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PROFILING OF LENGTH- AND DOSE-DEPENDENT ACTIVATION OF INNATE IMMUNE RESPONSES BY DIFFERENT DNA STRUCTURAL FORMS IN A THP-1 DUAL MONOCYTE MODEL

Background. DNA-containing therapeutics can trigger unintended immune responses, posing a significant obstacle to their development. Investigating how DNA length and structural forms influence immunological activation is critical. This study utilizes THP1-Dual cells, a convenient model that simultaneously monitors the NF- κ B pathway via secreted alkaline phosphatase (SEAP) and the interferon regulatory factor (IRF) pathway via Lucia luciferase. **Methods.** THP1-Dual cells were transfected with double-stranded (dsDNA) and single-stranded DNA (ssDNA) fragments of varying lengths (186, 1746, and 3897 bp). The dsDNA fragments were generated by PCR and purified. The ssDNA fragments were produced by digesting these PCR products with lambda exonuclease, followed by an additional purification step. Purified DNA samples were delivered via electroporation (Neon NxT, 1400 V, 20 ms) at a standard concentration of 20 μ g/mL, or 1.5–50 μ g/mL for dose-response assays. Following a 24-hour incubation in RPMI-1640 with 10% HI-FBS at 37 °C, SEAP and Lucia luciferase activities were quantified using colorimetric (QUANTI-Blue Solution, InvivoGen) and chemiluminescent (QUANTI-Luc 4 Lucia/Gaussia, InvivoGen) assays, respectively. The data were expressed as fold-change relative to mock-electroporated controls. **Results.** No measurable immune activation was observed for any ssDNA samples. In contrast, all dsDNA samples induced statistically significant, length-dependent activation. The 186 bp dsDNA elicited minimal activation (3-fold for NF- κ B; 5-fold for IRF). For longer fragments, both the 1746 bp and 3897 bp dsDNA induced ~50-fold activation of the NF- κ B pathway, whereas the IRF pathway demonstrated much higher activation at 260-fold for 1746 bp and 170-fold for 3897 bp. Dose-response assays for longer dsDNA fragments revealed a strong dose-dependent effect with a minimal effective concentration of ~6 μ g/mL. High DNA concentrations over 50 μ g/mL triggered cell death, preventing activation from reaching a plateau. The 186 bp dsDNA showed a similarly shaped but significantly lower dose-dependent response. **Conclusions.** Single-stranded DNA does not trigger immunogenic pathways in THP1-Dual cells, whereas double-stranded DNA induces strong, length-dependent activation. Longer dsDNA fragments exhibit a pronounced dose-dependent immune response that is ultimately constrained by cytotoxicity.

Keywords: THP-1 Dual, ssDNA, dsDNA, NF- κ B pathway, IRF pathway, immune activation.

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