Endosomal translocation of vertebrate DNA activates dendritic cells via TLR9-dependent and -independent pathways.

TLRs discriminate foreign from self via their specificity for pathogen-derived invariant ligands, an example being TLR9 recognizing bacterial unmethylated CpG motifs. In this study we report that endosomal translocation of CpG DNA via the natural endocytotic pathway is inefficient and highly saturable, whereas endosomal translocation of DNA complexed to the cationic lipid N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium methylsulfate (DOTAP) is not. Interestingly, DOTAP-mediated enhanced endosomal translocation of otherwise nonstimulatory vertebrate DNA or of certain noncanonical CpG motifs triggers robust dendritic cell activation in terms of both up-regulation of CD40/CD69 and cytokine production, such as type I IFN and IL-6. We report that the stimulatory activity of phosphorothioated noncanonical CpG oligodeoxynucleotides is TLR9 dependent, whereas phosphodiester DNA, such as vertebrate DNA, in addition trigger TLR9-independent pathways. We propose that the inefficiency of the natural route for DNA internalization hinders low affinity TLR9 ligands in endosomes to reach threshold concentrations required for TLR9 activation. Endosomal compartmentalization of TLR9 may thus reflect an evolutionary strategy to avoid TLR9 activation by self-DNA.