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Involvement of Extracellular Vesicles from Staphylococcus aureus in host cells manipulation

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1 Introduction

Extracellular vesicles (EVs) are **nanosized membrane-encapsulated particles** produced by almost **all living cells** and loaded with various molecules (**eg, lipids, nucleic acids, proteins**). They play important roles in **cell-to-cell communication** by transporting and transferring their cargo to recipient cells. EVs may have distinct activities, depending on **the producing cell, their functional charge and their mode of action** in recipient cells. EVs produced by bacterial pathogens contribute to **pathogenicity** as mediators of **host-pathogen interactions**. However, how pathogen-derived EVs act on host cells is still poorly documented.

2 Objectives

- Characterization of EVs produced by the methicillin-resistant *Staphylococcus aureus* strain N315
- Evaluation of their impact on the expression of several inflammatory genes, as well as their routes of entry into human non-phagocytic cells

3 Results

1 N315-derived EVs are nanosized spherical particles which carry proteins

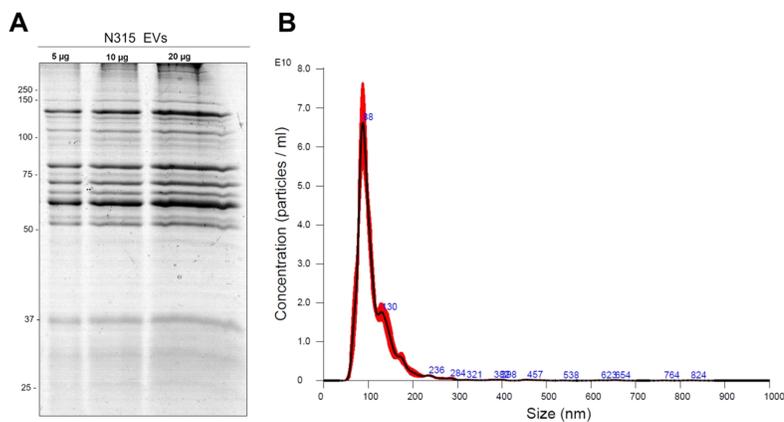


Figure 1 : To mimic infection, N315 strain was grown in RPMI + 10% LB medium and N315-derived EVs were purified by size exclusion chromatography from culture supernatants. **A/** The concentration of proteins present in N315-derived EVs samples was determined by Qubit device (Invitrogen, Carlsbad, CA, USA). 5µg, 10µg and 20µg of proteins thus quantified were migrated on SDS-PAGE gel 12 %. **B/** Analysis of EVs size and concentration by nanoparticle tracking analysis (NTA) showed that N315-derived EVs are particles around 85 to 115nm.

3 N315-derived EVs induce the expression of various TLR and immune genes in MG-63 cells

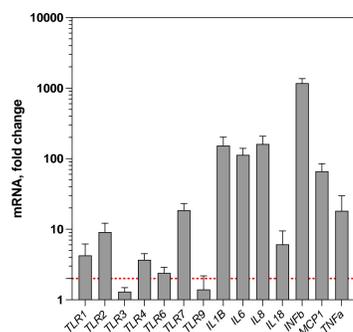


Figure 3 : MG-63 cells were incubated with 20 µg *S. aureus* EVs for 3h for RT-qPCR analyses. The data are presented as relative fold-change compared with control (without EVs incubation).

2 N315-derived EVs are internalized by MG-63 cells

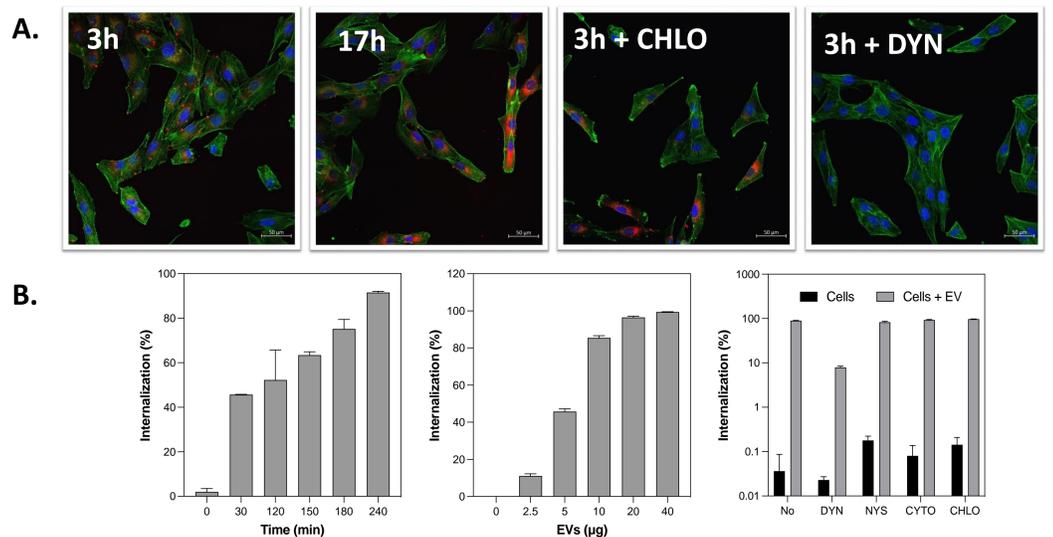


Figure 2 : **A/** Confocal microscopy of MG-63 cells (10^5) pretreated or not (1h before) with inhibitors Dynasore (80 µM) and Chlorpromazine (10 µM) incubated with 20 µg *S. aureus* DiI-labelled EVs (red) for 3h and 17h. These different inhibitors are used to block some possible internalization pathways. Cell nuclei were stained using Hoescht (blue) and the actin filaments from MG-63 cells were stained with Phalloidin (green). **B/** Flow cytometry indicates the EVs internalization percentage in MG-63 cells increasing by time and by concentration. Internalization of DiI-labelled EVs decreases for MG-63 cells pretreated with dynasore compared with control cells. DYN – Dynasore; NYS – Nystatin; CYTO – Cytochalasin; CHLO – Chlorpromazine.

4 The induced expression mediated by EVs of TLR7, INFβ and IL-6 genes is dependent on the internalization of EVs

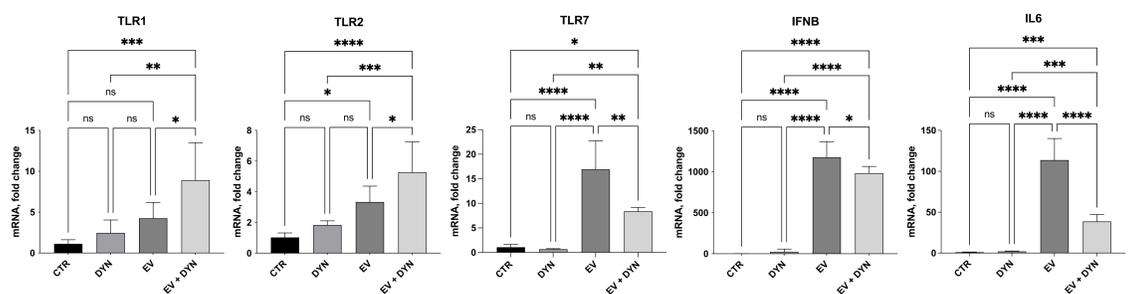
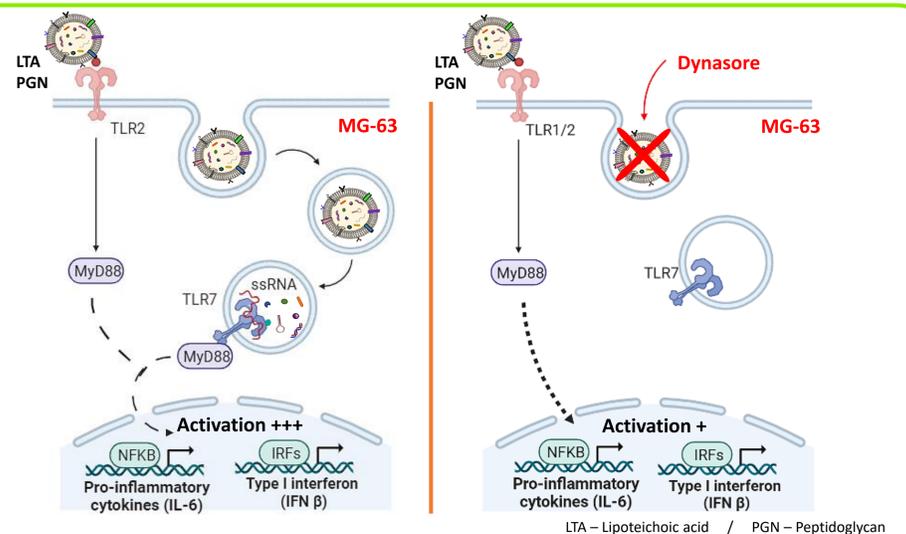


Figure 4 : MG-63 cells were incubated with 20 µg *S. aureus* EVs for 3h for RT-qPCR analyses. The data are presented as relative fold-change compared with control (without EVs incubation). Among the genes tested, the inhibition of the entry of EVs by dynasore alters the induced expression mediated by EVs of only *TLR1*, *TLR2*, *TLR7*, *INFβ* and *IL-6* genes. While the expression of *TLR1* and *TLR2* genes is induced in presence of EVs + DYN when compared to EVs alone, the expression of *TLR7*, *INFβ*, and *IL6* is lower when the entry of EVs is inhibited.

4 Conclusion

- ✓ EVs induce the expression of numerous immune and signaling genes such as *IL-6*, *IL-8*, *MCP-1*, *IL1β*, *TLR2*, *TLR4* and *TLR7*. Indeed, the expression of some drastically decreased when EV uptake was blocked, while others remained unchanged.
- ✓ N315-derived EVs can trigger both extracellular and intracellular signaling pathway in host cells, using different bacterial components.
- ✓ New insights into pathogenesis of *S. aureus* and potentially new avenues of treatment and prevention.



LTA – Lipoteichoic acid / PGN – Peptidoglycan