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Silver Nanoparticles Induce Degradation of the Endoplasmic Reticulum Stress Sensor Activating Transcription Factor-6 Leading to Activation of the NLRP-3 Inflammasome

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Jean-Christophe Simard[1], **Francis Vallieres, Rafael de Liz, Valerie Lavastre, and Denis Girard**[2]

From the Laboratoire de recherche en inflammation et physiologie des granulocytes, Institut National de la Recherche Scientifique-Institut Armand-Frappier, Université du Québec, Laval, Québec H7V1B7, Canada

Background: Some nanoparticles are known to induce endoplasmic reticulum (ER) stress and lead to cell death.
Results: Silver nanoparticles induce ATF-6 degradation, leading to activation of the NLRP-3 inflammasome and pyroptosis.
Conclusion: ATF-6 is an important target to silver nanoparticles.
Significance: Our results provide a new link between ER stress and activation of the NLRP-3 inflammasome.

In the past decade, the increasing amount of nanoparticles (NP) and nanomaterials used in multiple applications led the scientific community to investigate the potential toxicity of NP. Many studies highlighted the cytotoxic effects of various NP, including titanium dioxide, zinc oxide, and silver nanoparticles (AgNP). In a few studies, endoplasmic reticulum (ER) stress was found to be associated with NP cytotoxicity leading to apoptosis in different cell types. In this study, we report for the first time that silver nanoparticles of 15 nm ($AgNP_{15}$), depending on the concentration, induced different signature ER stress markers in human THP-1 monocytes leading to a rapid ER stress response with degradation of the ATF-6 sensor. Also, $AgNP_{15}$ induced pyroptosis and activation of the NLRP-3 inflammasome as demonstrated by the processing and increased activity of caspase-1 and secretion of IL-1β and ASC (apoptosis-associated speck-like protein containing a CARD domain) pyroptosome formation. Transfection of THP-1 cells with siRNA targeting NLRP-3 decreased the $AgNP_{15}$-induced IL-1β production. The absence of caspase-4 expression resulted in a significant reduction of pro-IL-1β. However, caspase-1 activity was significantly higher in caspase-4-deficient cells when compared with WT cells. Inhibition of $AgNP_{15}$-induced ATF-6 degradation with Site-2 pro-

medical healthcare. Silver nanoparticles (AgNP) are among the most commonly used NP in nanomedicine, mainly because of their potent antimicrobial properties, increasing the interest to use them for drug delivery (1). Indeed, silver ions and nanosilver were shown to be highly toxic for various types of microorganisms, including *Pseudomonas* spp. and *Escherichia* spp. (2, 3). Even if potential exposure of humans to AgNP is already high, it will certainly increase in the becoming years. Because the toxicity of AgNP in humans is not fully understood, it is highly relevant to investigate their mode of action at the cellular and molecular level in humans.

Endoplasmic reticulum (ER) stress leads to unfolded protein response, a major hallmark of cytotoxicity. To date, three ER stress sensors have been documented: protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE-1), and activating transcription factor 6 (ATF-6). IRE-1 and PERK both contain cytoplasmic kinase domains known to be activated by homodimerization and autophosphorylation in the presence of ER stressors (4–6). In the case of ATF-6, accumulation of unfolded proteins induces ATF-6 transition to the Golgi, where it is cleaved by two transmembrane proteins, Site-1 and Site-2 proteases (7). ATF-6 cleavage yields a cyto-