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ABSTRACT

Preventative immunotherapeutic treatments have been an area of great interest to combat infectious disease because of the ability to stimulate the host's immune system which prepares the host to fight pathogenic microbes. The immunotherapeutic approach requires the use of an immune stimulating molecule that is able to boost the host's immune response. A major problem exists that these immune stimulating molecules are often very expensive and require a large dose to be effective. To reduce the cost of using these molecules, a delivery system can be used which is able to lower the effective dose of the immune stimulant while not causing any toxic effects towards the host's health. In this study, the immune stimulating molecules synthetic unmethylated cytidine-phosphate-guanosine oligodeoxynucleotides were attached non-covalently to multi-walled carbon nanotubes. The use of carbon nanotubes as a delivery mechanism could result in a lower effective dose able to stimulate a protective immune response in a chicken model. In this study, we first assessed which of the non-covalant linkages was ideal for linking the immune stimulant to the carbon nanotubes. This was conducted by looking at which method of linkage would allow the best cellular proliferation and transcriptional activation of selected innate immune genes. Once an appropriate linkage method had been selected, cellular uptake studies were conducted to establish that cytidine-phosphate-guanosine oligodeoxynucleotides were delivered to intracellular target receptors. After cellular uptake was demonstrated, it was important that the carbon nanotubes linked to the immune stimulant do not cause toxicity towards the host. To measure toxicity, *in vitro* studies were conducted to observe cell viability post treatment with carbon nanotube linked immune stimulant. Further studies were conducted on any alterations to the immune stimulants' ability to activate immune cells by studying the pathway of macrophage activation. The protective ability of the molecules was then measured by the ability to protect chickens from a lethal challenge with *S. typhimurium*. Once the protective nature of the molecules was established, the mechanism of immune stimulation was examined by *in vivo* cell recruitment and *in vitro* cytokine production. These studies indicate that linking cytidine-phosphate-guanosine oligodeoxynucleotides to carbon nanotubes can lower the effective dose of the immune stimulant without altering the biological function of the molecule.

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LIST OF ABBREVIATIONS

AFM	Atomic Force Microscopy
ANOVA	Analysis of variance
AP	Activator protein
APC	Antigen presenting cell
BIEC	Bovine intestinal epithelial cell
cDNA	Complimentary deoxyribonucleic acid
CpG	Cytidine-phosphate-guanosine
DMEM	Dulbecco's modified eagle's medium
DMF	N,N-Dimethylformamide
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ERK	Extracellular receptor kinase
FBS	Fetal bovine serum
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HD11	Avian MC29 virus-transformed chicken macrophage cell
IFN-α	Interferon-alpha
IFN-β	Interferon-beta
IFN-γ	Interferon-gamma
Ig	Immunoglobulin
IKK	IκB kinase
IL	Interleukin
iNOS	Inducible Nitric-Oxide Synthase
IRAK1	Interleukin-1 receptor-associated kinase-1
IRF	Interferon regulatory factor
IκB	Phosphorylation inhibitor
LN	Lymph node
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
mDC	Myeloid dendritic cell

MDC	Monodansylcadaverine
MEK	MAPK/ERK kinase
MHC	Major histocompatibility complex
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MWCNT	Multi-walled carbon nanotube
MWCO	Molecular weight cut off
MyD88	Myeloid differentiation factor 88
NF-κB	Necrosis-factor-kappa-beta
NO	Nitric oxide
OD	Optical density
ODN	Oligodeoxynucleotide
p38	Protein 38
p65	Protein 65
PAL	Poly-allylamine hydrochloride
PAMP	Pathogen-associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pDC	Plasmacytoid dendritic cell
PEI	Polyethyleneimine
PKC	Protein kinase C
PRR	Pattern recognition receptor
PySE	Pyrenebutanoic acid, succinimidyl ester
qRT-PCR	Quantitative real time -polymerase chain reaction
RNA	Ribonucleic acid
SQ	Subcutaneous
ssODN	Single-stranded olgodeoxynucleotides
Th	Helper T cells
TICAM	Toll-like receptor adaptor molecule

TLR	Toll-like receptor
TNF	Tumour-necrosis factor
TRAF	Tumour-necrosis factor-α receptor activated factor