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 programed cell death and inflammation. It cleaves precursors of inflammatory cytokines into active cytokines.

Interlueken-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

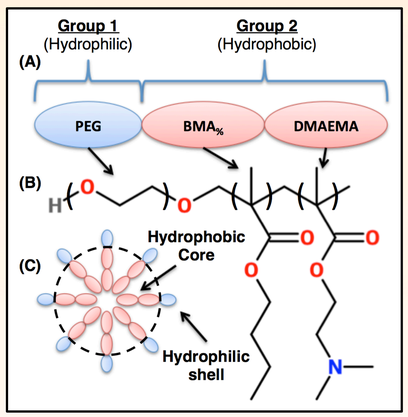

Figure 1: (A) Hydrophilic and hydrophobic groups of polymer. **(B)** Polymer structure. **(C)** Micelle structure.

OBJECTIVE

GOAL	HOW?	WHY?
To test various polymers for inflammasome activation...	...by quantifying the amount of IL-1β secretion...	...to gain insight on the inflammasome's role in the immune response to polymeric micelles.

METHODS

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

RESULTS

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

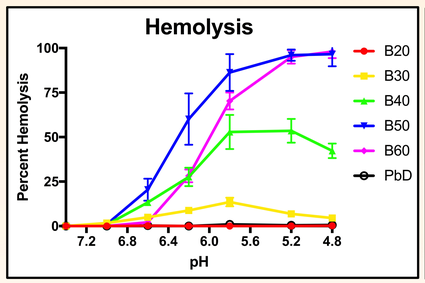

Figure 2: Hemolysis assay of polymeric micelles shows that endosomal escape of polymers increases at endosomal pH levels.

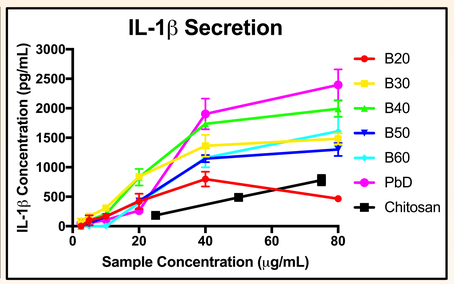

Figure 3: QUANTI-Blue assay detects SEAP from HEK-Blue cells and displays IL-1β secretion levels from THP-1 cells.

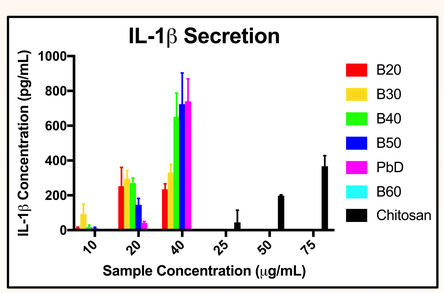

Figure 4: ELISA assay reveals more accurate IL-1β secretion levels and confirms reliability of QUANTI-Blue assay.

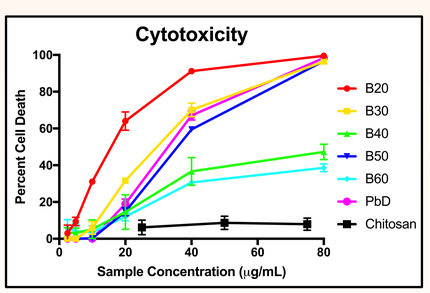

Figure 5: CellTiter-Glo assay shows increased cell death with increased sample concentration.

COMPARING RESULTS WITH NLRP3 GENE DEFFICIENT THP-1 CELLS (4 HR DOSING)

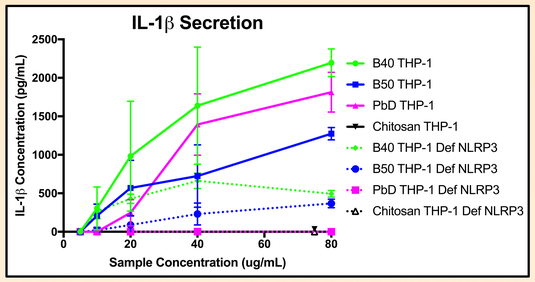

Figure 6: QUANTI-Blue assay confirms decreased IL-1β secretion levels in NLRP3 gene deficient cells.

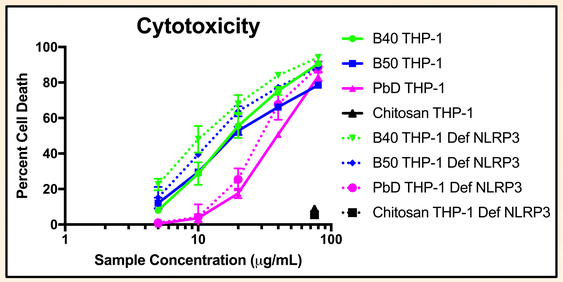

Figure 7: Increasing cytotoxicity results from CellTiter-Glo assay indicates that the polymers themselves contribute to cell death.

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 cytomerty

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