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Ι

ABSTRACT

Nippostrongylus brasiliensis is a rodent intestinal nematode with an important pulmonary migrating stage. Previous studies have observed a lack of TNF-a 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 LPSinduced pro-inflammatory mediators, TNF- α and NO, but not IL-1 β , 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- κ B 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 ($\approx 40\%$) 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-1 β , 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
- lg 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 0.1% Tween-20 in Tris buffered saline TBST 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 T regulatory Treg UC Ulcerative colitis Vascular cell adhesion molecule VCAM