**Apply for Oral Presentation**

**Nanosized manganese acts as a promising antigen delivery system and immune nanoadjuvant**

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**Background and aims.** Aluminum is one of the most widely used adjuvants in few decades, but it preferably promoted humoral immunity without Th1-mediated cellular immunity [1]. There are emerging attempts in exploring other potential metals, which can remedy the weaknesses of aluminum. Manganese as an essential trace metal has been considered to enhance humoral and cellular immunity [2]. However, the detailed adjuvant mechanism and functions of manganese are still unknown. Alternatively, nanocarriers can deliver antigens and act as adjuvants to enhance immune responses [3]. Hence, this study aims to investigate the immunoenhancing effect and molecular mechanism of nanoformulated manganese adjuvants.

**Methods.** This study constructed manganese nanoparticles with different dosages, L-Mn NPs, M-Mn NPs and H-Mn NPs. Flow cytometry and confocal microscopy were used to assess the ability of manganese nanoparticles to deliver and protect antigens, as well as their enhancing effects on the maturation and antigen cross-presentation of dendritic cells. Biotechniques including RT-PCR, Western blot, ELISA, and flow cytometry were employed to detect the regulation of cytokines, proteins, and signaling pathways by nanoparticles to elucidate their immunoenhancing mechanisms. Furthermore, *in vivo* studies were performed to validate the enhancing effects and mechanisms of different manganese nanoparticles on cellular and humoral immune responses.

**Results.** Manganese nanoparticles, but not Mn2+ or aluminium, not only promoted antigen uptake, cross-presentation and BMDC maturation, but also activated DC cells, CD4+/CD8+ T cell responses and antibody productions, uncovering that manganese nanoparticles amplified the immunoenhancing effect of Mn2+. Moreover, we uncovered that H-Mn NPs alleviated ferroptosis of immune cells to exert remarkable adjuvant effects via crosstalk with STING signaling. In contrast, H-Mn NPs triggered adverse effects in the ferroptosis of tumor, which initiated ferroptosis in cancer cells to prevent tumor progression, indicating the role of Mn NPs in ferroptosis depended on cell types and context. H-Mn NPs with high dosage of Mn notably modulated ferroptosis and STING/NLRP3 signaling as well as antigen-specific immune responses *in vitro* and *in vivo*, which were more potent than L-Mn NP and M-Mn NP, suggesting the adjuvant effects of manganese nanoparticles were highly dose-dependent.

**Conclusion/Discussion.** This study revealed the dose-dependent immunoenhancing effects and mechanisms of nanosized manganese, providing a theoretical and experimental foundation for the design of potential manganese nanoadjuvants.

**References:**

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