P16 & FP – **DNase I-Degraded Bacterial Genomic DNA Augments Activation of Human Toll-Like Receptor 9 by Synthetic DNA Oligonucleotides**

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Background: Human Toll-like receptor 9 (TLR9), an endosomal pattern recognition receptor of the innate immune system, responds to specific DNA motifs, such as dimers of unmethylated cytosine and guanine (CpG), which are more abundant in bacterial than human genomes. Previous studies demonstrated that synthetic DNA oligonucleotides (ODNs) of length 20-24nt directly activate TLR9 *in vitro*, and that TLR9 activation by ODNs can be augmented by co-stimulation with CpGcontaining short ODNs (sODNs) of length 2-7nt, which do not activate on their own.
Objective: We seek to determine and compare the role of genomic DNA from different bacterial species for human TLR9 stimulation, both as direct activators and as enhancers of TLR9 activation by other ligands.
Methodology: Genomic DNA of varying sequence-based predicted CpG concentrations from different bacterial species was isolated and treated with DNase I. Ramos Blue B cells were stimulated with combinations of synthetic oligonucleotides (ODN-2006, ODN-2006GC), short ODNs (TCGTT and TTTTT), and untreated or DNase I-digested bacterial genomic DNA. TLR9 response was measured as NF-κB induction via expression of the secreted embryonic alkaline phosphatase (SEAP) reporter gene.
Results: While stimulation with untreated bacterial genomic DNA induced less NF-κB than with ODN-2006, co-stimulation of even smaller amounts of ODN-2006, ODN-2006GC, and bacterial genomic DNA from different species with the sODN TCGTT enhanced TLR9 induction. In addition, DNase I-digested bacterial genomic DNA, which did not activate TLR9 on its own, augmented TLR9 activation by ODN-2006 at low concentrations. This “boost” effect of degraded DNA on TLR9 activation by ODN-2006 was dependent on the bacterial genomic CpG content.
Conclusion: The potential of microbial genomic DNA to activate human TLR9 may be dependent on a combination of direct stimulation by larger genomic DNA fragments and indirect enhancement of activation through other ligands by very short, degraded genomic DNA fragments.