines produced in the setting of bacterial infection as well as the manner in which foreign DNA is presented to the immune system.

In contrast to the situation in normal immunity, in SLE, bacterial DNA may drive the production of antibodies to conserved backbone determinants by a T-cell-dependent mechanism. The switch from T-cell independence to T-cell dependence may result from the presence of aberrantly expressed helper T cells that arise in SLE because of abnormalities of tolerance, T-cell activation, or the cytokine mileu. In this regard, SLE is associated with an expansion of DNA-binding B cells. These B cells can bind to DNA-protein complexes and serve as antigen-presenting cells. These cells may facilitate a T-cell-dependent response to DNA as well as to the attached proteins, a mechanism that does not require direct T-cell recognition of DNA.

As these considerations suggest, encounters with bacterial DNA may be particularly hazardous in SLE because this antigen displays both self- and non-self-determinants. The self-determinants are the backbone, while the non-self-determinants are the sequences. In SLE, responses to the self-determinants may reflect a general tendency for recognition of conformational epitopes as opposed to sequential epitopes. This tendency, which would heighten and broaden cross-reactivity, is also manifest in the response to protein autoantigens such as Sm, Ro, and La. Autoantibodies to these proteins bind to conserved sites on these antigens, leading to cross-reactivity with antigens from species such as rabbits or cows. Furthermore, autoantibodies in SLE are less likely to bind to peptidic determinants than antibodies induced in normal animals by intentional immunization with self-antigen (41).

In SLE, the preferential recognition of conformational determinants may reflect tolerance abnormalities that allow for the retention of B cells that would be deleted or anergized in normal individuals. These B cells may display V gene sequences that promote cross-reactivity and the binding to conformational determinants on self-antigen and foreign antigen. Evidence for this model comes from experiments assessing the response of NZB/NZW autoimmune mice to immunization with bacterial dsDNA. In these mice, bacterial DNA induces cross-reactive autoantibodies that bind to self-DNA as well as foreign dsDNA under conditions in which immunization elicits antibodies specific for bacterial dsDNA in normal mice. Importantly, the induced antibodies from the autoimmune mice differ from those from normal mice in certain sequences (e.g., CDR3 arginines) considered important for binding to dsDNA. CDR3 arginines occur rarely in antibodies from normal mice, possibly because their presence leads to DNA binding and the induction of tolerance (8, 9).

In addition to the production of autoantibodies to dsDNA, SLE may be associated with a diminution in the production of antibodies specific for bacterial DNA. As shown by immunoaffinity techniques, absorption of sera from hosts with SLE with mammalian DNA eliminates essentially all reactivity to both mammalian and bacterial DNA. In contrast, absorption of normal sera with mammalian DNA does not affect the response to bacterial DNA. These findings indicate a deficiency in antibodies specific for foreign DNA in SLE (29). This deficiency could be secondary to a shift toward recognition of conserved DNA determinants during ongoing autoimmune disease. Alternatively, the deficiency may be a primary abnormality and, indeed, a factor predisposing an individual to SLE. Thus, in the absence of a specific antibody response in SLE, bacterial DNA may persist in the system, leading to prolonged immune stimulation and the emergence of cross-reactive autoantibodies. This situation would be analogous to the induction of autoimmunity in animals by repetitive treatment with polyclonal B-cell activators such as lipopolysaccharide (14).

ROLE OF IMMUNOSTIMULATORY DNA IN INFECTION

The discovery of bacterial DNA's immunological properties has broad implications, both theoretical and practical. Certainly, the central dogma of SLE needs revision and the simple equation anti-DNA = autoimmunity needs to be discarded. In the future, any model of production of anti-DNA in SLE must take into account the immunological diversity of DNA and the existence of responses of anti-DNA in both normal immunity

as well as SLE. As studies with both humans and mice suggest, the difference in DNA recognition in normal immunity and SLE may reside at the level of specificity rather than responsiveness. As such, production of autoantibodies to DNA may represent a distortion in the response to an ordinarily active foreign antigen rather than the acquisition of the response to an ordinarily inactive self-antigen.

While NHS contains antibodies to many different bacterial and viral DNAs, the rules for antigenicity are not known. It is not clear why NHS binds well to DNAs from certain bacteria and not to DNAs from other bacteria. These differences could reflect differences in the number and structure of antigenic sequences, the content of ISSs, or the location and extent of contact of the bacterium with its host. In this regard, it is reasonable to inquire whether antibody responses to bacterial DNA could be used diagnostically, with elevation of antibody titers to a bacterial DNA antigen being indicative of infection. This serologic approach could be especially useful for evaluating infections caused by organisms that are difficult to culture.

The role of immunostimulatory DNA in the pathogenesis of infection is a topic of emerging interest. While purified bacterial DNA as well as synthetic ISSs have impressive immunostimulatory activities, the relevance of these activities to human disease is much less certain. Recent studies indicate, for example, that bacterial DNA can cause septic shock and can promote serious pulmonary inflammation when it is administered to animals (36, 37). Determining whether bacterial DNA exerts these effects during ordinary infection will be a major undertaking, since bacteria have many immunostimulatory molecules (e.g., lipopolysaccharide) with similar activity. If, during infection, bacterial DNA, alone or in synergy with other dangerous molecules, provokes harmful reactions, then the use of strategies that can speed its elimination may be worthwhile for future antimicrobial therapy.

The consequences of exposure to DNA to animals and humans may vary somewhat among species. Thus, under ordinary culture conditions in vitro, bacterial DNA or CpG motifs fail to stimulate human B cells, although they effectively trigger murine B cells. In both humans and mice, however, bacterial DNA can induce cytokine production (26, 27). These differences in response patterns may reflect differences in binding and uptake of DNA by cells as well as intrinsic differences in cell activation. The induction of cytokines by bacterial DNA nevertheless appears to be a common mode of action in both humans and animals, suggesting that foreign DNA can serve the same immediate role in promoting inflammation and inducing host defense in humans as well as animals.

IMPLICATIONS FOR DNA THERAPEUTICS

While the involvement of bacterial DNA in infection is speculative, the medicinal use of DNA will expose the host to ISSs. Indeed, these ISSs may be key to the success of some of these approaches. Among recent advances in DNA therapeutics, DNA vaccines have attracted enormous interest because of their potential to induce responses against a broad range of human pathogens. These vaccines are plasmids that encode a protein to be targeted for protective immunity. These vaccines are administered as naked DNA by the intramuscular or intradermal route and are taken up into cells and induce both CD4 and CD8 responses (24, 45). While the trafficking of the plasmids in vivo is poorly understood, vaccine responses ultimately involve bone marrow-derived antigen-presenting cells.

Since they are propagated in bacteria, vaccine vectors are potentially an important source of ISSs. These vectors display the bacterial pattern of DNA methylation and, in addition, have the bacterial sequences needed for replication or antibiotic resistance; other foreign DNA sequences may relate to the encoded protein. As such, these vectors can exert adjuvant properties and, through the mediation of IL-12, IFN-α/β, and IFN-γ, promote Th1 responses. As shown in recent studies, plasmid-borne ISSs may be key to the induction of cellular as well as antibody responses. Indeed, the potencies of these vaccines may reflect their ability to serve as internal adjuvants as well as provide an intracellular source of foreign protein for processing and presentation to T cells (18, 35).

Although ISSs can facilitate vaccination, they may also cause adverse reactions. These reactions include local inflammation, nonspecific immune stimulation, and skewing of responses to a Th1 pattern. Depending on the setting, these reactions could be detrimental and could, for example, potentiate autoimmunity or impair the response to infecting organisms. Furthermore, plasmid vaccines could induce the production of anti-DNA, although the outcome of any induced response would likely vary depending on the immune status of the host. In a normal individual, the vaccine could induce antibodies specific for the plasmid, which, like those in normal individuals, would be nonpathogenic. On the other hand, in an individual predisposed to autoimmunity, the induced antibodies could have cross-reactive autoantibody activity.

The likelihood of adverse reactions from a DNA vaccine appears low, however, since bacterial DNA is a normally encountered antigen and the amount of DNA used for vaccination is small. Indeed, initial experience with DNA vaccines in both animals and humans suggests that naked DNA is safe. Strategies involving other vaccine components, however, could be more problematic. Agents such as lipofectin, which can coat DNA and promote its uptake into cells, can amplify immunostimulatory effects and increase the likelihood of inflammatory or autoimmune reactions (52). In this regard, similar issues of safety pertain to the use of naked DNA for gene therapy.

Antisense agents are another innovative form of nucleic acid therapy that may provoke immunostimulatory effects. These agents are short oligonucleotides complementary to an mRNA sequence for a protein whose functional elimination would be therapeutic; mRNA binding by these oligonucleotides prevents translation or promotes degradation. The range of proteins postulated for use in antisense therapy is enormous and varies from oncogenes to viral proteins to cellular macromolecules (e.g., cytokines or adhesion molecules) whose overexpression can promote disease. Since phosphodiester oligonucleotides are rapidly degraded or have difficulty in penetrating cells, antisense agents are usually nucleic acid derivatives with modified backbones that resist degradation or that have an enhanced permeation ability (38, 44).

Because of its target sequence, an antisense agent could theoretically display an ISS and therefore induce nonspecific immune activation. Furthermore, some nucleic acid derivatives have immunostimulatory properties that may not simply reflect the display of an ISS. Phosphorothioates have been tested extensively for in vitro and in vivo antisense activities and have a sulfur substitution for one of the nonbridging oxygens in the phosphodiester backbone (44). In general, an ISS in phosphorothioate chemistry is much more active than the comparable phosphodiester. There is evidence, moreover, that sequences other than the classic CpG motifs may have immune-activating properties when they are synthesized as phosphorothioates (28). These activities may reflect the unique properties of the phosphorothioate backbone, the long half-lives of these compounds, and different patterns of intracellular trafficking. Since antisense agents can be used as antimicrobial or antiviral agents in infected individuals, the potential for synergistic in-

teraction with products such as endotoxin could also complicate their use (12).

CONCLUSION

In the past few years investigators have witnessed a revolution in the conceptualization of immune responses to DNA. With the recognition of the epitope structure and immunostimulatory properties of bacterial DNA, DNA has been transformed from a uniform and inert molecule into a powerful presence whose activities are extensive and pervasive. The coming years should be exciting as investigators elucidate these immune activities and develop techniques for their manipulation in the treatment and prevention of human disease.

ACKNOWLEDGMENTS

This work was supported by the VA Medical Research Service Merit Review grant and the VA Research Center on AIDS and HIV Infection.

REFERENCES

1. **Aarden, L. A., F. Lakmaker, and E. R. De Groot.** 1976. Immunology of DNA. IV. Quantitative aspects of the Farr assay. J. Immunol. Methods **11:**153–163.
2. **Bunyard, M. P., and D. S. Pisetsky.** 1994. Characterization of antibodies to bacterial double-stranded DNA in the sera of normal human subjects. Int. Arch. Allergy Immunol. **105:**122–127.
3. **Casperson, G. F., and E. W. Voss, Jr.** 1983. Specificity of anti-DNA antibodies in SLE. I. Definition and gross specificity of antibody populations in human SLE plasma. Mol. Immunol. **20:**573–580.
4. **Casperson, G. F., and E. W. Voss, Jr.** 1983. Specificity of anti-DNA antibodies in SLE-II. Relative contribution of backbone, secondary structure and nucleotide sequence to DNA binding. Mol. Immunol. **20:**581–588.
5. **Emlen, W., D. S. Pisetsky, and R. P. Taylor.** 1986. Antibodies to DNA: a perspective. Arthritis Rheum. **29:**1417–1426.
6. **Foster, M. H., B. Cizman, and M. P. Madaio.** 1993. Nephritogenic autoantibodies in systemic lupus erythematosus: immunochemical properties, mechanisms of immune deposition, and genetic origins. Lab. Invest. **69:**494–507.
7. **Fredriksen, K., A. Skogsholm, T. Flaegstad, T. Traavik, and O. P. Rekvig.** 1993. 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. **38:**401–406.
8. **Gilkeson, G. S., A. M. M. Pippen, and D. S. Pisetsky.** 1995. Induction of cross-reactive anti-dsDNA antibodies in preautoimmune NZB/NZW mice by immunization with bacterial DNA. J. Clin. Invest. **95:**1398–1402.
9. **Gilkeson, G. S., D. D. Bloom, D. S. Pisetsky, and S. H. Clarke.** 1993. 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_H CDR3 arginines. J. Immunol. **151:**1353–1364.
10. **Gilkeson, G. S., J. P. Grudier, D. G. Karounos, and D. S. Pisetsky.** 1989. Induction of anti-double stranded DNA antibodies in normal mice by immunization with bacterial DNA. J. Immunol. **142:**1482–1486.
11. **Gilkeson, G. S., J. P. Grudier, D. G. Karounos, and D. S. Pisetsky.** 1989. The antibody response of normal mice to immunization with single-stranded DNA of various species origin. Clin. Immunol. Immunopathol. **51:**362–371.
12. **Hartmann, G., A. Krug, K. Waller-Fontaine, and S. Endres.** 1996. Oligodeoxynucleotides enhance lipopolysaccharide-stimulated synthesis of tumor necrosis factor: dependence on phosphorothioate modification and reversal by heparin. Mol. Med. **2:**429–438.
13. **Hergersberg, M.** 1991. Biological aspects of cytosine methylation in eukaryotic cells. Experientia **47:**1171–1185.
14. **Izui, S., N. M. Zaldivar, I. Scher, and P.-H. Lambert.** 1977. Mechanism for induction of anti-DNA antibodies by bacterial lipopolysaccharides in mice. I. Anti-DNA induction by LPS without significant release of DNA in circulating blood. J. Immunol. **119:**2151–2156.
15. **Karounos, D. G., J. P. Grudier, and D. S. Pisetsky.** 1988. Spontaneous expression of antibodies to DNA of various species origin in sera of normal subjects and patients with systemic lupus erythematosus. J. Immunol. **140:**451–455.
16. **Kataoka, T., S. Yamamoto, T. Yamamoto, E. Kuramoto, Y. Kimura, O. Yano, and T. Tokunaga.** 1992. Antitumor activity of synthetic oligonucleotides with sequences from cDNA encoding proteins of *Mycobacterium bovis* BCG. Jpn. J. Cancer Res. **83:**244–247.
17. **Klinman, D. M., A.-K. Yi, S. L. Beaucage, J. Conover, and A. M. Krieg.** 1996. CpG motifs present in bacterial DNA rapidly induce lymphocytes to secrete interleukin 6, interleukin 12, and interferon γ. Proc. Natl. Acad. Sci. USA **93:**2879–2883.
18. **Klinman, D. M., G. Yamshchikov, and Y. Ishigatsubo.** 1997. Contribution of CpG motifs to the immunogenicity of DNA vaccines. J. Immunol. **158:**3635–3639.
19. **Kredich, N. M., J. S. Skyler, and L. J. Foote.** 1973. Antibodies to native DNA in systemic lupus erythematosus. Arch. Intern. Med. **131:**639–644.
20. **Krieg, A. M.** 1995. CpG DNA: a pathogenic factor in systemic lupus erythematosus? J. Clin. Immunol. **15:**284–292.
21. **Krieg, A. M., A.-K. Yi, S. Matson, T. J. Waldschmidt, G. A. Bishop, R. Teasdale, G. A. Koretzky, and D. M. Klinman.** 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature **374:**546–549.
22. **Madaio, M. P., S. Hodder, R. S. Schwartz, and B. D. Stollar.** 1984. Responsiveness of autoimmune and normal mice to nucleic acid antigens. J. Immunol. **132:**872–876.
23. **Messina, J. P., G. S. Gilkeson, and D. S. Pisetsky.** 1991. Stimulation of in vitro murine lymphocyte proliferation by bacterial DNA. J. Immunol. **147:**1759–1764.
24. **Pardoll, D. M., and A. M. Beckerleg.** 1995. Exposing the immunology of naked DNA vaccines. Immunity **3:**165–169.
25. **Pisetsky, D. S.** 1992. Anti-DNA antibodies in systemic lupus erythematosus. Rheum. Dis. Clin. N. Am. **18:**437–454.
26. **Pisetsky, D. S.** 1996. Immune activation by bacterial DNA: a new genetic code. Immunity **5:**303–310.
27. **Pisetsky, D. S.** 1996. The immunologic properties of DNA. J. Immunol. **156:**421–423.
28. **Pisetsky, D. S., and C. Reich.** 1993. Stimulation of *in vitro* proliferation of murine lymphocytes by synthetic oligodeoxynucleotides. Mol. Biol. Rep. **18:**217–221.
29. **Pisetsky, D. S., and D. M. Drayton.** 1997. Deficient expression of antibodies specific for bacterial DNA by patients with systemic lupus erythematosus. Proc. Assoc. Am. Phys. **109:**237–244.
30. **Pisetsky, D. S., G. G. Gilkeson, and E. W. St. Clair.** 1997. Systemic lupus erythematosus. Diagnosis and treatment. Med. Clin. N. Am. **81:**113–128.
31. **Radic, M. Z., and M. Weigert.** 1994. Genetic and structural evidence for antigen selection of anti-DNA antibodies. Annu. Rev. Immunol. **12:**487–520.
32. **Robertson, C. R., and D. S. Pisetsky.** 1992. Specificity analysis of antibodies to single-stranded micrococcal DNA in the sera of normal human subjects and patients with systemic lupus erythematosus. Clin. Exp. Rheum. **10:**589–594.
33. **Robertson, C. R., G. S. Gilkeson, M. M. Ward, and D. S. Pisetsky.** 1992. 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. **62:**25–32.
34. **Sabbaga, J., O. G. Pankewycz, V. Lufft, R. S. Schwartz, and M. P. Madaio.** 1990. Cross-reactivity distinguishes serum and nephritogenic anti-DNA antibodies in human lupus from their natural counterparts in normal serum. J. Autoimmun. **3:**215–235.
35. **Sato, Y., M. Roman, H. Tighe, D. Lee, M. Corr, M.-D. Nguyen, G. J. Silverman, M. Lotz, D. A. Carson, and E. Raz.** 1996. Immunostimulatory DNA sequences necessary for effective intradermal gene immunization. Science **273:**352–354.
36. **Schwartz, D. A., T. J. Quinn, P. S. Thorne, S. Sayeed, A.-K. Yi, and A. M. Krieg.** 1997. CpG motifs in bacterial DNA cause inflammation in the lower respiratory tract. J. Clin. Invest. **100:**68–73.
37. **Sparwasser, T., T. Miethke, G. Lipford, K. Borschert, H. Hacker, K. Heeg, and H. Wagner.** 1997. Bacterial DNA causes septic shock. Nature **386:**336–337.
38. **Stein, C. A., and Y.-C. Cheng.** 1993. Antisense oligonucleotides as therapeutic agents—is the bullet really magical? Science **261:**1004–1012.
39. **Stollar, B. D.** 1994. Molecular analysis of anti-DNA antibodies. FASEB J. **8:**337–342.
40. **Stollar, D., L. Levine, and J. Marmur.** 1962. Antibodies to denatured deoxyribonucleic acid in lupus erythematosus serum. II. Characterization of antibodies in several sera. Bioch. Biophys. Acta **61:**7–18.
41. **Tan, E. M.** 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv. Immunol. **44:**93–151.
42. **Termaat, R.-M., K. J. M. Assmann, H. B. P. M. Dijkman, F. V. Gompel, R. J. T. Smeenk, and J. H. M. Berden.** 1992. Anti-DNA antibodies can bind to the glomerulus via two distinct mechanisms. Kidney Int. **42:**1363–1371.
43. **Tokunaga, T., H. Yamamoto, S. Shimada, H. Abe, T. Fukuda, Y. Fujisawa, Y. Furutani, O. Yano, T. Kataoka, T. Sudo, N. Makiguchi, and T. Suganuma.** 1984. Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. I. Isolation, physicochemical characterization, and antitumor activity. J. Natl. Cancer Inst. **72:**955–962.
44. **Uhlmann, E., and A. Peyman.** 1990. Antisense oligonucleotides: a new therapeutic principle. Chem. Rev. **90:**544–584.
45. **Ulmer, J. B., J. J. Donnelly, S. E. Parker, G. H. Rhodes, P. L. Felgner, V. J. Dwarki, S. H. Gromkowski, R. R. Deck, C. M. DeWitt, A. Friedman, L. A. Hawe, K. R. Leander, D. Martinez, H. C. Perry, J. W. Shiver, D. L. Montgomery, and M. A. Liu.** 1993. Heterologous protection against influenza by injection of DNA encoding a viral protein. Science **259:**1745–1749.
46. **Valerio, R. D., K. A. Bernstein, E. Varghese, and J. B. Lefkowith.** 1995. Murine lupus glomerulotropic monoclonal antibodies exhibit differing specificities but bind via a common mechanism. J. Immunol. **155:**2258–2268.

47. **Vlahakos, D., M. H. Foster, A. A. Ucci, K. J. Barrett, S. K. Datta, and M. P. Madaio.** 1992. Murine monoclonal anti-DNA antibodies penetrate cells, bind to nuclei, and induce glomerular proliferation and proteinuria *in vivo*. J. Am. Soc. Nephrol. **2:**1345–1354.
48. **Wu, Z.-Q., D. Drayton, and D. S. Pisetsky.** 1997. Specificity and immunochemical properties of antibodies to bacterial DNA in sera of normal human subjects and patients with systemic lupus erythematosus (SLE). Clin. Exp. Immunol. **109:**27–31.
49. **Yamamoto, S., E. Kuramoto, S. Shimada, and T. Tokunaga.** 1988. *In vitro* augmentation of natural killer cell activity and production of interferon-α/β and -γ with deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. Jpn. J. Cancer Res. **79:**866–873.
50. **Yamamoto, S., T. Yamamoto, S. Shimada, E. Kuramoto, O. Yano, T. Kataoka, and T. Tokunaga.** 1992. DNA from bacteria, but not from vertebrates, induces interferons, activates natural killer cells and inhibits tumor growth. Microbiol. Immunol. **36:**983–997.
51. **Yamamoto, S., T. Yamamoto, T. Kataoka, E. Kuramoto, O. Yano, and T. Tokunaga.** 1992. Unique palindromic sequences in synthetic oligonucleotides are required to induce INF and augment INF-mediated natural killer activity. J. Immunol. **148:**4072–4076.
52. **Yamamoto, T., S. Yamamoto, T. Kataoka, and T. Tokunaga.** 1994. Lipofectin of synthetic oligodeoxyribonucleotide having a palindromic sequence of AACGTT to murine splenocytes enhances interferon production and natural killer activity. Microbiol. Immunol. **38:**831–836.