THE IMMUNE RESPONSE INDUCED BY TOLL-LIKE RECEPTOR LIGANDS IS DIFFERENTIALLY REGULATED BY TIOPRONIN MONOLAYER-PROTECTED SILVER NANOPARTICLES

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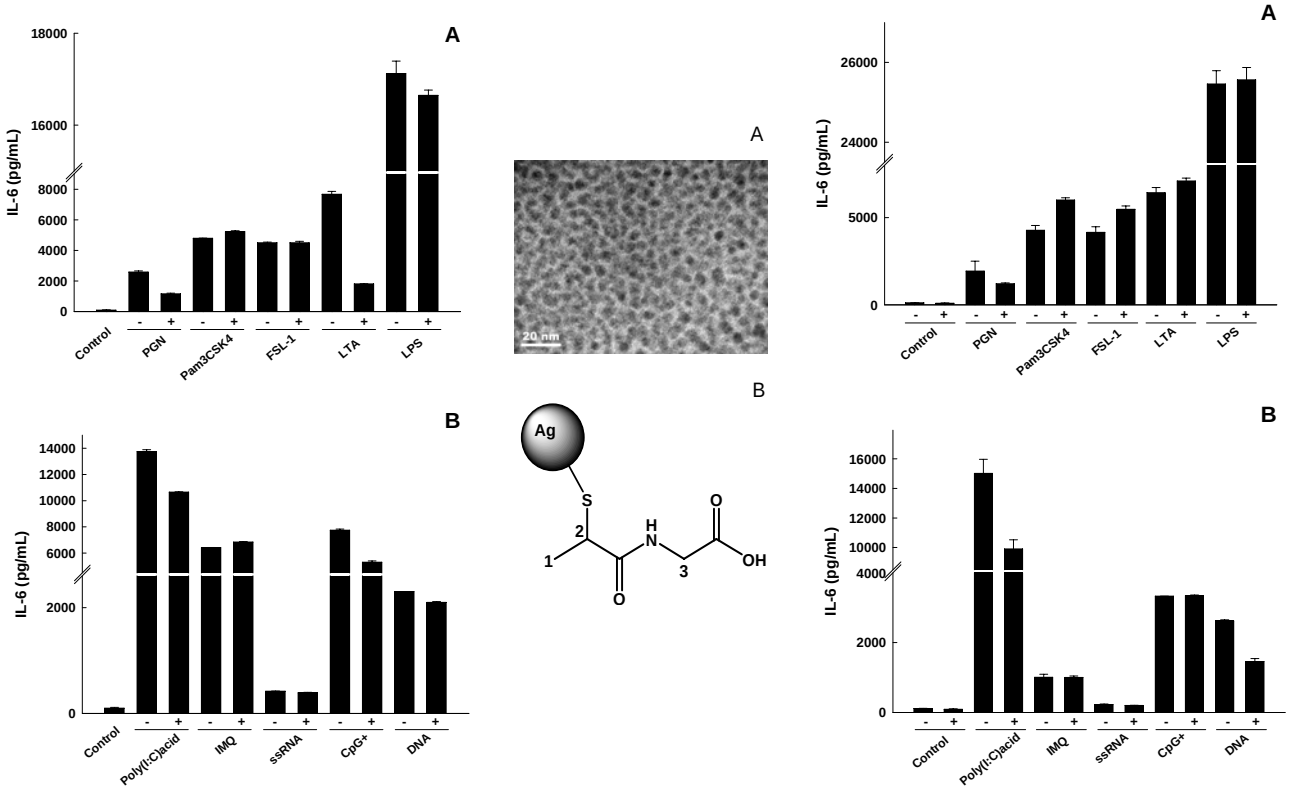
The immune system is one of the most dynamic body components in determining our state of health or disease {Pozo, 2008 #2908}. Capped silver nanoparticles that can be coupled to a variety of molecules and biomolecules are of great interest due to their potential applications in biomedicine. However, there are no data about their toxicity or functional effects on a key innate immune response –such as IL-6 secretion– after the engagement of the main group of pathogen-associated molecular patterns receptors, i.e., the Toll-like receptors. Tiopronin capped silver (Ag@tiopronin) nanoparticles of a narrow sized distribution (≈5 nm) were synthesised and characterised by TEM, FTIR, Raman, ^{1}H-NMR and TOCSY. Cytotoxicity was determined by LDH and MTT assays in Raw 264.7 macrophages. IL-6 was measured by ELISA. Ag@tiopronin nanoparticles have a narrow size distribution (≈5 nm), high solubility and stability in aqueous environment with no cytotoxicity in terms of mitochondrial function or plasma-membrane integrity at concentrations as higher as 200 μg/10^{6} cells. Ag@tiopronin nanoparticles were not pro-inflammatory agents, but remarkably they specifically impaired the IL-6 secretion mediated by TLR2, TLR2/6, TLR3, or TLR9 stimulation in co-treatment experiments. However, in pre-treatment experiments, nanoparticles enhanced the susceptibility of macrophages to inflammatory stimulation mediated by TLR2/1 and TLR2/6 specific ligands while severely impaired the IL-6 secretion activated by the TLR3 or TLR9 ligands. Therefore, contrary to what is found for bare silver nanoparticles, Ag@tiopronin nanoparticles are non-cytotoxic to macrophages. Ag@tiopronin nanoparticles showed direct and indirect effects on TLR signalling of a high degree of specificity, without pro-inflammatory effects by themselves {Castillo, 2008 #3055}. These effects have to be born in mind when using bioconjugates of Ag@tiopronin nanoparticles for future medical applications.

References:

1 Pozo, D. (2008) Immune-based disorders: the challenges for translational immunology. J Cell Mol Med 12, 1085-1086

2 Castillo, P. M., Herrera, J. L., Fernández-Montesinos, R., Caro, C., Zadarenko, A. P., Mejias, J. A. and Pozo, D. (2008) Tiopronin monolayer-protected silver nanoparticles modulates interleukin-6 secretion mediated by Toll-like receptor ligands. Nanomedicine 3, 627-635

Figure 1 (left & right panels)



Differential regulation of TLR-mediated IL-6 production in Raw 264.7 macrophages in the presence of tiopronin silver nanoparticles. A. *Left.* TLRs located on the cell surface. B. *Left.* TLRs located in the endocytic compartment. Tiopronin silver nanoparticles exposure modulates the TLR-mediated IL-6 responsiveness in Raw 264.7 macrophages. A. *Right.* TLRs located on the cell surface. B. *Right.* TLRs located in the endocytic compartment. A. *Center.* EM image of Ag@tiopronin particles. The image was obtained with a high resolution CM200 Philips-FEI microscope. The sample was prepared by drying a drop of an aqueous solution of nanoparticles (1 mg/mL) on a copper grid. B. *center.* Scheme of a tiopronin molecule adsorbed on an Ag nanoparticle (not to scale) with the numbers of the tiopronin atoms used for the NMR interpretation. Patent Nr. P2008-2831.