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

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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 (GCTAGACGTTAGCGT) or a control (GCTAGAGCTTAG GCT) phosphorothioate ODN and challenged 3 days later with 10^5 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 10^4 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

FIG. 1. Effect of CpG ODN on *C. albicans* challenged normal mice. Normal 6- to 8-week-old female BALB/C mice were treated with CpG ODN and then challenged with 10^5 CFU *Candida* using Animal Care and Use Committee-approved protocols. Survival was monitored for 3 weeks. Differences in survival rates were evaluated using chi-square and Wilcoxon rank-sum tests. (A) Fifteen or more mice/group were injected intraperitoneally with CpG ODN (◆; 50 µg, ■; 100 µg) and challenged intravenously with *Candida* 3 days later. The survival of CpG-treated animals was significantly shorter than that of controls treated with control ODN or with phosphate-buffered saline (□, $P < 0.05$). The survival of the two control groups was statistically indistinguishable. The survival of mice treated with 100 µg of CpG ODN was significantly shorter than those treated with 50 µg ($P < 0.05$). (B) Fifteen or more mice/group were injected intraperitoneally with 100 µg of control or CpG ODN from 7 days before to 1 day after a challenge with *Candida*. Data represent the median survival time plus the standard deviation. **, $P < 0.01$. MST, mean survival time; Cont, control. (C) Mice treated with 100 µg of ODN or phosphate-buffered saline (PBS) were challenged with *Candida*. The numbers of fungal cells in the kidney and spleen 3 days after infection are shown. Data represent the mean plus the standard error of the mean of six independently studied mice/group in two independent experiments. Statistical significance was evaluated using a two-tailed Student *t* test (**, $P < 0.001$; *, $P < 0.05$).

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FIG. 2. Survival of CpG ODN-treated IL-12 KO mice challenged with *Candida*. IL-12 KO mice (≥ 10 /group) were treated with 100 μ g of control ($\square$) or CpG ($\blacksquare$) ODN and then challenged with 10^5 CFU of *Candida*. **, $P < 0.001$. PBS, phosphate-buffered saline.

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 autoimmune 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.

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