

# Toxic effects of nanoparticles on cells are modulated by their exposure scenarios

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# Exposure of murine primary macrophages to silver nanoparticles: fate of NPs inside the cells and functional consequences




**Acute exposure** (one dose, consequences 24h latter),

This protocol reproduces what would happen in the event of an *accidental* exposure to a high dose of NPs

**But**

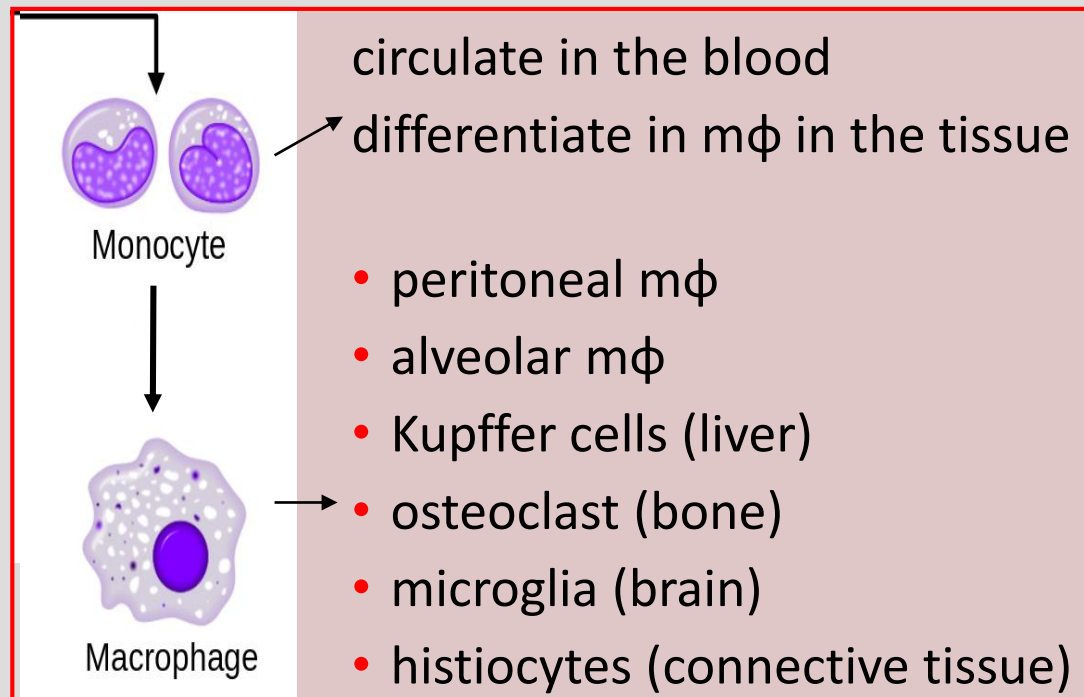
- ❖ Are the observed effects reversible after few days?
- ❖ What are the consequences of repeated exposures to lower doses?

## Exposure protocols

- ❖ Acute exposure : 1 dose for 24 h (5 $\mu$ g/ml)  
analysis at 24 h  
 *accidental exposure scenario*
  
- ❖ Repeated exposure : same dose but delivered as  $\frac{1}{4}$  dose every day for 4 days  
(1.25  $\mu$ g/ml per day; 4 days)  
analysis at the end of day 4  
 *chronic exposure scenario*
  
- ❖ Reversibility : 1 dose for 24 h (5  $\mu$ g/ml)  
cells rinsed and cultured for 72h w/o NPs  
analysis at the end of day 4  
 *recovery scenario*

## Bone marrow-derived macrophages

- ❖ bone marrow (femurs and tibiae) from 6- to 8-week-old C57BL/6 mice
- ❖ differentiation for 10 days in a M-CSF containing medium
- ❖  $30\text{-}50 \times 10^6$  macrophages per mouse at D10



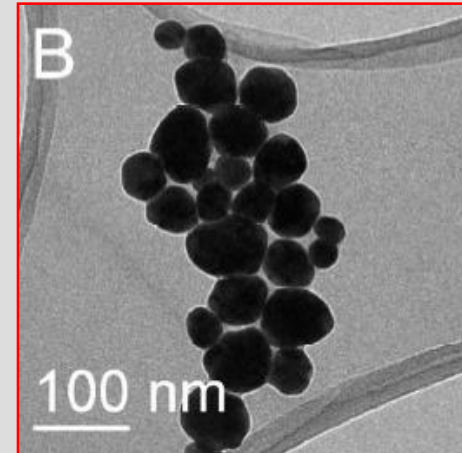
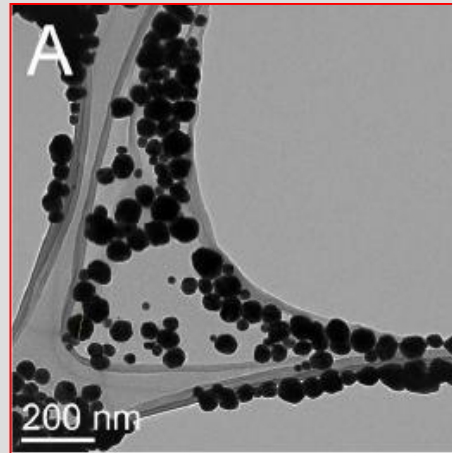
- ❖ phagocytosis and destruction of pathogens and abnormal cells
- ❖ antigen presentation
- ❖ inflammation signaling (cytokine signaling)

**Sentinel cells, first line of defense upon uptake of NPs**

## Silver nanoparticles

- ❖ Commercial Silver Nanoparticles (Sigma):  
< 100 nm , spherical, PVP40-coated NPs
- ❖ TEM:  $59 \pm 18$  nm (water)

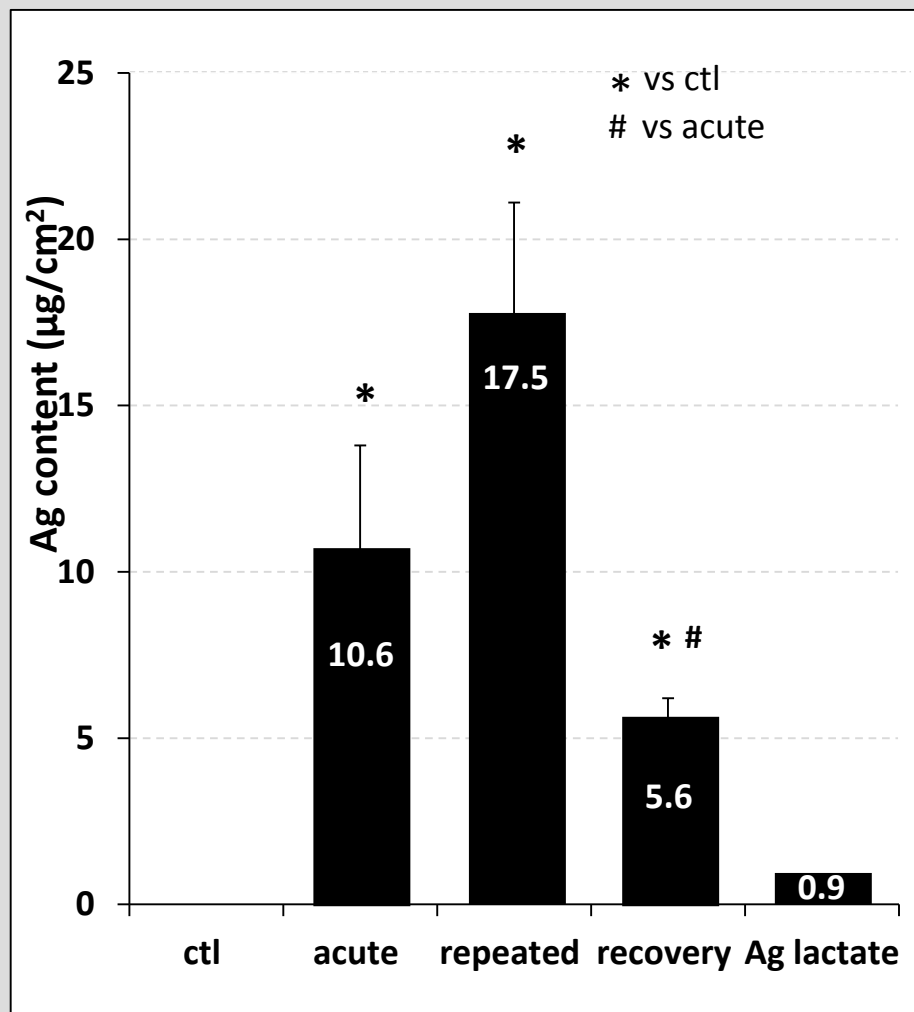
*Transmission Electron Microscopy*



- ❖ DLS:  $95.7 \pm 4,06$  nm (water/culture medium)
- ❖ no aggregation in medium for at least 4 days
- ❖ Zeta potential : -22.1 mV.

Collaboration: Nathalie Herlin  
CEA-Saclay (DRF/IRAMIS/NIMBE/LEDNA)

## Intracellular Ag content



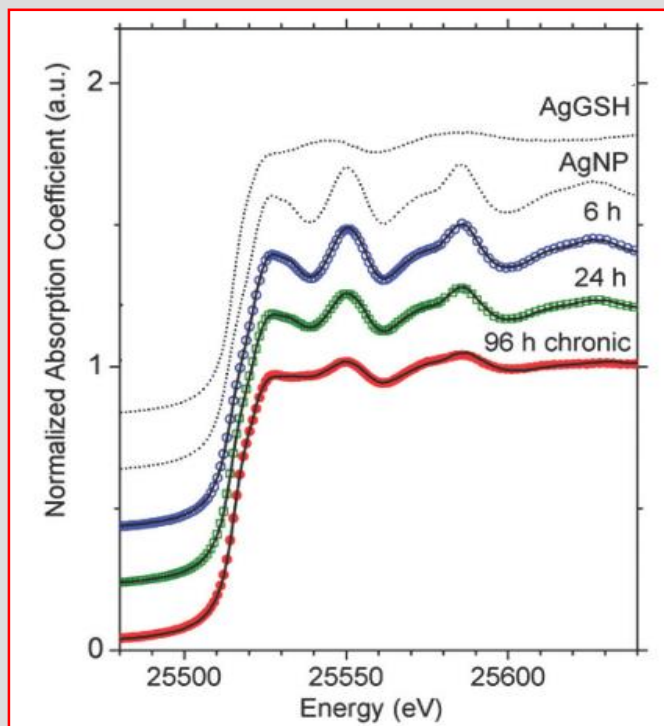
*particle-induced X-ray emission (PIXE)*

- ❖ intracellular Ag accumulation in cells exposed to NPs
- ❖ Ag content significantly lower when macrophages are allowed to recover for 72h in a NP-free medium



**cells are able to eliminate Ag**

## Probing the fate of Ag-NPs *in cellulo*



*X ray absorption spectroscopy  
(Ag K-edge XANES spectra)  
ESRF Grenoble*

Exposure to Ag-NPs	Ag-NPs fraction (%)	Ag-GSH fraction (%)
6 h acute (1 x 5 $\mu\text{g}/\text{ml}$ )	90,6 $\pm$ 8	9,4 $\pm$ 8
24 h acute (1 x 5 $\mu\text{g}/\text{ml}$ )	61,1 $\pm$ 4	38,9 $\pm$ 4
96h repeated (4 x 1,25 $\mu\text{g}/\text{ml}$ )	27,3 $\pm$ 8	72,7 $\pm$ 8

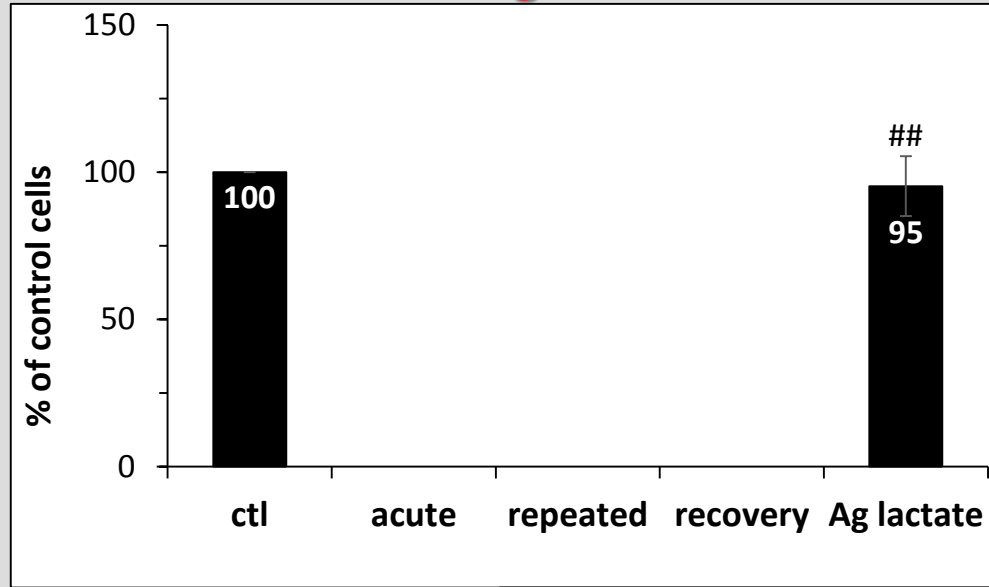
**Ag<sup>+</sup> is progressively released from Ag-NPs  
and is recombined with GSH**

Collaboration: Giulia Veronesi ESRF / CEA-Grenoble (DRF/BIG/LCBM)  
G. Veronesi *et al.*, Nanoscale 2015

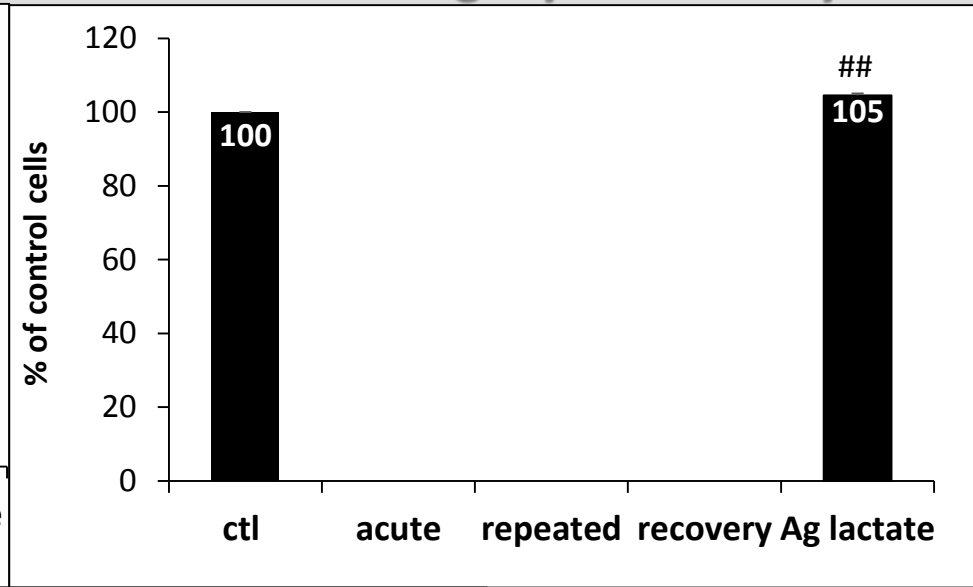
**What are the biological consequences?**



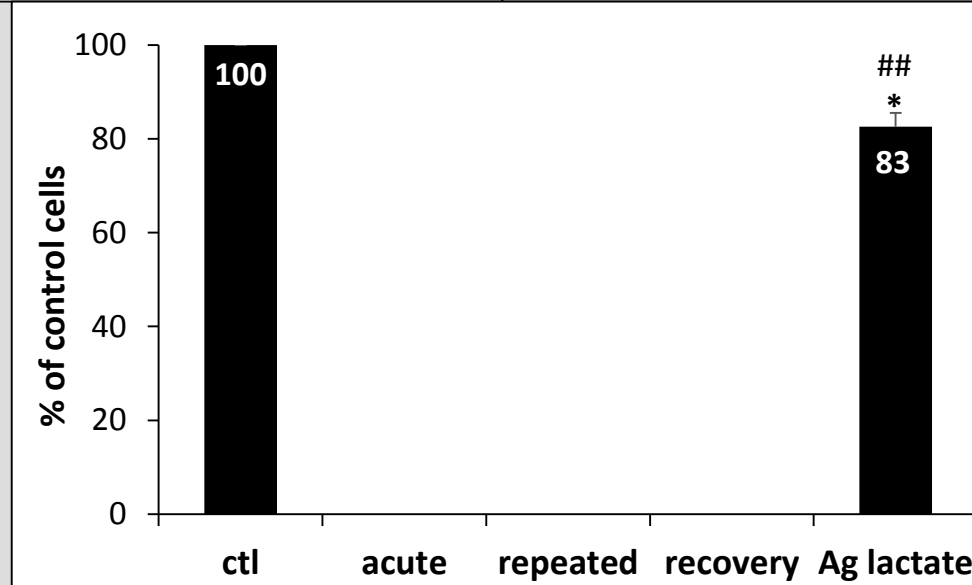
**Intracellular glutathione level**



**Phagocytosis ability**

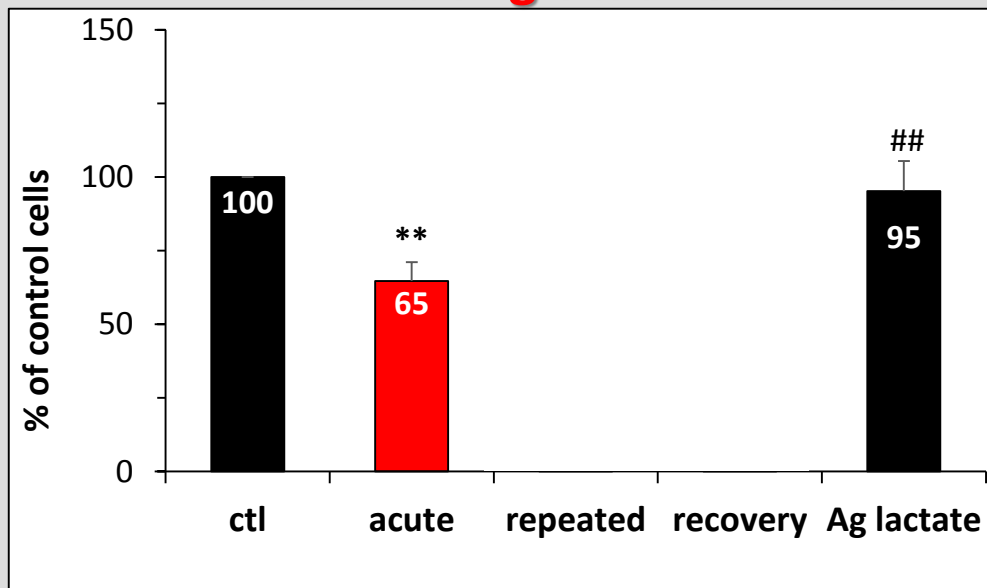


**mitochondrial transmembrane potential**

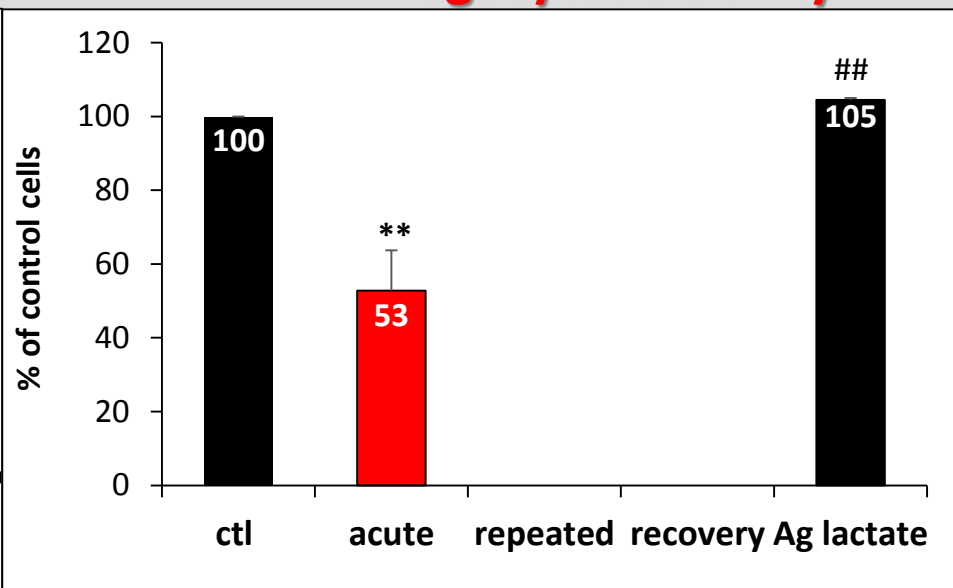


\*: vs ctl  
#: vs acute

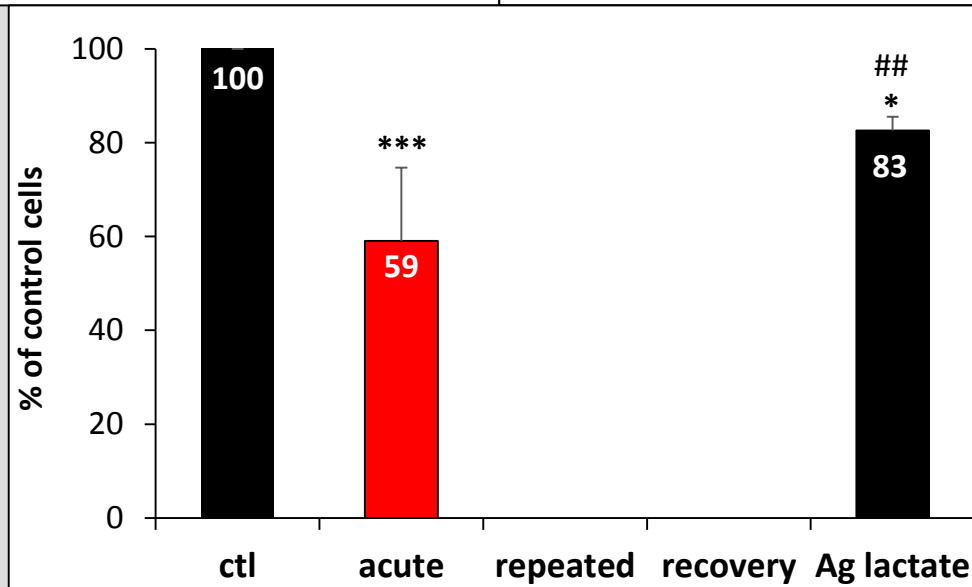
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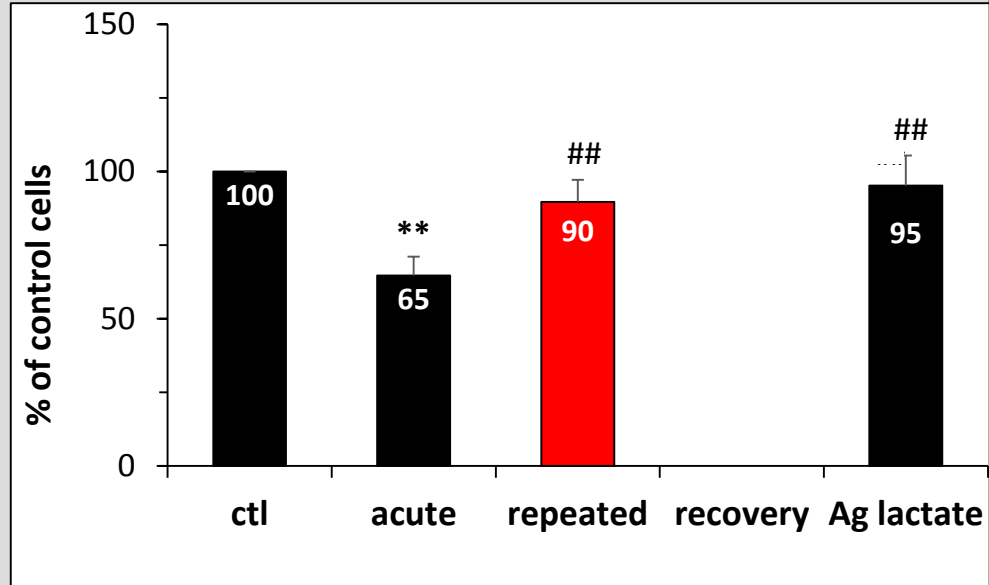


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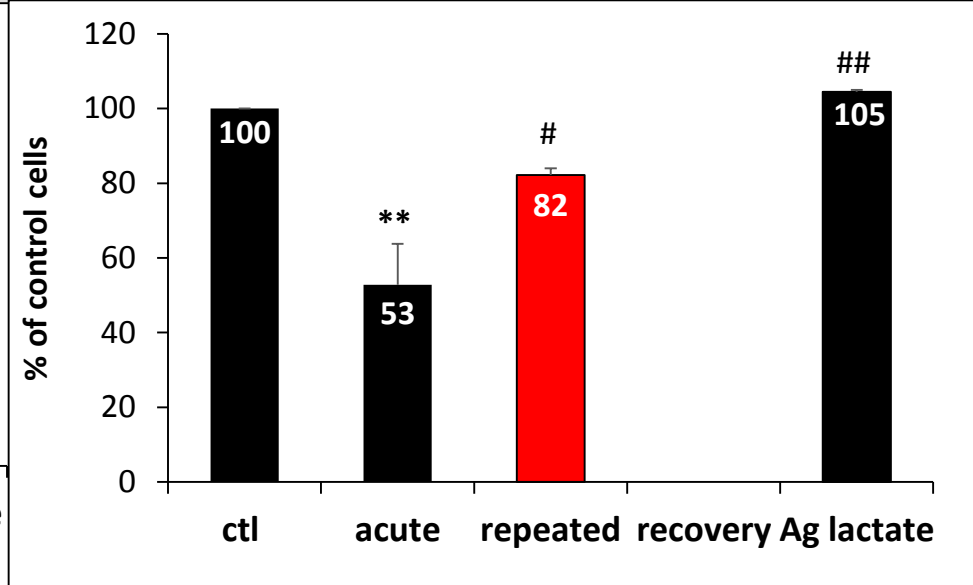


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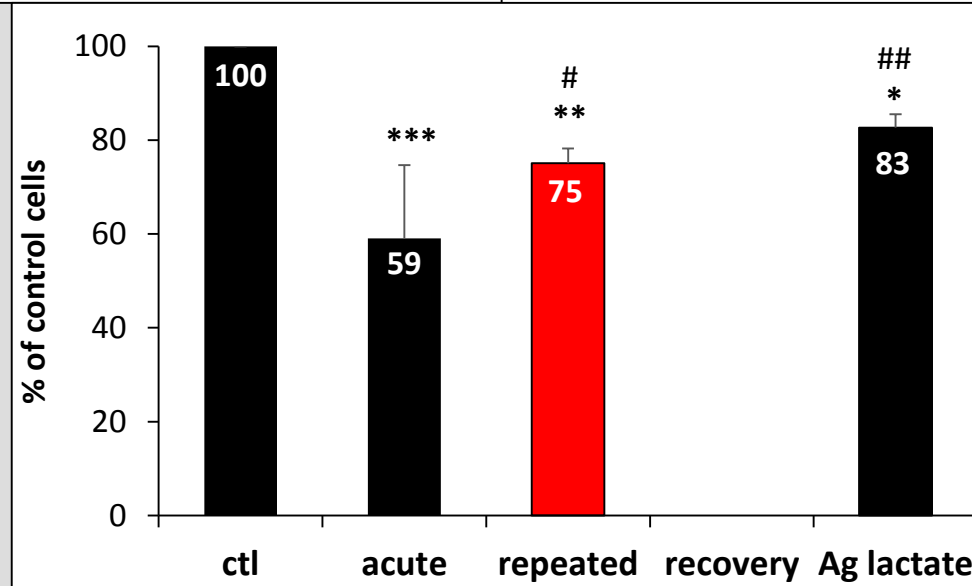
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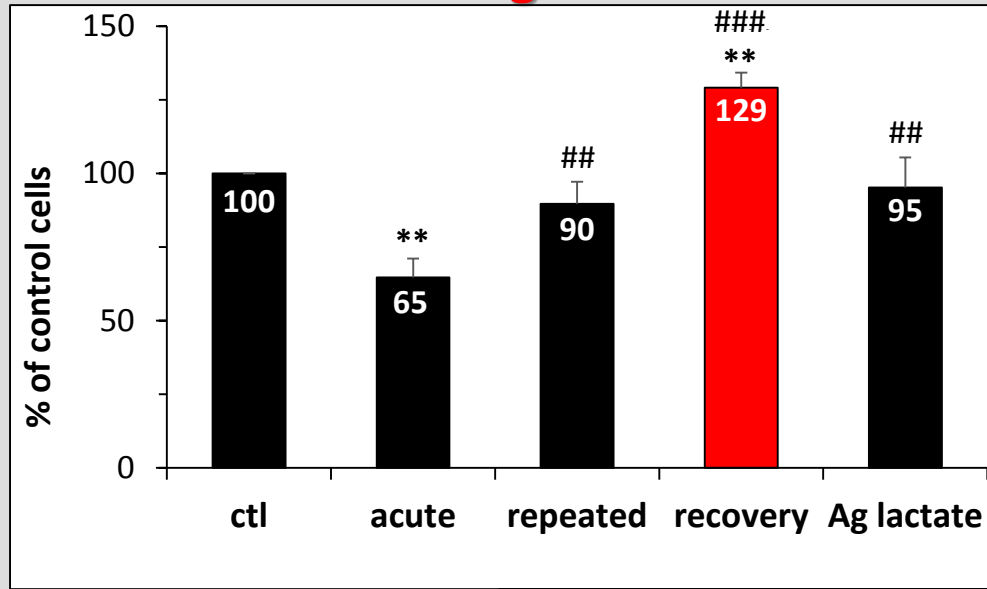


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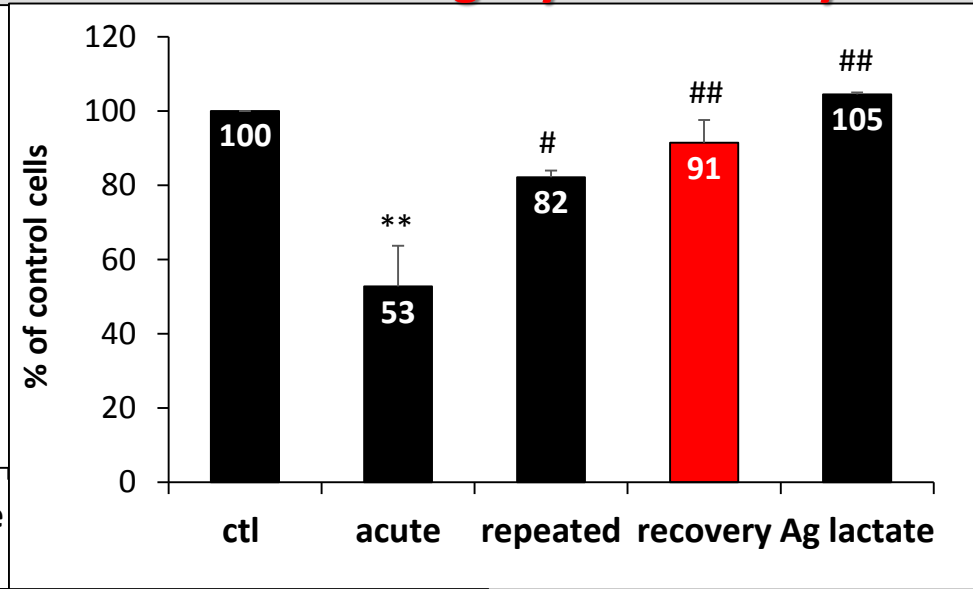


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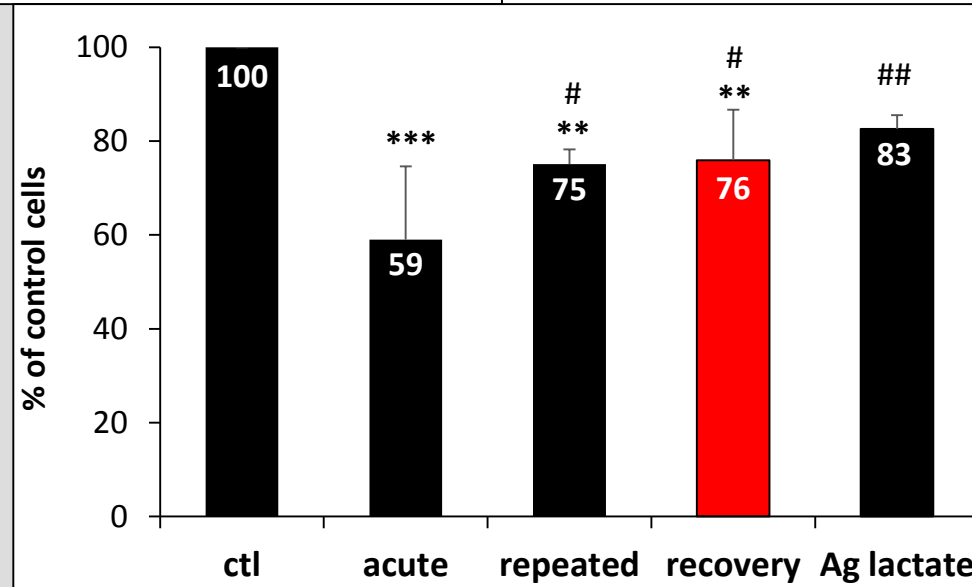
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**Phagocytosis ability**



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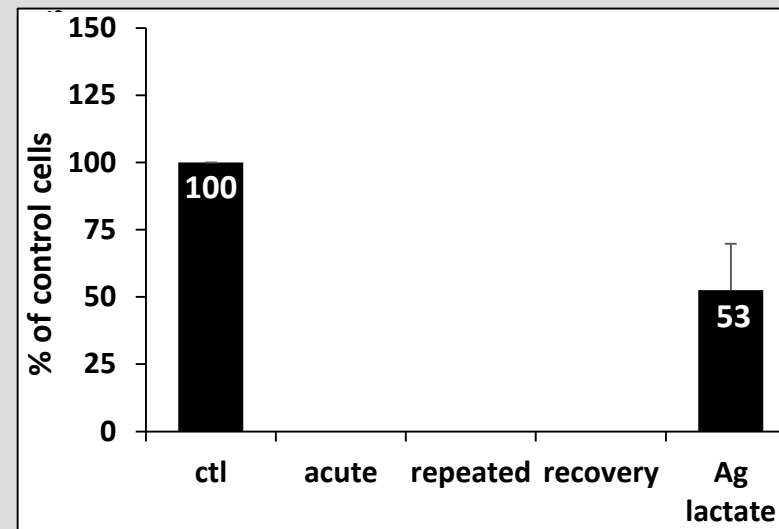


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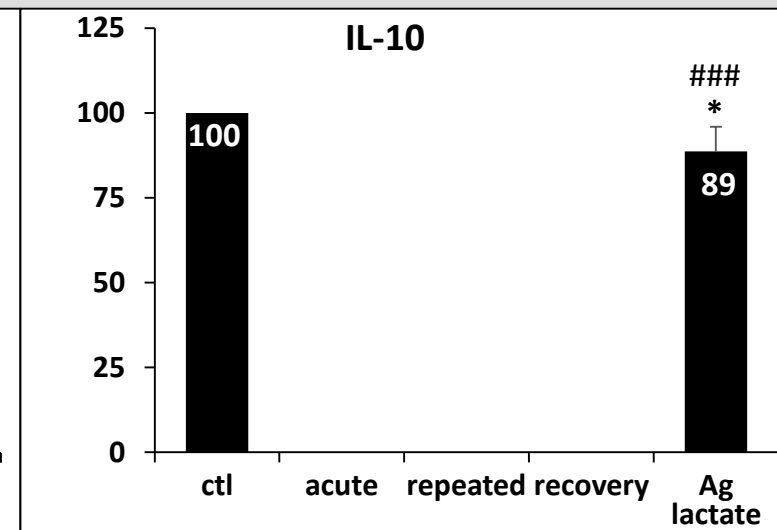
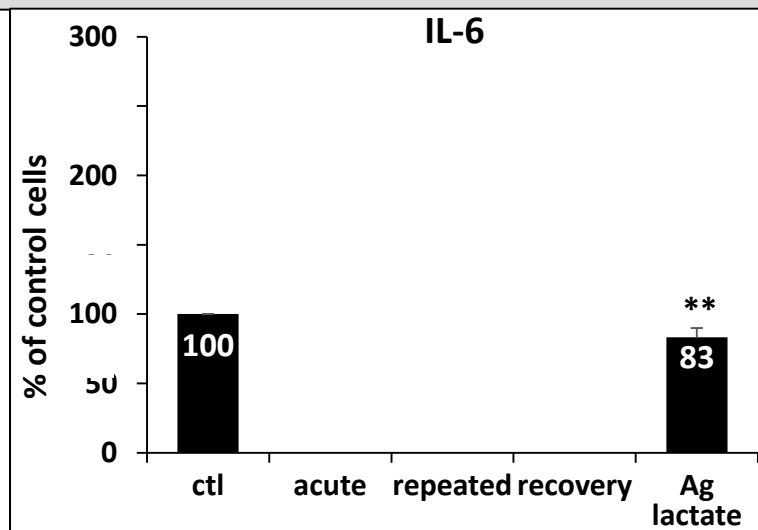
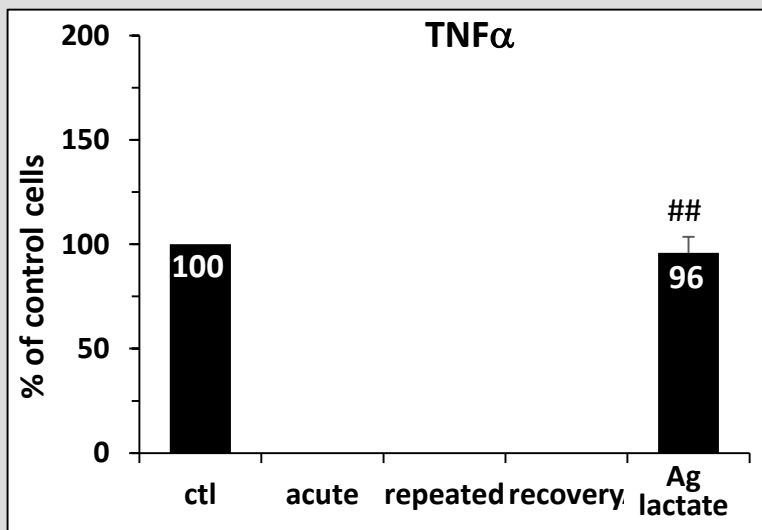
## Capacity to face up to a bacterial attack

- 1- exposure to Ag-NPs (≠ scenario)
- 2- O/N activation by LPS (0,1 µg/ml)
- 3- measure of the quantity of secreted NO and cytokines

\*: vs ctl  
#: vs acute



NO production



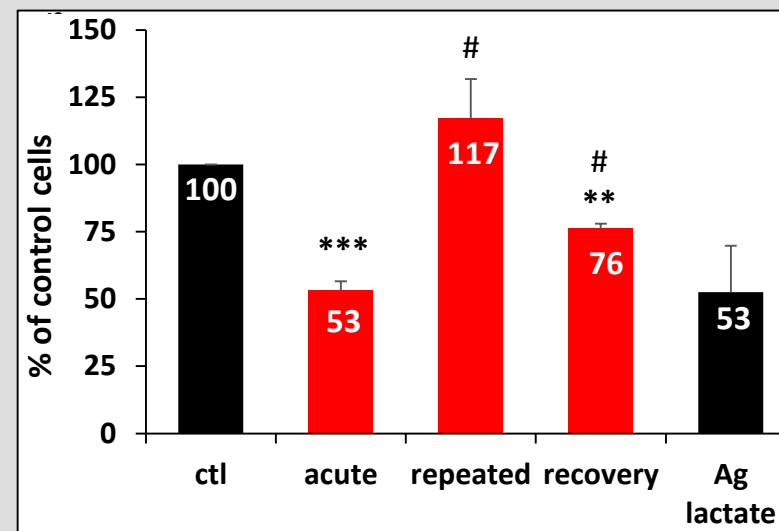
Pro-inflammatory cytokines

Anti-inflammatory cytokines

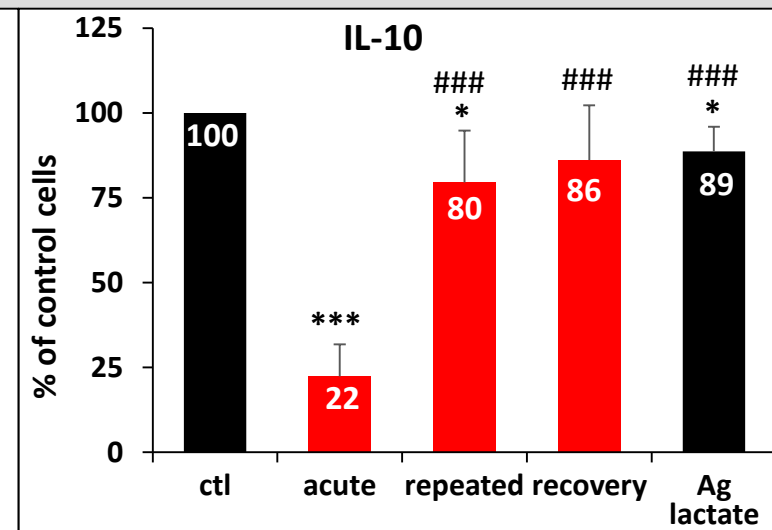
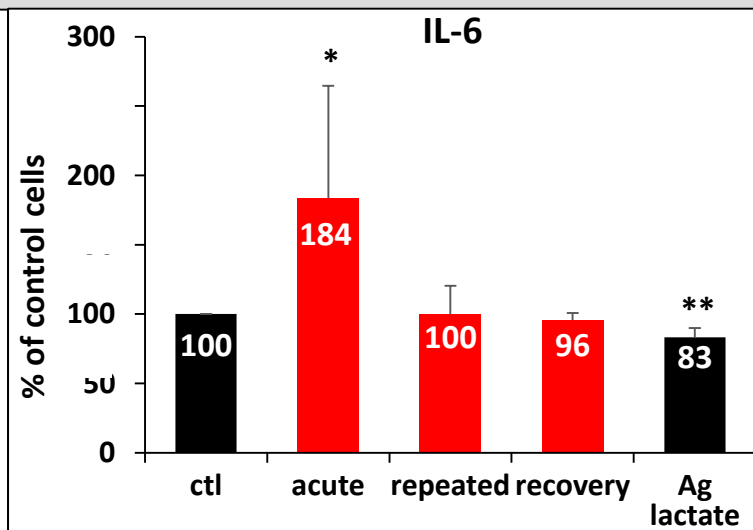
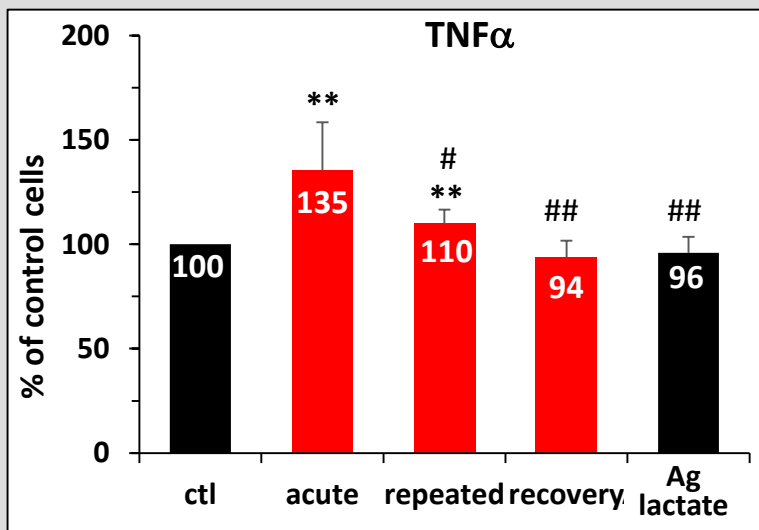
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NO production



Pro-inflammatory cytokines

Anti-inflammatory cytokines

❖ **An acute exposure to Ag-NPs deeply affects macrophage functions**

**BUT**

- ❖ when cells are allowed to **recover in a NP-free medium**, they do eliminate Ag rapidly and by doing this, they recover their functions
- ❖ If the same dose of NPs is **delivered in a four-split dose**, similar amounts of Ag are internalized , and a higher proportion of Ag is recombined with thiol-containing ligands. All the macrophage functions tested are only poorly affected
- Acute response: massive release and re-precipitation of  $\text{Ag}^+$  with thiol-containing ligands (GSH), compromising the cellular functions.
- Long term response to repeated low concentrations: the initial dose is low enough not to disturb the cellular functions too severely. The cells are still able to react by increasing the production of thiols scavengers (MTs, GSH)

## Thanks to

- ❖ All the members of the ProMD team
  - ❖ Giulia Veronesi (LCBM/BIG/CEA-Grenoble) for the *X ray absorption spectroscopy* studies
  - ❖ Marie Carrière (LAN/INAC/CEA-Grenoble) for the *PIXE* studies
  - ❖ Nathalie Herlin-Boime (CEA Saclay) for the *characterization of the Ag-NPS by TEM*
- 
- Labex Serenade
  - ANSES
  - CEA Toxicology Program