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ABSTRACT

Nippostrongylus brasiliensis is a rodent intestinal nematode with an important pulmonary migrating stage. Previous studies have observed a lack of TNF-α production and minimal recruitment of neutrophils, which led us to the belief that anti-inflammatory mechanisms could be active in the lung stage. In this study, lipopolysaccharide (LPS) stimulated alveolar macrophages (NR8383) or rat lungs were used as *in vitro* or *in vivo* inflammation models respectively. Both live *N.brasiliensis* larvae and NES significantly reduced the production of LPS-induced pro-inflammatory mediators, TNF-α and NO, but not IL-1β, in NR8383 cells. The inhibition of TNF-α production was related to the heat-labile and trypsin-sensitive fraction of NES concentrate. 1-D protein gel of NES concentrate revealed that the molecular weights of proteins are between 6kDa and 100kDa. Glycoproteins were found abundant in NES concentrate. The inflammatory processes, including NF-κB translocation and TNF-α gene transcription were significantly inhibited by NES and/or NES concentrate. *In vivo*, we observed a significant reduction of neutrophil recruitment (≈ 40%) by NES on a background of LPS (100ng/ml) induced inflammation. This reduction was associated with the significant inhibition in gene transcriptions of proinflammatory mediators TNF-α, IL-1β, iNOS, ICAM-1 and MIP-2 in bronchoalveolar lavage (BAL) cells. The down-regulation of pro-inflammatory mediators and inflammatory processes observed in this study suggests that *N.brasiliensis* larvae and/or NES are capable of modifying the normally potent LPS inflammatory response, both *in vitro* and *in vivo*. This study and planned future studies could be fundamental in developing anti-inflammatory agents with immune-active molecules in *N.brasiliensis*-derived products.

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ABBREVIATIONS

2-ME	2-mercaptoethanol
AAMs	Alternatively activated macrophages
Ac-TMP	*Ancylostoma caninum* tissue inhibitor of metalloproteinase
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
BCA	Bicinchoninic Acid
BSA	Bovine serum albumin
CAMs	Classically activated macrophages
CD	Crohn's disease
cDNA	Complementary deoxyribonucleic acid
DALYs	Disability-adjusted life-years
DC	Dendritic cells
DEPC	Diethyl pyrocarbonate
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotides
DTT	Dithiothreitol
E. coli	*Escherichia coli*
ECL	Enhanced Chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked-Immuno-Sorbent Assay
ES	Excretory-secretory
FBS	Fetal bovine serum
FCS	Fetal calf serum
FITC	Fluorescein isothiocynanate
GAPDH	Glyceraldehydes-3-phosphate

GMFI	Geometric median fluorescence intensity
GSH	L-γ-glutamyl-L-cysteinylglycine, reduced from
GSSG	L-γ-glutamyl-L-cysteinylglycine, oxidised form
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HRP	Streptavidin-horseradish peroxidase conjugate
IBD	Inflammatory bowel disease
ICAM	Intercellular adhesion molecule
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL-10	Interleukin-10
IL-13	Interleukin-13
IL-17	Interleukin-17
IL-1RI	Type I IL-1 receptor
IL-1RII	Type II IL-1 receptor
IL-1β	Interleukin-1 beta
IL-4	Interleukin-4
IL-5	Interleukin-5
iNOS	Inducible nitric oxide synthase
IRS	insulin receptor substrate
L3	Third stage larvae
LBP	Lipopolysaccharide binding protein
LDH	Lactate dehydrogenase
LFA	lymphocyte function-associated antigen
L-G	L-glutamine
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase

MD-2	Myeloid differentiation protein-2
MIP-2	Macrophage inflammatory protein-2
MMP	Matrix metalloproteases
mRNA	Messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
MWCO	Molecular weight cut off size
NADH	Nicotinamide adenine dinucleotide
NED	Napthylethylenediamine dihydrochloride
NES	*N.brasiliensis* L3 larvae excretory-secretory products
NF-κB	Nuclear factor-κB
NK	Natural killer
NO	Nitric oxide
NOS	Nitric oxide synthase
OD	Optical density
OPT	O-phthaldehyde
P/S	Penicillin/ streptomycin
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PECAD	Platelet endothelial cell adhesion molecule
PES	Polyethersulphate
PMSF	Phenylmethylsulphonyl fluoride
PRRs	Pattern recognition receptors
Q-PCR	Quantitative real time PCR
RNA	Ribonucleic acid
RPMI	Roswell park memorial institute medium
RT	Room temperature

RT-PCR	Reverse transcription-polymerase chain reaction
SDS PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SOD	Superoxide dismutase
STAT	Signal transducer and activators of transcription
T1D	Type 1 Diabetes
TBE	Tris-Borate-EDTA
TBS	Tris buffered saline
TBST	0.1% Tween-20 in Tris buffered saline
TFA	Trifluoroacetic acid
TGF-β	Transforming growth factor-beta
Th1	T helper type 1
Th17	T helper type 17
Th2	T helper type 2
TIMP	Tissue inhibitor of metalloproteases
TIR	Toll/Interleukin-1 receptor
TLR	Toll like receptor
TNFR	Tumour necrosis factor receptor
TNF-α	Tumour necrosis factor alpha
Treg	T regulatory
UC	Ulcerative colitis
VCAM	Vascular cell adhesion molecule