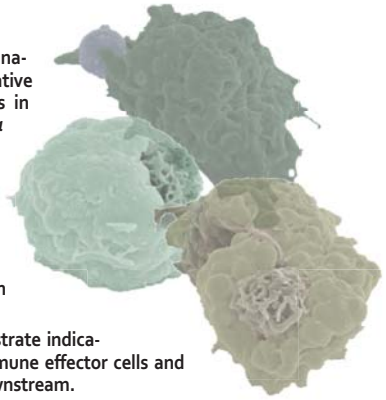
Of Worms and Men

Inflammatory and Acute phase responses to NANOSILVER

Yuya Hayashi[1,2] (yuya@inano.au.dk)
Teodora Miclaus[1]
Péter Engelmann[4]
Herman Autrup[3]
Janeck Scott-Fordsmand[2]
Duncan S Sutherland[1]

[1] iNANO Interdisciplinary Nanoscience Center
[2] Department of Bioscience - Terrestrial Ecology
[3] Department of Public Health
Aarhus University, DENMARK
[4] Department of Immunology and Biotechnology
University of Pécs, HUNGARY

Aims

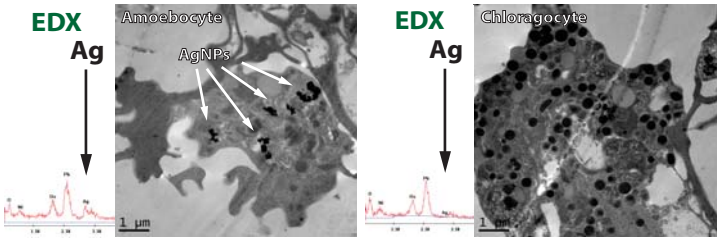


Our previous study suggested that silver nanoparticles (AgNPs) can induce oxidative stress and subsequent immune responses in coelomocytes of the earthworm *Eisenia fetida* and in the human acute monocytic cell line THP-1 (Hayashi et al. 2012, also see "Putative mode of action" on the right.

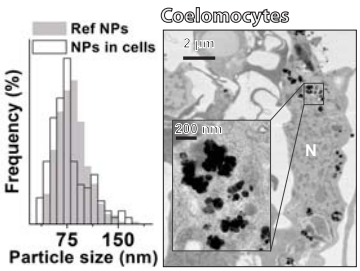
The present study seeks to extend the current understanding of innate immunity towards AgNPs, with a particular focus on cell-to-cell communication.

We here report on our early results to illustrate indications of the signalling events between immune effector cells and acute-phase responders (liver-like cells) downstream.

A mixed cell population model



Earthworm coelomocytes are characterised by two distinct cell populations. Amoebocytes are immune effectors, some of which belong to a class of active phagocytes. Chloragocytes are originated from sessile chloragogenous tissues around the gut being a functional homologue to vertebrate liver systems.



Amoebocytes proved capable of AgNP uptake with no apparent size restriction (size found in the cells = 79 ± 35 nm, original particle size = 83 ± 22 nm). This is in contrast to chloragocytes that seemed negative for AgNP uptake (Hayashi et al. 2012).

We therefore hypothesised that AgNPs can have an indirect impact on chloragocytes through cell communications signalled from amoebocytes, the scavenger of AgNPs.

Concluding remarks

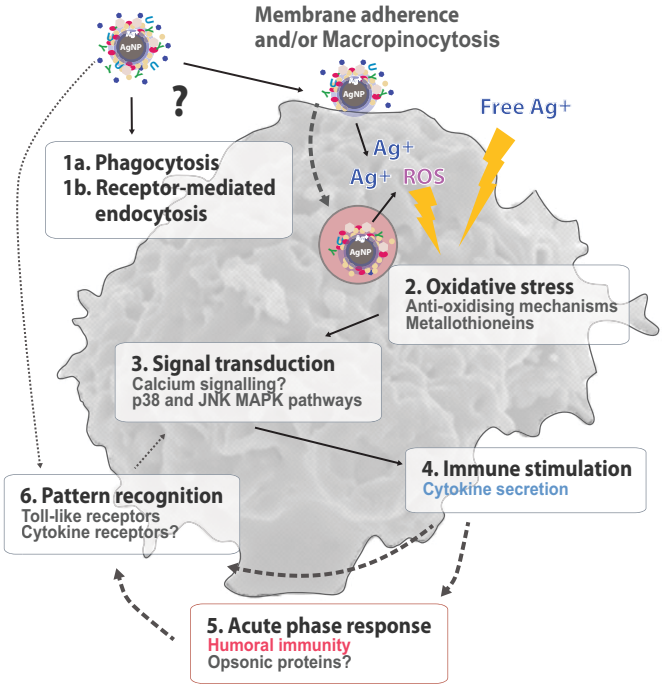
Our preliminary results support selective uptake of AgNPs by the phagocytic population of earthworm coelomocytes (amoebocytes). This coincided with morphological changes (degranulation) in the liver-like cell population (chloragocytes) at non-cytotoxic concentrations of AgNPs.

Flow-cytometric protein expression analysis on the two populations revealed a positive correlation between the differential regulation of cytokines (TNF-like proteins) in amoebocytes and of humoral immune-active molecules (lysenin) in chloragocytes.

Further comparisons to human monocultures await elucidation to determine whether a similar scenario exists for mononuclear phagocyte systems and the liver in humans.

Reference Hayashi et al. 2012. Earthworms and Humans in Vitro: Characterizing Evolutionarily Conserved Stress and Immune Responses to Silver Nanoparticles. *Environ. Sci. Technol.* 46. 4166–4173.

Putative mode of action



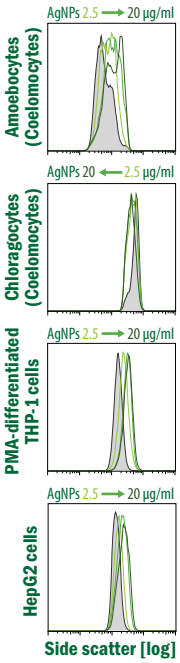
Putative mode of action of AgNPs in immune-competent cells. The schematic is constructed after Hayashi et al. (2012). Molecular biomarkers were probed in a time-resolved manner (1, 3 and 6 h) and suggest a pattern shift from oxidative stress responses to immune functions following in vitro exposure to AgNPs (NanoAmor, PVP-capped, 83 ± 22 nm).

The focus of the current study is on inflammation and acute phase reactions. Our approach is to examine signal transduction and cytokine production in phagocytic cells that trigger acute phase responses in liver(-like) cells at non-cytotoxic concentrations of OECD AgNPs (NanoComposix, citrate-capped, 75 ± 4 nm).

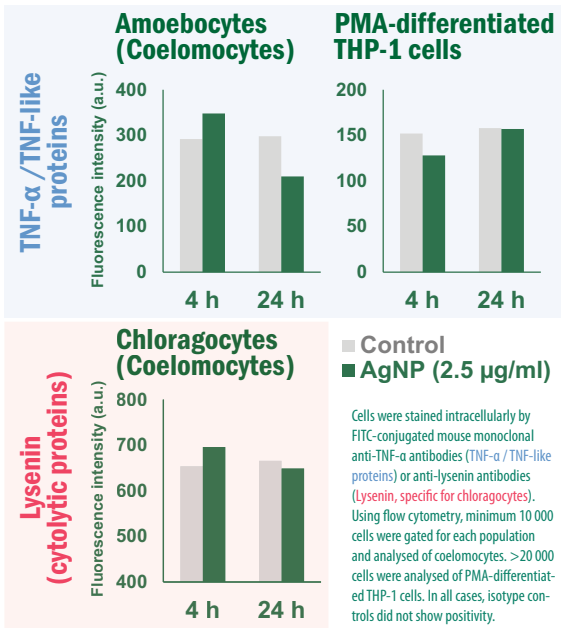
Preliminary results

Uptake

(Side scatter analysis)



Protein expression



Cells were stained intracellularly by FITC-conjugated mouse monoclonal anti-TNF-α antibodies (TNF-α / TNF-like proteins) or anti-lysenin antibodies (Lysenin, specific for chloragocytes). Using flow cytometry, minimum 10 000 cells were gated for each population and analysed of coelomocytes. >20 000 cells were analysed of PMA-differentiated THP-1 cells. In all cases, isotype controls did not show positivity.

Preferential accumulation of AgNPs in phagocytic cells. As previously suggested, phagocytic cells (amoebocytes and differentiated THP-1 cells) preferentially accumulated AgNPs following 24 h exposure. HepG2 cells (human hepatoma cell line) accumulated AgNPs to a lesser extent, while chloragocytes did not, but degranulated (a sign of stress responses).

Communication between amoebocytes and chloragocytes? Amoebocytes scavenge AgNPs, but chloragocytes seem to respond indirectly to AgNPs. In support of this hypothesis, we observed a positive correlation between TNF-like proteins and lysenin expression, possibly a sign of cell-to-cell interactions. Further studies will include comparisons to human monocultures (PMA-differentiated THP-1 cells and HepG2 cells).