7 CpG Oligonucleotides as Immune Adjuvants

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7.1 Introduction

In recent years, it has become increasingly clear that effective vaccination strategies require activation of both the innate and the acquired arms of immune defenses. Vaccines comprising purified protein antigens have been found to induce little or no immune response unless the vaccine also contains components with the ability to activate antigen-presenting cells (APCs). Upon activation, the APCs upregulate their expression of costimulatory molecules such as B7–1 and B7–2, whose expression is essential for the optimal induction of acquired immune responses. Recent studies have demonstrated that many adjuvants have direct stimulatory effects on APCs.

It is now widely accepted that APCs possess pattern recognition receptors (PRRs), which give them a broad ability to detect molecular structures present in many pathogens, but not in host molecules. For example, vertebrates have evolved PRRs to detect microbial structures
such as lipopolysaccharide (LPS), high mannose proteins, and viral double-stranded RNA structures (Dempsey et al. 1996; Kumar et al. 1997).

Although DNA has usually been thought of primarily for its function of encoding genetic information, recent studies have also uncovered a structural difference between vertebrate and prokaryotic DNA which allows the detection of the latter by host APCs (Krieg et al. 1995). Tokunaga et al. (1984) were the first to demonstrate the specific immunostimulatory effect of bacterial genomic DNA for activating natural killer (NK) cells and interferon (IFN) secretion (Yamamoto et al. 1988, 1992b). B cell proliferation and immunoglobulin secretion is also specifically stimulated by bacterial but not vertebrate DNA (Messina et al. 1991). It is now clear that these potent immune stimulatory activities of bacterial DNA are due to its content of unmethylated CpG dinucleotides in particular base contexts (Krieg et al. 1995). In contrast to bacterial DNA, in which CpG dinucleotides are unmethylated and are generally present at the expected random frequency of approximately 1/16 bases, it has long been recognized that CpG dinucleotides are usually methylated at the 5 position of the cytosine and “suppressed” in vertebrate genomes, which contain only about 1/4 as many CpG dinucleotides as would be predicted if base utilization was random (Bird 1987). Furthermore, the base context of CpG dinucleotides in vertebrate genomes is not random; CpGs are most frequently preceded by a C and/or followed by a G (Han et al. 1994). If bacterial DNA is methylated with CpG methylase, which converts it into a form more similar to vertebrate DNA, the immune stimulatory activities are lost (Krieg et al. 1995). The immune stimulatory effects of bacterial DNA can be mimicked using synthetic oligodeoxynucleotides (ODN) containing one or more unmethylated CpGs in appropriate base contexts (Table 1). Immune stimulatory CpG ODN can be synthesized using either the native phosphodiester backbone or certain highly nuclease resistant backbones, such as the phosphorothioate backbone, which can greatly improve the immune stimulatory effects by increasing the stability and cellular uptake of the CpG ODN (Zhao et al. 1993, 1996; Krieg et al. 1995, 1996).