CpG Oligodeoxynucleotides Increase the Susceptibility of Normal Mice to Infection by Candida albicans

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Received 28 February 2005/Returned for modification 4 April 2005/Accepted 19 April 2005

Synthetic oligodeoxynucleotides containing CpG motifs trigger an innate immune response that typically increases host resistance to infection. Yet CpG treatment reduces the resistance of normal mice to Candida albicans infection. This effect is mediated by CpG-induced interleukin-12, indicating that CpG-dependent cytokine production may have adverse consequences for the host.

Recognition of bacterial DNA by Toll-like receptor 9 triggers an innate immune response in which murine lymphocytes, dendritic cells, and macrophages are stimulated to functionally mature and produce a variety of proinflammatory and Th1 cytokines (15, 12, 7, 20). CpG oligodeoxynucleotide (ODN) treatment typically improves host resistance to infection by bacterial, viral, and parasitic pathogens (13, 14, 26).

Candida albicans is present in the normal flora of the skin, mucous membranes, and gastrointestinal tract. It causes opportunistic infection of immunosuppressed patients, with mortality ranging from 40 to 50% despite modern antifungal drug therapy (17, 18). Candida infection can be eliminated by strong Th1 responses (4). Since CpG ODNs promote Th1 immunity (even in immunosuppressed individuals) (24), their ability to protect against yeast infection was examined.

CpG ODNs accelerate death from C. albicans in normal mice. BALB/C mice were treated with an endotoxin-free CpG (GCTAGACGTAGGCT) or a control (GCTAGAGCTTAG) ODN and challenged 3 days later with 105 CFU of C. albicans strain SC5314 (kind gift of Brad Spellberg, University of California Los Angeles). Unexpectedly, the CpG-treated mice succumbed to infection nearly twice as rapidly as normal controls (Fig. 1A, P < 0.01). This effect was dose dependent and sequence specific, since a control ODN had no effect on mortality (Fig. 1A).

Administering a CpG ODN to mice during the period from 3 days prior to 1 day after Candida infection significantly reduced their mean survival time (Fig. 1B, P < 0.01). CpG ODN treatment also increased susceptibility to a low-dose Candida challenge, converting an otherwise sublethal 104 CFU dose to one that caused 100% mortality (P < 0.05, data not shown).

The severity of infection was monitored by culturing serial 10-fold dilutions of homogenized kidney and spleen preparations (the primary loci of Candida infection) on Sabouraud dextrose infusion agar plates (23). Results show that the number of fungi in the organs of CpG-treated animals is significantly higher than in control mice. (Fig. 1C, P < 0.01).

Role of CpG-induced interleukin-12 (IL-12) production on susceptibility to Candida infection. CpG ODNs trigger the production of multiple Th1 cytokines, including IL-12 (6). Preliminary studies showed that mice treated with CpG DNA and then challenged with Candida had serum IL-12 levels nearly fourfold higher than non-CpG-treated controls (P < 0.001, data not shown). Previous reports indicate that high levels of...
serum IL-12 can increase susceptibility to Candida infection (16). To explore whether CpG-induced IL-12 contributed to the susceptibility of ODN-treated mice to yeast infection, CpG DNA was administered to IL-12 knockout (KO) mice. In marked contrast to the effect observed in normal mice, CpG treatment of IL-12 KO animals significantly prolonged survival following a Candida challenge (Fig. 2, P < 0.01). Thus, in the absence of IL-12, the innate immune response elicited by CpG ODN enhanced host resistance to Candida.

Analysis. CpG ODNs have complex effects on the immune system, triggering an immunomodulatory cascade characterized by the production of multiple proinflammatory and Th1 cytokines and chemokines (12, 6, 2). This response may be of benefit in the treatment of allergy, cancer, and certain infectious diseases (10, 8, 9, 3). Yet CpG-induced immune responses may have negative consequences, such as exacerbating autoimmune disease (21, 25, 22, 1). Current findings indicate that the cytokine production elicited by CpG ODNs may also reduce host resistance to certain pathogens, such as C. albicans.

Lavigne et al. documented that elevated levels of IL-12 can exacerbate Candida infection (16). Current results indicate that CpG treatment of normal mice significantly increases IL-12 production and concomitantly increases susceptibility to Candida. Consistent with a cause-effect relationship, when CpG ODNs were administered to IL-12 KO mice, resistance to Candida was increased rather than diminished (Fig. 2). While suggesting that CpG-induced IL-12 was responsible for the greater susceptibility of normal mice to Candida infection, given the diverse effects of CpG ODN in vivo, additional factors could also contribute to this outcome.

The observation that CpG treatment reduces the survival of normal mice while increasing the survival of IL-12 KO mice challenged with Candida emphasizes the complexity of the host response elicited by immunomodulatory ODNs. Similar complexity was observed in studies of CpG-treated mice challenged with the Friend retrovirus: animals treated with CpG DNA after a challenge were resistant to virus-induced leukemia, while those treated prior to a challenge showed increased susceptibility to viral infection and elevated rates of leukemia (19).

CpG ODNs show promise as vaccine adjuvants, as immunoprotective agents, and in the treatment of allergy and cancer (11, 8, 9, 3). Data from clinical trials suggest they can be used safely in humans (8, 5, 11), despite concern that they might promote the development of autoimmunity disease (21, 25, 22, 1). Current findings indicate that CpG ODNs may also increase susceptibility to certain infectious diseases. Thus, careful clinical testing is needed to clarify the benefits and risks of CpG ODN therapy.

**REFERENCES**


Editor: T. R. Kozel