

In Vivo Activity of Inhibitors of TLR7 and TLR9 in Normal and Pathological Contexts

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We and others have reported oligonucleotide (ODN) sequences that can inhibit TLR9 activation by CpG-containing immunostimulatory sequences (ISS) *in vitro*. We have recently developed series of novel ODN-based inhibitors of TLR signaling. These ODNs, we have termed immunoregulatory DNA sequences (IRS), include inhibitors of TLR9, inhibitors of TLR7 and most interestingly, sequences that inhibit signals through both receptors. When co-injected with ISS *in vivo*, IRS were shown to inhibit inflammation through a reduction in serum cytokine responses. The IRS did not need to be injected at the same site, demonstrating that rapid, systemic inhibition can be readily achieved. In normal mice, ISS stimulation does not induce death; however, when pre-treated with D-galactosamine (D-Gal), mice become highly susceptible to inflammation and die rapidly upon injection of ISS. Strikingly, injection of IRS prevented death of D-Gal-sensitized mice in a dose dependant manner while inactive control-ODN did not. In addition, we have found that IRS can also block TLR7 activation *in vivo*. Finally, the *in vivo* ability of IRS was tested in the lupus-prone (NZB x NZW) F1 mice. In this setting, we have demonstrated that IRS could decrease lupus-like symptoms, including decreased proteinuria levels, dsDNA autoantibody levels and overall renal involvement. These data strengthen the potential for such ODNs in therapeutic arenas with respect to autoimmune diseases such as systemic lupus erythematosus.