ADDRESSING THE IMMUNE SYSTEM: MACROPHAGES RESPONSES TOWARDS AU NANOPARTICLE CONJUGATES.

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There has been a rapid proliferation of technologies based on nanoparticle (NP) conjugates for diagnostic and therapeutic uses in biomedicine, and some of them are at different stages of preclinical development. Such advances require an in-depth understanding of NP evolution in biological media as well as of their specific interaction with cells and living systems. This is particularly true for the immune system, which is responsible for maintaining body integrity and preventing external invasion.

The first line of defence, typically triggering a protective inflammatory response within minutes, is carried out by the innate immune system. The key feature of innate immune cells enabling them to detect and categorize infection is their repertoire of pattern-recognition receptors (PRRs), which bind certain general types of molecules expressed by broad classes of pathogens. In fact, what seems to distinguish a pathogen as non-self by the innate immune system cells is the structural conformation and spatial distribution of its molecules and not the recognition of specific protein sequences.

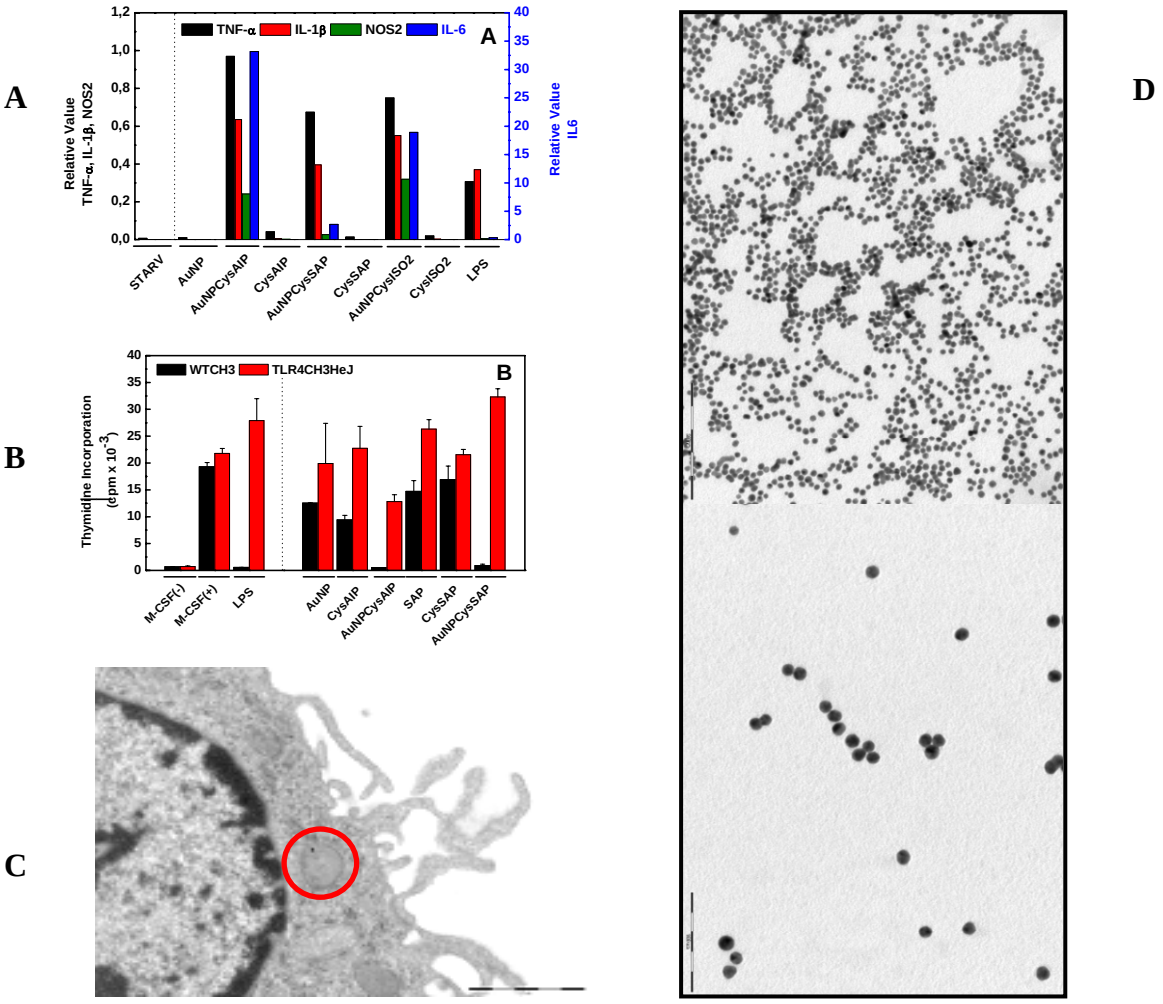
Based on these principles, we used Au NP conjugates as scaffolds for biomolecules to test the response of macrophage cells. These cells play key roles in the innate (phagocytosis) and adaptive immune system (stimulation of lymphocytes). In order to analyze the activation of bone marrow macrophages, production of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) as well as the induction of nitric oxide synthase were measured by Real-Time PCR after exposure to Au NP conjugates and controls. In addition, activation of macrophages was also measured analyzing the inhibition of proliferation. Both experiments indicate that Au NP-peptides conjugates induce macrophage activation while neither the peptide nor the citrate-coated nanoparticles do activate macrophages, even at concentrations thousand times higher, showing the biological relevance of conjugation.

The observed macrophage activation occurs concomitantly with that of Toll-like Receptor 4 (TLR 4) which is described to be involved in the recognition of Lipopolysaccharide (LPS) or Heat Shock Protin 60 (HSP60) from bacteria. The activation of macrophages via TLR 4 was confirmed by the absence of activation when macrophages came from a knock-out strain unable to express TLR4. If TLR4 is required for nanoparticle-mediated activation, it is critical to exclude LPS contamination. We discarded the possible presence of LPS by using a Limulus Lysate

assay and polymyxin B. Thus, while LPS-inhibited proliferation is reversed in the presence of PMB, we detected no similar changes in the proliferation of AuNP-peptide conjugates, thus indicating that their effect was not due to LPS contamination.

Moreover, it has been suggested that TLR4 activation results in the onset of phagocytosis and Au NP-peptide conjugated internalization. The presence of Au NP in vesicles within short time frames indicates that they are internalized actively via specific receptors, rather than a passive mechanism such as pinocytosis. No NP were observed when they have not been conjugated to peptides

All in all helps determine a small number of principles that should be taken into account when working with nanomedical devices in vivo.



A: mRNA levels (as measured by real-time PCR) of TNF-α, IL-1β, IL-6 and NOS2 in relation to β-actin of macrophages stimulated for 6 hours with LPS.
B: Macrophages from WT C3H and mutant TLR4 C3H/HeJ mice were stimulated with different substances, and proliferation was determined
C: TEM image of macrophage revealing internalization at 6 hours
D: TEM images of citrate-coated gold nanoparticles. The bar indicates 200 nm (up) and 100nm (down).