CHAPTER 9

Agonists of Toll-Like Receptor 9:
Modulation of Host Immune Responses with Synthetic Oligodeoxynucleotides

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Innate immunity is the body's first line of defense against invading microbes. This component of our immune system relies on highly conserved pattern-recognition receptors (PRRs) to distinguish different pathogens.1 Toll-like receptors (TLRs) are one class of PRR1-2 of which ten (TLR1-10) have now been identified in mammals.1-3 TLRs recognize pathogen-associated molecular patterns (PAMPs) and initiate the appropriate immune responses. These entail the activation of signal cascades leading to the secretion of cytokines, the activation of cell-surface molecules and the production of pathogen-specific immunoglobulins (Ig).4 As TLRs are constitutively expressed on immune cells and are responsive to synthetic ligands, they provide us with a rational way with which to modulate the immune system; a strategy which can be seen as quite distinct from conventional vaccination.

At least three TLRs, TLR3, 7, and 9 recognize and respond to nucleosides, nucleotides, and oligo- and polynucleotides of natural and/or synthetic origin (Fig. 1). TLRs 3 and 9 recognize nucleic acid molecular patterns that are present in microbes but not mammals.5-8 TLR3 recognizes viral RNA, synthetic polyI.polyC, and synthetic double-stranded (ds) RNA.9 Short interfering (si) RNAs can also activate the interferon (IFN) system.10,11 TLR7 recognizes small synthetic nucleoside-like fused-ring heterocyclic molecules7 whilst TLR9 is specific for d(CpG) dinucleotides in specific sequence contexts (CpG motifs), whether in bacterial, plasmid or synthetic DNA.5 Bacterial and synthetic CpG DNAs have direct mitogenic effects on B cells.12,13 These DNAs can stimulate NK-cell activity in vitro and activate macrophages, dendritic cells (DCs), and monocytes, causing them to secrete cytokines, chemokines and to express cell surface molecules.14-16 In vivo, CpG DNAs induce splenomegaly in mice (enlargement of the spleen)17,18 accompanied by splenic B cell proliferation, the up-regulation of class-II major histocompatibility complex (MHC class II) antigens, the increased synthesis of RNA and DNA and the elevated production of cytokines and chemokines.18

CpG DNA does not directly activate T and NK cells as these cells do not express TLR9. However, CpG DNA can indirectly augment the DC mediated stimulation and cytokine production of other immune cells.19 The expression of TLR9 in immune cells is required for these activities, but a direct interaction between CpG DNA and TLR9 has not yet been demonstrated. The ability of CpG DNA to induce strong innate then acquired immune responses is a clear indication of its potential as a pharmacophore and natural adjuvant.20-23
Figure 1. Three of the ten TLR family members that are known to recognize pathogen-associated nucleic acid or small nucleoside-like heterocyclic molecules. TLR 3 recognizes double-stranded viral and synthetic polyl.polyC RNAs. TLR 7 recognizes a number of synthetic nucleosides and small nucleoside-like molecules. No naturally occurring ligand for TLR 7 has yet been identified.* TLR 9 recognizes d(CpG) dinucleotides in specific sequential contexts (CpG motifs) in bacterial DNA, plasmid DNA, and synthetic oligodeoxynucleotides. TLR 9 also recognizes the synthetic YpG, CpR, YpR, and R'pG motifs discussed in the text. Key signaling components are shown. The activated transcription factors up-regulate the expression of a number of cytokines, chemokines, and costimulatory molecules.

* Since going to press murine TLR7 and human TLR8 have been shown to recognize ssRNA, both of viral and endogenous origin (Heil F et al. Science 2004; 303:1526-1529. Diebold SS et al. Science 2004; 303:1529-1531.)