

## Immune Response to DNA in Systemic Lupus Erythematosus

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### Abstract

Antibodies to DNA occur prominently in systemic lupus erythematosus and have been extensively studied as probes for underlying immune disturbances. These antibodies have features of DNA antigen drive. While previous models for this response posited DNA as simple and inert, recent studies have indicated that DNA is immunologically diverse and, depending upon sequence and backbone structure, can stimulate or suppress immune responses. In particular, bacterial DNA is immunologically potent and can function as both an adjuvant and immunogen, eliciting in normal individuals antibodies to sites exclusive to bacterial DNA. In mice genetically predisposed to autoimmunity, however, bacterial DNA can elicit anti-DNA autoantibodies under conditions in which mammalian DNA is inactive. These findings suggest that foreign DNA can serve as a trigger for anti-DNA responses, with SLE reflecting a disturbance in antibody specificity and a shift from binding of sequential to backbone determinants. In contrast to bacterial DNA, mammalian DNA can suppress certain immune responses and prevent macrophage cytokine production. To the extent that self-DNA drives responses in SLE, anti-DNA production in this disease may reflect a failure of this suppression. The recognition of DNA's immune activities thus suggests novel possibilities for disease pathogenesis.

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Antibodies to DNA are prototypic autoantibodies that are the serological hallmark of systemic lupus erythematosus. These antibodies represent markers of diagnostic and prognostic significance and play an important role in the pathogenesis of lupus nephritis, a major cause of morbidity and mortality in this disease. Because of the close association of anti-DNA with SLE, these antibodies have been extensively studied genetically and molecularly on the assumption that understanding a diagnostic marker would reveal key steps in disease pathogenesis. Indeed, the focus on anti-DNA as a probe for autoimmunity has placed these antibodies among the best characterized responses, both normal and aberrant, in all of immunology [1,2].

SLE = systemic lupus erythematosus

While the antibody response to DNA has been studied in exquisite detail, the nature of DNA as an antigen has remained more obscure. Indeed, DNA was long viewed as an immunologically simple and inert molecule whose immunologic activity was confined to the setting of SLE. Whereas protein autoantigens have been defined as arrays of amino acid determinants of varying sequence and structure, DNA was dichotomized into single stranded and double stranded conformations. According to this conceptualization, these conformations were strongly influenced by charge, with anionic phosphate groups being a major determinant of antigenicity [3].

An important limitation in understanding the antigenic properties of DNA in SLE concerned the previous lack of systems in normal immunity for comparison. As shown in numerous studies published in the past, anti-DNA antibodies appear exclusive to SLE and are rarely encountered in normal individuals. While this finding established anti-DNA as a diagnostic marker, it deprived investigators of a comparator response to assess the difference between DNA recognition in SLE and normal immunity. Further limiting analysis of anti-DNA binding was the absence of induced models of SLE. Even when presented as protein complexes in adjuvant, mammalian DNA fails to elicit a significant response in normal or autoimmune animals. These observations, by strengthening the association of anti-DNA with SLE, have suggested that immune recognition of DNA requires immunologic abnormalities unique to patients with SLE [4].

### Changes in the conceptualization of DNA

In the last decade, studies from a number of disciplines have provided a new perspective on the immune properties of DNA, leading to novel concepts of the mechanisms of anti-DNA expression. Central to this new perspective is the realization that DNA is not uniform in its immunologic properties and that, because of sequence microheterogeneity, DNA can exert both positive and negative effects on the immune system. Furthermore, serological studies have shown clearly that antibodies to DNA occur in normal individuals as well as in patients with SLE, with differences in the specificity of antibodies for sequential versus conformational determinants critical to pathogenicity.

As shown both *in vitro* and *in vivo*, DNA – depending on base sequence and species origin – can exert powerful immunostimulatory effects that rival those of endotoxin in range and potency. Thus, DNA from bacteria can induce the production of cytokines that include interferon- $\alpha/\beta$ , interleukin 6 and 12,

tumor necrosis factor- $\alpha$  and IFN- $\gamma$  among others. In addition, DNA from bacteria is mitogenic in mice and can stimulate B cell activation and polyclonal antibody production. These activities result from short six-base motifs called CpG motifs or immunostimulatory sequences that have the general structure of two 5' purines, an unmethylated CpG motif and two 3' pyrimidines [5–9].

CpG motifs occur much more commonly in bacterial DNA than mammalian DNA for two main reasons. In mammalian DNA, CpG sequences occur less frequently than predicted by base composition, a phenomenon known as CpG suppression. Furthermore, in mammalian DNA, cytosine is commonly methylated in this position, presumably as a mechanism for gene regulation [10–12]. While the origin of CpG suppression and cytosine methylation remains speculative, bacterial DNA, because of its content of CpG motifs, possesses a code for foreignness that can serve as a danger signal to activate innate immunity.

The presence of CpG motifs in bacterial DNA is only one feature that distinguishes bacterial DNA from mammalian DNA. The other concerns the large number of differences in coding sequence between prokaryotic and eukaryotic DNA. As such, bacterial DNA displays key properties predicted for an effective immunogen: it has a built-in adjuvant and displays a host of sequential epitopes. Furthermore, DNA is a polymer that can display repeating epitopes and has the capacity for receptor cross-linking.

While the immunogenicity of bacterial DNA is now evident, the existence of antibodies to bacterial DNA was essentially missed until 1988, when studies from my laboratory unequivocally demonstrated the presence of such antibodies in normal human sera. Using solid-phase assays with a panel of natural DNA antigens, we showed that normal human sera bind to some but not all bacterial DNA while lacking reactivity to mammalian DNA. The anti-DNA in normal human sera are selective for certain bacterial DNA, suggesting recognition of non-conserved sequential determinants as opposed to backbone structures [13]. Furthermore, the anti-DNA in normal human sera bind with high affinity, display the immunoglobulin G isotype and occur at titers similar to that of patients with SLE. These features are suggestive of an antigen-driven response [14–16]. Table 1 compares the response to DNA in normal human sera and SLE sera.

The immunogenicity of bacterial DNA has been amply verified in experimental animals. In these immunization experiments, DNA was complexed to methylated bovine serum albumin as a carrier and administered in complete Freund's adjuvant. Under these conditions, *Escherichia coli* dsDNA induced a robust antibody response whereas calf thymus dsDNA failed to induce a response. The antibodies induced by the *E. coli* DNA bound selectively to bacterial DNA and lacked cross-reactive autoantibody activity to mammalian DNA. These

**Table 1. Properties of anti-DNA**

	<b>Normal</b>	<b>SLE</b>
Antigen specificity	Bacterial DNA	Mammalian and bacterial DNA
Strand specificity	ss and ds	ss and ds
Isotype	IgG2	IgG1 and IgG3
Light chain	$\kappa$ predominance	$\kappa$ and $\lambda$
Effect of ionic strength on binding	Resistant	Sensitive

antibodies thus resemble the antibodies in normal human sera in their selectivity [17–19].

While immunization with bacterial DNA fails to induce an autoantibody response in normal mice, pre-autoimmune NZB/NZW show a different pattern of response. In these animals, immunization with *E. coli* dsDNA leads to the production of antibodies that show autoantibody activity and bind both calf thymus and *E. coli* dsDNA. Under these conditions, immunization of normal mice would lead to antibodies binding only bacterial DNA. These findings suggest that the autoimmune state is associated with a shift in the pattern of DNA recognition, with normal animals, like normal humans, responding to non-conserved sequential determinants while autoimmune mice respond to conserved conformational determinants [20].

An interesting feature of these immunization experiments concerns the failure of mammalian DNA to induce autoantibody production by immunization of NZB/NZW mice. This failure is surprising since these mice are destined to produce anti-DNA autoantibodies spontaneously and can be induced to produce such antibodies by immunization with bacterial DNA. While the CpG motifs of bacterial DNA could promote autoimmunity in this setting, an alternative possibility for the failure of mammalian DNA immunization concerns the ability of self-DNA to inhibit responses. As now shown *in vitro*, mammalian DNA, rather than being neutral or inert, has immunosuppressive potential and can prevent the expression of cytokines such as IL-12 and IFN- $\gamma$ . Like immune stimulation, suppression by mammalian DNA results from sequence motifs and may occur with certain animal viral DNA as well as mammalian chromosomal DNA [21–23].

The sequences in mammalian DNA leading to suppression have not been well defined. Studies with synthetic oligonucleotides (ODN) suggest, however, that suppression can result from CpG repeats, runs of deoxyguanosine bases as well as CpG dinucleotides preceded by cytosine or followed by guanosine [21–23]. Furthermore, suppression can be enhanced by modifications of the DNA backbone, with phosphorothioate ODN displaying greater activity than phosphodiester ODN. This suppression can affect immune activation induced by bacterial DNA and, in some systems, lipopolysaccharide [23].

dsDNA = double stranded DNA

IL = interleukin

IFN = interferon

As these considerations suggest, self-DNA may serve as a "safety" molecule and limit immune responses in settings where inflammation leads to cell death and release of self-antigen that could stimulate autoreactivity. While the mechanisms of inhibition by mammalian DNA are not yet known, these findings suggest that mammalian DNA may fail to induce antibody production because this molecule hinders key steps in immune responses. Just as CpG motifs promote immune responses to bacterial DNA, sequences on mammalian DNA may inhibit immune responses to this molecule.

## Implications for SLE

While providing a plausible model for how bacterial DNA can drive anti-DNA production, these observations suggest an uncertain and ambiguous role for self-DNA. Since bacterial DNA can effectively stimulate anti-DNA production in normal individuals, it has the potential to induce anti-DNA responses in patients with SLE, albeit with a shift in specificity to backbone determinants. Support for this possibility comes from immunization experiments with NZB/NZW mice as well as from serological studies on patients with SLE. Thus, as shown in absorption experiments, sera from SLE patients, while containing abundant quantities of cross-reactive anti-DNA autoantibodies, lack antibodies specific for bacterial DNA [24].

Although the absence of antibodies to bacterial DNA in SLE could reflect a type of immunodeficiency, the more likely explanation for this serological finding relates to the impact of the autoimmune state on antibody specificity. Because of tolerance defects in SLE, the B cell repertoire in patients may have an array of specificities different from that of normal individuals where tolerance cleans the repertoire of autoreactive precursors or makes them anergic. In SLE, however, because of failed tolerance, B cells binding to self-DNA may be available and be preferentially expressed whether the stimulating antigen is foreign or self-DNA. Support for this possibility comes from the molecular analysis of induced anti-DNA antibodies in normal and autoimmune mice [25,26]. With an immune system poised for autoreactivity and cross-reactivity, foreign antigens may be much more likely to induce autoimmunity than in a normal individual.

In SLE, the anti-DNA response does not occur alone. Rather, it is part of a response to nucleosomes and is linked to the response to other chromatin components such as histones [27]. To reconcile the role of bacterial DNA and the role of self-DNA in this response requires several assumptions: a) bacterial DNA may initiate and promote the response to self-DNA but is not the exclusive driving antigen; b) bacterial DNA could facilitate the response to nucleosomes by expanding cross-reactive DNA B cells that bind nucleosomes through DNA and serve as antigen-presenting cells for histones; c) DNA in the form of nucleosomes may differ in immunologic (stimulatory or suppressive) activity from free DNA; d) the presence of cross-reactive anti-DNA may influence the immunological properties of stimulatory as well as inhibitory DNA; and e) cells from

patients with SLE may differ in their response to suppressive DNA than cells from normal individuals.

These assumptions have not been experimentally tested yet but provide a fertile field for future investigation. The recognition of DNA's immunologic properties provides a wide range of possibilities for how foreign and self-DNA can impinge on the immune system in SLE and modulate autoreactivity. It is too early to know whether the stimulatory activity of foreign DNA and suppressive activity of self-DNA are relevant to SLE. It is not too early, however, to ask these questions.

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