The Effects of Exosomal Derived TSG-6 on Microglia Activation

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Background

- Microglia are macrophages located in CNS and retina
- Regulate neuronal function and clearance of cell debris for neuronal regeneration and growth
- “Disease associated microglia” (DAM) are overactive microglia in neurodegenerative states and brain injuries that release pro-inflammatory cytokines and phagocytose viable neurons
- Distinguished by increased expression DAM genes and phagocytic activity
- Traumatic brain injury (TBI) causes visual deficits and is observed to increase levels of overactive DAM.

- Stem cell therapies are being developed as therapeutics for TBI visual deficits.

- Human mesenchymal stem cells (MSC) can be stimulated with inflammatory cytokines to secrete secretomes (conditioned medium).

- MSC conditioned media shown to decrease phagocytosis of mouse microglia in-vitro, decrease visual deficits in TBI model mice.

- Prior studies suggest within the secretome, exosomes (transport vesicle) containing anti-inflammatory protein TSG-6 are therapeutic.
Hypothesis

• Exosomal derived TSG-6 will decrease activation of stimulated microglia

Methods

• Cell line: HMC3 human fetal microglia commercial line
• Exosomes: containing TSG-6, collected from adipose stem cell media
• Conditions:
  • APOE transfected HMC3
  • LPS IFN-γ stimulated HMC3
• Experiment:
  • Phagocytosis Assay
  • PCR Gene Expression
Exosomal collection

A

siRNA

ASC Culture

Cytokine treatment

Collect Samples

-24h

0h

24h

48h

siControl

siTSG-6

IFNγ / TNFα

ASC-CCM (50 µg/ml)

B

Ultracentrifugation

+ +

anti-TSG-6

anti-TIMP1

Exosome (50 µg/ml)

C

DAPI
dil-ASC-Exo

IBA-1

Positive GM130 Flot-1 ICAM ALIX CD81

CD63 EpCAM ANXA5 TSG101 Blank Positive

Intensity (%)

Size (nm)
Phagocytosis Assay

Attachment 24 h → Exosome (20 ng/ml) incubation → LPS (100 ng/ml) & IFN-γ (20 ng/ml) Or APOE (20 ng/ml), with exosomes → Fluorosphere incubation → Imaging

Control

LPS IFN-γ

LPS IFN-γ + exosomes

DAPI
Flourosphere
Di-O

APOE

APOE + exosomes

HMC3 Phagocytosis Activity

Fluorosphere intensity/area (AU) (mean ± SD)

Control  LPS IFN  LPS IFN + exosomes

p < 0.05  p < 0.005

HMC3 Phagocytosis Activity

Fluorosphere intensity/area (AU) (mean ± SD)

Control  APOE  APOE + exosomes

p < 0.0005  p < 0.0005
PCR Gene Expression

LPS (100 ng/ml) & IFN-γ (20 ng/ml)  
Or APOE (20 ng/ml), with exosomes  
24 h  
Cell lysate collection  
RNA isolation  
cDNA conversion  
PCR

![Graphs showing fold change in gene expression](image)
Conclusions:

• LPS IFN-\(\gamma\) and APOE stimulation significantly increased phagocytic activity, exosomal derived TSG-6 significantly decreased phagocytic activity

• LPS IFN-\(\gamma\) and APOE stimulation increased DAM gene expression, exosomal derived TSG-6 showed decreased DAM gene expression

Future Directions:

• Adjust stimulation period and exosome concentration for gene expression
• Utilize engineered cells containing isolated TSG-6 exosomes
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References


