Enhancement of immune responses by co-delivery of a CpG oligodeoxynucleotide and tetanus toxoid in biodegradable nanospheres.

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Abstract

Synthetic oligodeoxynucleotides (ODN) consisting of unmethylated bacterial DNA sequences with CpG motifs are potent immunological adjuvants. Immunostimulatory CpG sequences are species-specific. Optimal CpG sequences specific for humans, rodents, livestock, and companion animals have been reported. Nearly all of these reports describe the use of soluble forms of CpG ODN and antigens. We investigated the co-delivery of CpG ODN and antigens in biodegradable nanospheres as an alternative approach for immunization using tetanus toxoid (TT) as the model antigen and ODN 1826 as the model CpG sequence. TT and CpG ODN were co-encapsulated in poly(D,L-lactic-co-glycolic acid) nanospheres. Separate groups of C57BL/6 mice were subcutaneously immunized twice with TT and CpG ODN in nanospheres (test group), TT alone in nanospheres, TT alone in nanospheres mixed with CpG ODN in solution, TT and CpG ODN both in solution (reference group), TT alone in solution, and alum adsorbed TT. T cells isolated from the test group showed strong antigen-specific T cell proliferation ex vivo (stimulation index=45). This was significantly (P<0.0001) higher than that observed for T cells isolated from the reference group. The T cell proliferation of the test group was associated with higher levels of interferon gamma secretion (IFN-gamma 2694.7+/−41.1 pg/ml) than that of the reference group (814.7+/−50.2 pg/ml). Interleukin 4 (IL-4) secretion, if any, was below the detection limit (<13 pg/ml) in all the groups. Anti-sera obtained from the test group also showed very high total IgG titers (end point titers, 2560000) that were 16 times higher than the reference group. Similarly, differences of 8-fold for IgG1 and IgG3, and 5-fold for IgG2b titers were observed. Noticeably, the antibody response induced in the alum-TT group was far less (total IgG, end point titers 160000) than that obtained in the TT-CpG ODN nanospheres group. Overall, the results show that co-delivery of CpG and TT resulted in induction of both T helper type 1 and type 2 (Th1 and Th2) immune responses with a bias towards Th1 type. These results suggest that the co-delivery of CpG ODN adjuvants and antigens in nanospheres is a more efficient approach for immunization than the use of CpG ODN and TT in solution.

Keywords: Tetanus toxoid; CpG; Oligodeoxynucleotides; Nanospheres; Alum; Adjuvant