

AUTHENTICATION OF KEY BIOLOGICAL AND/OR CHEMICAL RESOURCES

Key resources for this proposal include:

- 1) PGP peptides: We purchase both PGP and N- α -PGP from Bachem (Bubendorf, Switzerland) at a >99% purity. Beyond the company's strict quality control, each batch of the synthesized peptide undergoes rigorous quality control assessment in our laboratory including mass spectrometry signature via electron spray ionization tandem mass spectrometry to assess for appropriate peptide size and sequence. In addition, bioactivity of peptides is assessed for each batch via neutrophil chemotaxis assays. A PGP peptide-dose curve is tested with 10 ug/ml for N- α -PGP or a dose of 100 ug/ml for PGP each giving 50% increase of PMN chemotaxis. If a discrepancy is noted on either of these results, the PGP peptide is discarded.
- 2) Exosomes: PMNs supernates will be purchased from Zen-Bio. We will obtain donor-specific information. Exosomes are purified from PMN supernates using ultracentrifugation and are enumerated on our NanoSight 300. Little donor variability has been observed between donors. These nanoparticles are validated as exosomes, based on size, exosomal markers such as CD63, Alix, TSG 101, morphology by EM and density by appropriate gradients. PMN origin is validated with CD66b.
- 3) Immunoreagents (ie antibodies) will be purchased from commercial sources. These reagents will be tested with validated targets (ie recombinant proteins for antibodies), prior to use in the outlined experiments.
- 4) All other reagents including ZPP, fMLP, and buffers are standard laboratory reagents available from numerous commercial vendors.