Exploring the effects of pH responsive polymeric nanoparticles on inflammasome activation

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BACKGROUND

INFLAMMATORY IMMUNE RESPONSE

NLRP3 Inflammasome Activation

Inflammasomes are multi-protein oligomers formed in cells that respond to cellular distress by promoting the maturation and secretion of pro-inflammatory cytokines.

Caspase-1 Activation

Caspase-1 is an enzyme that plays an essential role in programmed cell death and inflammation. It cleaves precursors of inflammatory cytokines into active cytokines.

Interleuken-1β (IL-1β)

IL-1β is an inflammatory cytokine that induces fever, mediates the inflammatory response, and modifies adaptive immune cells.

pH RESPONSIVE POLYMERIC NANOPARTICLES

- Stable micelles form at a pH of 7.4
- Micelles destabilize at the endosomal pH of 5.8
  - Polymers breach endosome membrane and enter cytosol
- Hydrophobic group compositions:
  - BMA% ranging from 20 to 60
  - B20 = 20% BMA and 80% DMAEMA

RESULTS

IL-1β SECRETION IN THP-1 CELLS (24 HR DOSING)

Seeding
THP-1 cells were seeded at a concentration of 80,000 cells/well

Differentiation
The THP-1 cells were differentiated into macrophages using 100 nM PMA

Priming
The macrophages were primed with 100 ng/mL LPS in media for 3 hours

Dosing
The macrophages were dosed with several polymeric micelles at various concentrations

Reporting
50,000 cells/well of HEK-Blue cells were plated with the dosed macrophage supernatant (19:1 ratio)

Testing
Hemolysis, QUANTI-Blue, ELISA, and CellTiter-Glo assays were performed to characterize the cells

OBJECTIVE

GOAL
To test various polymers for inflammasome activation...

HOW?
...by quantifying the amount of IL-1β secretion...

WHY?
...to gain insight on the inflammasome’s role in the immune response to polymeric micelles.

CONCLUSION

IMPLICATIONS OF RESULTS
- The polymeric micelles cause endosomal escape
- The IL-1β secreted is caused by inflammasome activation
- Extended dosing times are not necessary for IL-1β secretion
- The polymeric micelles are cytotoxic

FUTURE STEPS
- Study effects of inflammasome inhibitor on THP-1 cells
- Test lysosome rupture using flow cytometry

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