Delineation of a CpG Phosphorothioate Oligodeoxynucleotide for Activating Primate Immune Responses In Vitro and In Vivo[1]

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Abstract

Oligodeoxynucleotides (ODN) containing unmethylated CpG dinucleotides within specific sequence contexts (CpG motifs) are detected, like bacterial or viral DNA, as a danger signal by the vertebrate immune system. CpG ODN synthesized with a nuclease-resistant phosphorothioate backbone have been shown to be potent Th1-directed adjuvants in mice, but these motifs have been relatively inactive on primate leukocytes in vitro. Moreover, in vitro assays that predict in vivo adjuvant activity for primates have not been reported. In the present study we tested a panel of CpG ODN for their in vitro and in vivo immune effects in mice and identified in vitro activation of B and NK cells as excellent predictors of in vivo adjuvant activity. Therefore, we tested >250 phosphorothioate ODN for their capacity to stimulate proliferation and CD86 expression of human B cells and to induce lytic activity and CD69 expression of human NK cells. These studies revealed that the sequence, number, and spacing of individual CpG motifs contribute to the immunostimulatory activity of a CpG phosphorothioate ODN. An ODN with a TpC dinucleotide at the 5' end followed by three 6 mer CpG motifs (5'-GTCGTT-3') separated by TpT dinucleotides consistently showed the highest activity for human, chimpanzee, and rhesus monkey leukocytes. Chimpanzees or monkeys vaccinated once against hepatitis B with this CpG ODN adjuvant developed 15 times higher anti-hepatitis B Ab titers than those receiving vaccine alone. In conclusion, we report an optimal human CpG motif for phosphorothioate ODN that is a candidate human vaccine adjuvant.