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# In ovo delivery of CpG-ODN protects against avian infectious laryngotracheitis virus (ILTV) infection

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Pathogen associated molecular patterns (PAMPs) are recognized by the Toll-like receptors (TLRs) leading to the induction of host innate immune responses. One of the PAMPs that triggers avian cells with TLR21 receptor (homolog of TLR9 in mammals) to release pro-inflammatory cytokines is oligonucleotides (ODN) containing unmethylated CpG motifs. Although the anti-viral effects of CpG ODN have been well reported for other avian host-viral models, its potential as an antiviral agent is yet to be explored against infectious laryngotracheitis virus (ILTV) when CpG ODN is delivered in ovo. In this study, we investigated whether CpG ODN can be used as an innate immune stimulant leading to anti-viral response against ILTV in vivo. Here, we delivered CpG ODN in ovo into embryo day (ED) 18 eggs so that CpG ODN is available at the respiratory mucosa. Then, we infected the CpG ODN and control ODN treated eggs at ED19 and observed the ILTV genome load in the respiratory tract at ED20. We observed that in ovo delivery of CpG ODN marginally inhibits ILTV infection which was associated with expansion of macrophage numbers in these tissues. A subset of CpG ODN treated and control eggs were allowed to hatch and then challenged with ILTV on the day of hatch. We observed that CpG ODN treatment in ovo protects chickens from clinical disease induced by post-hatch infection with ILTV. The protection against ILTV in CPG ODN treated chickens was associated with increase in macrophage and natural killer (NK) cell number in the respiratory mucosa and mRNA expression of other pro-inflammatory molecules. The data suggest that CpG ODN might be a potential innate immune stimulant with antiviral activity that can be used against ILTV infection in chickens.

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