The Role of Neutrophil Extracellular Traps (NETs) in Beryllium-Induced Disease

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Background

Beryllium is in high demand as a technological and industrial material because of its high melting point, low density, and strength. People occupationally exposed to beryllium are at risk of beryllium sensitization (BES), which may progress to the fatal granulomatous lung disease known as chronic beryllium disease (CBD). Genetic susceptibility to CBD involves a specific allele in the HLA-DR β-chain gene that allows beryllium to induce a sterile inflammatory response and recruit neutrophils to the lungs. Neutrophils kill microbes by phagocytosis, degranulation, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs). Neutrophils use NETs to catch microbes by releasing their chromatin, inflammatory cytokines, and granulocytic proteins once activated. The goal of my project was to determine if beryllium exposure induced NET formation.

Hypothesis

Beryllium induces human neutrophils to produce NETs through ROS.

Materials and Methods

• Peripheral blood neutrophils were separated from mononuclear cells and plasma with Ficoll. The red blood cells were sedimented in 3% dextran and lysed.
• Flow cytometry determined neutrophil purity.
• The purified neutrophils were incubated with one or more of the following treatments for four hours:
  - C-RPMMI media
  - 25 nM PMA to induce NETosis
  - 200 mg/mL BeOH to induce necrosis
  - 12.5 μg/mL superoxide dismutase (SOD)
• The pellets of the treated neutrophils were stained with anti- elastase and Hoescht for analysis by fluorescent microscopy.
• Enzyme Linked Immunosorbent Assays (ELISA) quantified the amount of NETs in the supernatant of the treated neutrophils.

Results

Figure 1: Purified neutrophil viability decreases after cryopreservation.

Figure 2: Viability assay of post-treated pellets using flow cytometry contain live neutrophils.

Figure 3: Beryllium induces the release of NETs in the presence of inhibitors.

Figure 4: ELISAs detect less NETs in beryllium-treated neutrophils than the neutrophils incubated in media.

Conclusions

• Cryopreserved neutrophils do not maintain viability.
• NET quantification in response to BeOH, as of yet, cannot be determined.
• Beryllium particles attract extracellular DNA, keeping any possible NETs in the plasma with Ficoll. The red blood cells were sedimented in 3% dextran and lysed.
• The high cell death in PMA + SOD treated cells indicated this SOD may be toxic to the cells or not membrane permeable to oxidize the damaging superoxides.
• NETs are still formed in microscopy in the presence of ROS inhibitors.

Future Experiments

• Re-suspending beryllium into the supernatant will ensure that any NETs adhered to the cation will be measured.
• Staining the treated neutrophils with Sytox Orange will only stain extracellular DNA and can be quantified with a fluorescent plate reader.
• Time course microscopy of neutrophils incubated with beryllium, ROS inhibitors and controls will determine the NETotic differences between the treatments.
• Further studies can compare the NET and IL-1B differentiation between healthy individuals, BeS, and CBD patients.

References


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