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,

Antiviral cytokines and viral anticytokine strategies

Cytokine	Host Receptor	Mode of Action	Viral Defense Strategy (with example)
Interferon-α/β	Type I interferon-R	Induce antviral state	Soluble receptor mimic (vaccinia B18R)[9] Inhibit induced effectors (multiple virus inhibitors of PKR and 2',5'-A synthetase)[10]
Interferon-γ	Type II interferon-R	Induce antiviral state Immunoregulator (pleiotropic) Apoptosis of infected cells?	Soluble receptor mimic (myxoma T7)[11] Inhibit induced effectors (as for interferon α/β)[10]
TNF-α/β (LT-β?)	Type I, II TNF-R	Cytolysis/apoptosis Immunoregulator (pleiotropic) Proinflammatory activities	Soluble receptor mimic (Shope fibroma virus T2)[12] Inhibit signal transduction (adenovirus Elb-19K)[13]
Il-1b	Il-1-R	Synergize with TNF Proinflammatory activities	Soluble receptor mimic (vaccinia B15R)[14] Inhibit ligand processing (cowpox crmA)[15]
Chemokines (Il-8, etc.)	Serpentine-R (C-C/C-X-C, etc.)	Leukocyte recruitment? Chemotactic modulation?	Membrane receptor mimic (cytomegalovirus US28)[16,17]
FAS-L	FAS	Apoptosis of infected cells?	Inhibit signal transduction (cowpox crmA)[18]
CD40-L	CD40	Macrophage activation? Cytolysis/apoptosis of infected cells?	???

previous experiments with vaccinia constructs that overexpress known antiviral cytokines, such as interferon-γ or TNF-α, indicate that expression of these and other immunoregulatory ligands can cause dramatic attenuation of vaccinia spread and pathogenicity[8]. The results presented by Ruby *et al.* seem to place CD40L in the same category as the other antiviral cytokines listed in the table. Specifically, CD40L was faithfully expressed at the surface of cells infected *in vitro* with VV-CD40L and the recombinant virus was dramatically attenuated *in vivo* in three classes of immunodepleted mice (nude, SCID and sublethally irradiated). Furthermore, virus was rapidly cleared from infected ovaries in infected nude mice, a favored replication site for wild-type vaccinia. The apparent lackof B- or T-cell involvement in CD40L-mediated virus attenuation is surprising, and raises the question of how a cytokine that was originally defined as a ligand for B cell activation can function in the absence of either cell class.

The mechanism of CD40L-dependent antiviral activity is currently undefined, but several intriguing possibilities exist. The kinetics of VV-CD40L clearance in nude mice is relatively rapid and selective reductions in infectious virus within ovarian tissues are observed within the first 24 hours of infection. This makes activation and recruitment of NK cells an unlikely mechanism, though the stimulation of resident macrophages by the vaccinia-expressed CD40L cannot be excluded. It is also possible that surface CD40L is released by proteolysis from virus-infected cells to act as a soluble ligand within infected tissues, a situation reminiscent of TNF release from its membrane-bound precursor. Another possibility is that when CD40L is expressed from infected CD40$^+$ cells (for example, epithelial cells) the autocrine or juxtacrine signalling response could be directly cytotoxic or apoptotic. Although in B cells CD40 stimulation is anti-apoptotic, it is not clear how virus infection in other classes of CD40L responsive cells might tip the balance between life and death. Given the known complicity of the two current superstars in this ligand superfamily — TNF and FAS ligand — in apoptosis and/or necrosis, a similar role for CD40 and CD40L in aborting virus replication directly at the early stages of virus replication is highly appealing. In this regard, the demonstration by Ruby *et al.* that membrane bound CD40L, when exposed to uninfected mouse L929 cells, inhibits subsequent replication of HSV is significant. Could these cells be deficient in virus replication because of a supervening apoptotic response?

Which brings us to the last point. The larger DNA viruses in particular have not been sitting idly by while vertebrate hosts have evolved their pantheon of antiviral effectors. Each of the known antiviral cytokines discovered to date is the target of one or more anticytokine strategies by viruses themselves (see table). It is known that the vaccinia virus used by Ruby *et al.* has been highly attenuated by virtue of more than two centuries of passage outside a native vertebrate host, and hence is now relatively nonpathogenic (but highly immunogenic) in immunocompetent hosts. Given the breadth and spectrum of viral gene products already known to interact and subvert host cytokines and other immune effector molecules, can a viral anti-CD40/CD40L mechanism be far behind?

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