Sex-based differences in inflammatory responses to silver nanoparticles

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Abstract

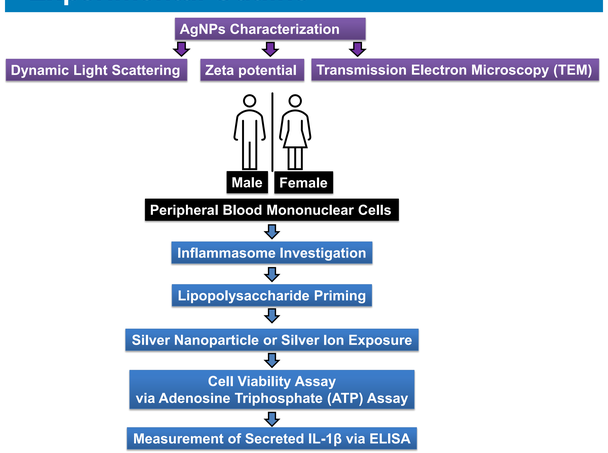
It has been increasingly important to distinguish sex when evaluating the biocompatibility of nanomaterials. Increasing evidence has been reported demonstrating the distinguishable differences in male and female innate and adaptive immune systems. Silver nanoparticles (AgNPs) have been incorporated into consumer, healthcare, and industrial products to serve as an anti-microbial agent. However, emerging health concerns involving continuous exposure to these AgNPs have been associated to reports demonstrating the proinflammatory and toxic effects of AgNPs. To date, the effects of AgNPs on the basic immunological functions have not been thoroughly evaluated to compare the potential sex-based differences. Thus, the goal of the study aims to investigate the sex-based differences in inflammasome activation after exposure to AgNPs on peripheral blood mononuclear cells (PBMCs) from 6 male and 6 female healthy human donors. These PBMCs were obtained from younger donors (ages 20 – 30 years old) and older donors (ages 40 – 51 years old) for comparison purposes. AgNPs (30 nm) were characterized for their hydrodynamic size by dynamic light scattering and their primary size by transmission electron microscopy. PBMCs were exposed to AgNPs (30 nm) for a period of 6- and 24-hours. Following exposure, IL-1β was monitored by ELISA to determine the inflammasome activity. The 6-hour exposure to AgNPs cause significantly more inflammasome activation in the females. This induction was still observed after 24-hours but was determined not be statistically significant. The young males had more inflammasome activity after 6-hours when compared to the old males; however, this difference was not observed after 24-hours. Interestingly, both age groups had similar inflammasome activation after 6-hours, but the young females had more inflammasome activity after the 24-hour exposure than the older females. The results demonstrate a distinct sex- and age-based difference in inflammasome activation by AgNPs. These findings provide more information that may help evaluating the potential sex-based health risks associated with AgNPs for improving women's and men's health.

Background

Inflammasome

- Complex structure of cytoplasmic proteins that is assembled in response to molecules and pathogens as part of the innate immune system which is responsible for the maturation of IL-1β [1].
- Inflammasome activation plays a protective role, but dysregulation has been associated to the pathogenesis of inflammatory diseases [1].
- Sex-based differences in inflammasome activity have been reported in different models but no work has been performed with AgNPs [2, 3].
- Silver nanoparticles have been shown to activate the inflammasome in human monocytes and PBMCs [4, 5].

Experimental Outline



Physical Characterization

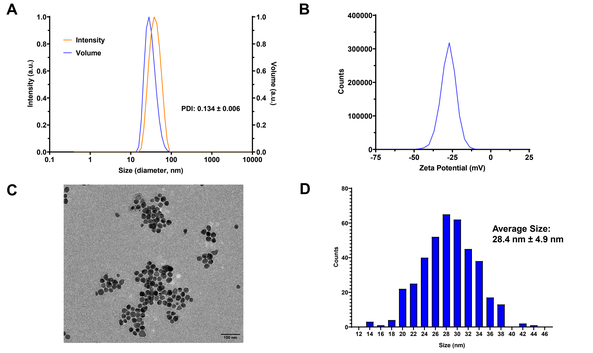


Figure 1. Physical characteristics of AgNPs. Hydrodynamic size and zeta potential was measured using a Malvern Zetasizer. (A) The hydrodynamic size was determined using DLS for the intensity and volume size distributions. Data was normalized to the peak size measured. (B) Zeta potential measurement. (C) Representative TEM image obtained using a TEM. (D) Size distribution analysis measured for TEM images.

Ag ions

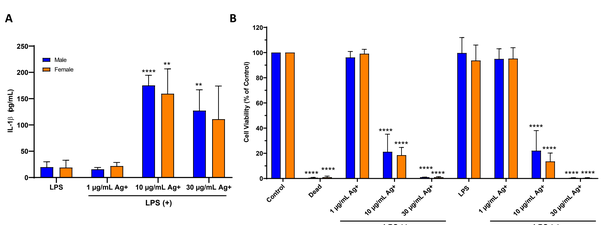


Figure 2. Inflammasome and cell viability . PBMCs from male and female donors were primed with LPS for 4-hours prior to 6-hour Ag ion exposure (n=3). (A) IL1-β levels measured by ELISA (B) Cell viability was determined by an ATP assay. **p<0.01 and ****p<0.0001 versus LPS priming controls.

Cell Viability

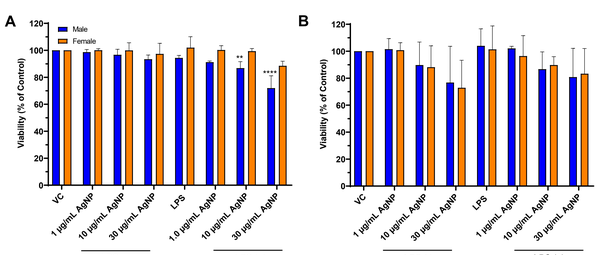


Figure 3. Cell viability. The cell viability of PBMCs from male and female donors with or without a 4-hour LPS priming, followed by a 6- and 24-hour of AgNPs exposure (n=3). Viability was determined at end points determined by an ATP assay. (A) 6-hour. (B) 24-hour. **p<0.01 and ****p<0.0001 versus controls (VC).

Inflammasome Activation

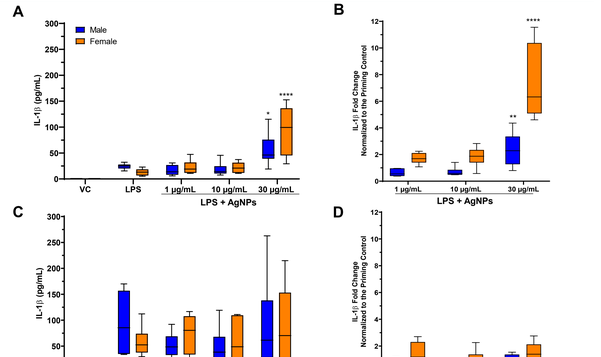


Figure 4. Inflammasome induction. PBMCs from 6 male and 6 female donors were primed with LPS prior to a 6- and 24-hour AgNPs exposure. The IL1-β levels measured by ELISA. The average of each independent experiment (n=6) is depicted in box-and-whisker plots for comparison (A) 6-hour AgNPs exposure (C) 24-hour AgNPs exposure. Data were normalized to the LPS priming controls to determine the fold-change of the IL-1β associated to the AgNPs. (B) 6-hour AgNPs exposure (D) 24-hour AgNPs exposure. Vehicle control (VC). *p<0.05, **p<0.01, and ****p<0.0001 versus LPS priming controls.

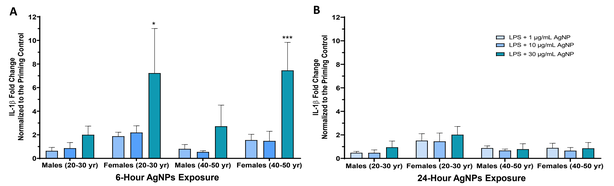


Figure 5. Sex and age analysis of inflammasome induction. IL-1β data from male and female donors were further divided into distinct age ranges, n=3 independent experiments for each age group. (A) 6-hour AgNP exposure. (B) 24-hour AgNP exposure. *p<0.05 and ***p<0.001 versus LPS priming controls.

Inflammasome Statistical Assessment

Initial multi-level mixed effects model

$$Inflammasome = Age + Sex + Time + Exposure + Time \times Exposure + Sex \times Time$$

Significant interaction: Sex and Time. Model stratified.

Simplified model: $Inflammasome = Age + Sex + Time + Exposure$

Table 1. Statistically significant results

6-Hour Statistically Significant Results						
Fixed Effects	Levels	Estimate (Fold)	Standard Error	DF	t Value	p-value
Sex[a]	Female	1.7982	0.4360	42	4.12	**0.0002**
AgNPs Exposure[b]	1 μg/mL AgNP	0.1726	0.6166	42	0.28	0.9046
	10 μg/mL AgNP	0.2688	0.6166	42	0.44	0.7983
	30 μg/mL AgNP	3.8516	0.6166	42	6.25	**<.0001**
24-Hour Statistically Significant Results						
Age Groups[c]	40-51 Years Old	-0.2724	0.1311	41	-2.08	**0.0441**
Sex[a]	Female	0.3658	0.1311	41	2.79	**0.0080**
AgNPs Exposure	1 μg/mL AgNP	-0.08921	0.1876	41	-0.48	0.6368
	10 μg/mL AgNP	-0.1841	0.1832	41	-1.00	0.3210
	30 μg/mL AgNP	0.1710	0.1832	41	0.93	0.3561

[a]Male is the baseline comparison group.
[b]LPS is the baseline comparison exposure.
[c]The 20-30 years of age category is the baseline comparison group.

Summary of Results

Physical characterization of AgNPs

- AgNPs hydrodynamic size was 35.9 nm with a -25 mV zeta potential (Fig. 1A,B).
- The average primary size of AgNPs by TEM was 28.4 nm (Fig. 1C,D).

Inflammasome induction by AgNPs and Ag ion exposure

- Ag ions induce inflammasome activation similarly in both sexes for the 10 and 30 μg/mL Ag ion 6-hour exposures (Fig. 2A).
- PBMCs show a significant increase in IL-1β with the 30 μg/mL AgNPs after 6-hours when compared to the lipopolysaccharide (LPS) priming controls (Fig. 4B).
  - Female PBMCs exhibit an average 7.3-fold increase in IL-1β
  - Male PBMCs exhibit an average 2.4-fold increase in IL-1β

Statistical modeling for significant sex-based differences (Table 1)

- Female PBMCs are estimated to have 1.8-fold more inflammasome activation than male PBMCs for the 6-hour exposure when controlling for Age and AgNPs Exposure.
- There is a significant sex-based difference with the 6-hour 30 μg/mL AgNPs treatment.
- Female PBMCs continue to have significantly higher inflammasome activation than male PBMCs for the 24-hour exposure.
- PBMCs from donors ages 20–30 yr have statistically more inflammasome activation than PBMCs from donors ages 40-51 yr at 24-hour exposure.

Cytotoxicity of AgNP and Ag ions

- Ag ions are extremely cytotoxic to the PBMCs with significant cell death (>80%) for both sexes, indicating that there is low dissociation of the AgNPs in this model (Fig. 2B).
- AgNP exposure caused concentration-dependent cytotoxicity in PBMCs of both sexes at each exposure time, with males showing significant cell death after 6 hours (Fig. 3).

Conclusions and Future Directions

- Female PBMCs have higher inflammasome activity induced by AgNPs than male PBMCs.
- Ag ions activate inflammasome and caused significant levels of cell death. However, very low dissociation of AgNPs is expected in this model.
- The underlying mechanism for the differences in the inflammasome activation by AgNPs for the male and females will be investigated.

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Disclaimer

This poster reflects the views of the authors and does not necessarily reflect those of the U.S. Food and Drug Administration.

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