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A 'senseless' immune response to DNA

Antisense oligonucleotides can have dramatic effects, but a recent paper suggests that the mechanism isn't always based on nucleotide sequence interactions.

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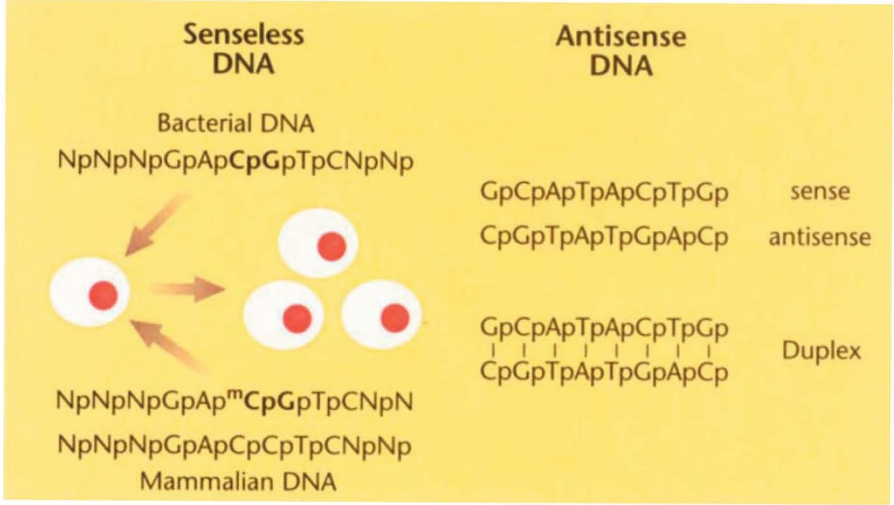
DNA serves as the sequence-based message that controls all cellular functions, yet there are relatively few instances of DNA functioning in a pharmacological setting. However, the conceptual framework holding that DNA operates exclusively as a genetic code-based intracellular signal is shattered by a report by Krieg *et. al.* in the April 6 issue of *Nature*[1]. The data unveiled in the paper lead to the conclusion that mammalian immune systems can 'recognize' bacterial DNA (because it contains unmethylated CpG dinucleotides — fancy molecular talk for a cytosine base that is followed by a guanosine base; see figure) and react with an immune response stronger than the response to lipopolysaccharide (LPS). Unlike most biologic activities of DNA, which involve mRNA transcription (sense effects) or hybridization to the sense or coding strand (antisense effects), this immune stimulation is influenced by unmethylated CpG motifs and is thus largely sequence-independent (senseless). Clearly this work has important implications for many fields of research, including infectious disease, immunology, rheumatology and pharmacology.

What is the evidence that B cells respond to CpG motifs in bacterial DNA? Krieg and collaborators show that (1) bacterial DNA stimulates B-cell proliferation, whereas mammalian DNA does not; (2) methylation of bacterial DNA by CpG methylase eliminates the effect; (3) alteration of DNA sequence within the CpG consensus region inhibits the effect, whereas mutations outside the CpG methylation region have little effect. This is convincing circumstantial evidence, but proof requires the identification of the mechanism or mechanisms through which CpG oligonucleotides elicit the B-cell proliferative response. The proliferative response to DNA is impressive, with 95% of B cells responding to bacterial DNA[1,2]. It is intriguing to speculate that much of the immune response to bacteria and viruses may be a consequence of B-cell activation by DNA, acting in concert with immune stimulation by other bacterial molecules such as LPS and teichoic acid. Further research is necessary to determine whether systems have evolved to present bacterial or viral DNA fragments to B cells and other immune cells in an efficient and orderly fashion.

The discovery that mammalian immune cells undergo a programmed response to bacterial DNA places certain rheumatologic diseases in a new light, such as systemic lupus erythematosus (SLE), in which patients harbour antibodies against DNA. These pathologic immune responses to DNA have generally been thought to constitute autoimmune responses to human DNA or an artefactual response to bacterial DNA. However, if mammals have evolved an immune response against DNA, then these rheumatologic disorders may result from inadequate fine-tuning of a physiologically appropriate immune response to DNA. Recent studies of lupus-prone NZB-NZW mice have demonstrated that these mice develop antibodies to bacterial DNA that cross-react with eukaryotic DNA, whereas normal mice develop only specific antibacterial antibodies following immunization with bacterial DNA[3]. Perhaps SLE, scleroderma, and mixed connective tissue disease represent subtle alterations in the usual immune response to bacteria, resulting in immune responses that 'backfire' in an autoimmune rheumatologic disorder.

Although the Krieg paper presents an orderly series of experiments pursuing clear-cut hypotheses, the actual history of this research is less systematic and thus far more interesting. Krieg and collaborators originally described immune stimulation by oligonucleotides that were designed to be antisense oligonucleotides[4]. Antisense oligonucleotides affect cells by hybridizing to target sequences within mRNA, thus destabilizing the target mRNA and ultimately decreasing translation to protein (see figure). However, when Krieg and collaborators carefully analysed the effects of non-antisense oligonucleotides — and performed studies probing the mechanism — they discovered that the effects were not due to antisense.

Immune stimulation has been reported in a number of antisense studies. I suspect that many, if not most, of these are due to the CpG effect described by Krieg and not a result of the antisense mechanism assumed by the authors. Krieg has a list of 18 publications showing immune stimulation by oligonucleotides that contain CpG motifs but were presumed to be due to antisense[1].



Schematic comparison of CpG immune stimulation (left) with antisense gene inhibition (right). The complete stimulatory DNA sequence is shown with the CpG motif presented in bold[1]. The two lower sequences are from mammalian DNA, which do not stimulate B-cell proliferation due to either methylation of CpG (shown as mCpG) or the absence of CpG sequences (which are underepresented in mammalian DNA). The right portion of the figure shows a sense (coding) sequence duplexed with its complementary antisense sequence, which leads to destabilization of the sense mRNA and inhibits translation.

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What are the implications of this work for therapeutic uses of DNA, specifically oligonucleotides? The CpG containing oligonucleotides employed in the Krieg paper are potent stimulators of B-cell proliferation and may be useful as immune adjuvants or stimulants in instances of immunosuppression or in certain malignancies. This work also has implications for antisense oligonucleotide therapeutics, because it provides a well-documented example of a presumed antisense effect that was not really an antisense effect. Previous reviews have summarized nonspecific toxicities of oligonucleotides[5,6], but the CpG effect is nefarious because it shows some sequence specificity. Another example of a non-antisense effect that shows some sequence specificity is an alteration of Sp-1 activity by certain modified oligonucleotides[7].

How can we be sure that an observed effect of a nucleotide is an antisense effect or a CpG ('senseless') effect? I have become convinced that there is no salvation in oligonucleotide research: just because some previous investigators have demonstrated that an effect is due to antisense does not mean that an investigator can assume that their effect with a different oligonucleotide or different cell system is due to antisense. It is important to use four distinct approaches to demonstrate an antisense effect: (1) employ multiple control oligonucleotides of varying sequences (2) demonstrate inhibition of the target gene by definitive molecular studies (not just immunostaining) (3) reverse the antisense effect by competing with a complementary oligonucleotide[8] and (4) rule out alternative explanations such as the CpG effect. Despite these concerns and caveats numerous well-documented reports present examples of antisense effects, including naturally occuring examples where antisense regulates cellular functions. Clearly these effects occur, but they must be distinguished from both toxic artefacts and physiologically relevant "senseless" DNA effects like the immune response to CpG motifs in DNA.

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Getting to know you: Viruses meet CD40 ligand

The coevolution of viruses and their hosts has given rise in both to elaborate molecular attack and defence mechanisms (pages 437–441).

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There is little doubt that vertebrates and the viruses that infect them have spent a fair bit of time getting to know each other. For example, Frank Fenner has estimated that New World rabbits have coevolved with endogenous poxviruses since the early Pleistocene, some twenty million years[1], and there is no reason to suspect that this kind of longevity is atypical for many host/virus interactions. Given such extended time scales for mutual R&D, it is not surprising that each party has evolved counteractive strategies to ensure that its opponent doesn't achieve complete dominance.

For example, a critical component of the host immune response to viral infection is the cytokine network, which plays a major role in orchestrating the events of virus recognition, active clearance and, later, acquired immunity. Recent studies have identified an important subset of cytokines that figure prominently in antiviral activity (see table). In turn, viruses have adapted specific subversive strategies to guarantee their own survival (see refs 2–5 for recent reviews). To this impressive list of antiviral cytokines we can now add a new contender, namely the ligand for CD40, a recently discovered member of the growing tumour necrosis factor (TNF) superfamily. In this issue of *Nature Medicine*[6], Janet Ruby's group provides evidence that CD40-ligand (CD40L), originally described as an antigen-induced cell surface glycoprotein on activated T lymphocytes, participates in the antiviral response to vaccinia infection in immunocompromised mice. These results are unexpected and raise new questions about how viral infections are managed by the host cytokine network. To understand how this expands the current picture of the antiviral immune response, a brief summary of the history of CD40 and CD40L is in order.

CD40 was originally described in the mid 1980s as a B-lymphocyte surface marker that, when stimulated with anti-CD40 antibody, promotes B-cell growth, isotype switching and the development of memory B cells (reviewed in ref. 7). However, CD40 was later shown to be present on many other cell types as well, including epithelial cells, monocytes/macrophages and haematopoetic progenitors. The ligand for this new receptor, called gp39 or CD40L, was cloned in 1992 and found to be expressed as a type II membrane protein on the surface of activated T cells. Although originally defined as an important costimulatory molecule involved in T-cell-dependent B cell activation, the presence of CD40L on the surface of other lymphoid cells, such as $CD8^+$ T cells, NK cells and monocytes, suggests other biological roles remain to be uncovered. The importance of the data presented in the recent work by Ruby *et al.* lies in the demonstration that CD40L can exert a powerful antiviral effect, even in mice lacking a functional repertoire of T or B lymphocytes.

To arrive at this conclusion, Ruby *et al.* expressed murine CD40L from recombinant vaccinia virus vectors and assessed virus propagation and pathogenicity in mice with severely compromised immune capabilities. Although vaccinia is relatively apathogenic in vertebrate hosts with intact cellular immunity, immunocompromised mice can be lethally infected. However,