Does the Host Cell Matter ?
Inter-patient Variability in Intraosteoblastic Survival of *Staphylococcus aureus*

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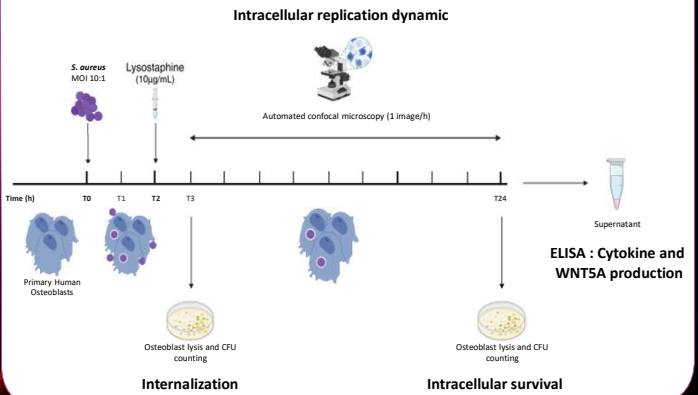
Introduction and Objective

Bone and joint infections are serious conditions often caused by ***Staphylococcus aureus***, leading to **chronicity** and treatment failure. A major reason for this **persistence** is the bacteria's ability to **survive inside osteoblasts**. While **many studies use cell lines**, **primary osteoblasts provide a more realistic model** of human responses and **show variability between donors**.

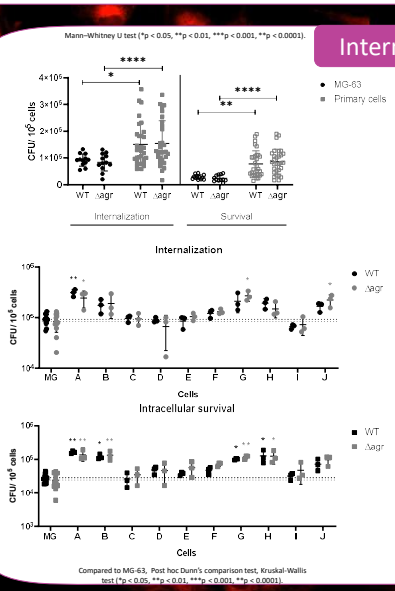
We compared ***S. aureus* internalization**, **intracellular survival**, and **replication** inside **primary osteoblasts from multiple donors** and the MG-63 cell line. We also assessed the production of **inflammatory cytokines** (IL-6, RANTES/CCL5) and bone homeostasis mediator **WNT5A** during infection.

Materials & Methods

Primary human osteoblasts isolated from **bone explants of 10 donors** were infected in vitro with **SH1000** wild-type (**WT**) and SH1000 **Δagr** strains.



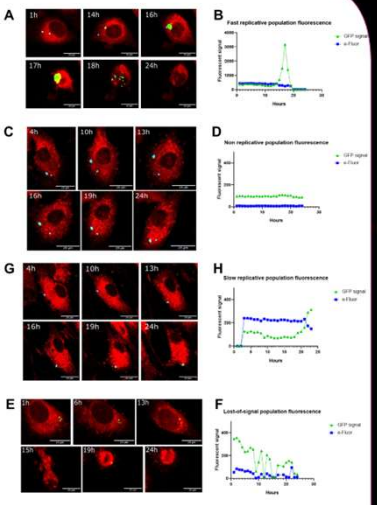
Internalization and intracellular survival



Primary osteoblasts internalize more *S. aureus* and promote its survival better than MG-63 cells

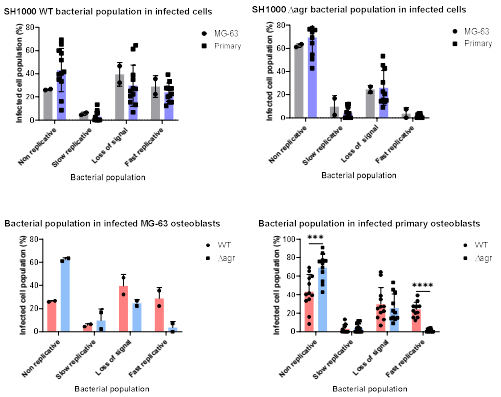
- Primary osteoblasts showed significantly **higher internalization and intracellular survival** for both WT and Δagr strains compared to MG-63. No differences were observed between strains within the same cell type.
- **Inter-donor variability** was observed for both **internalization** and **survival**. Donor **A** consistently showed higher internalization and survival compared to MG-63. At 24h p.i., four donors (**A, B, G, H**) had greater intracellular burden than MG-63 for both strains. Variability was donor-dependent, not strain-dependent.

Intracellular replication dynamic



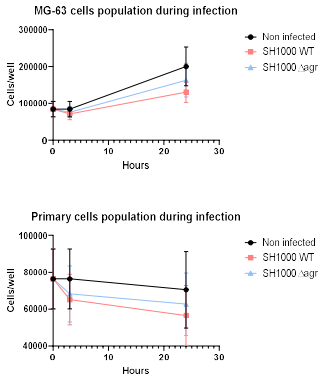
***agr* deletion promotes non-replicative intracellular population**

- **WT infections:** Primary cells harbored more non-replicative bacteria, while MG-63 had more loss-of-signal events.
- ***Δagr* infections:** Non-replicative bacteria dominated in both cell types; replicative forms were rare.
- ***agr* impact:** In both cell types, *Δagr* significantly increased non-replicative and reduced fast-replicative populations.



Mann–Whitney U test (*p < 0.05, **p < 0.01, ***p < 0.001, **p < 0.0001).

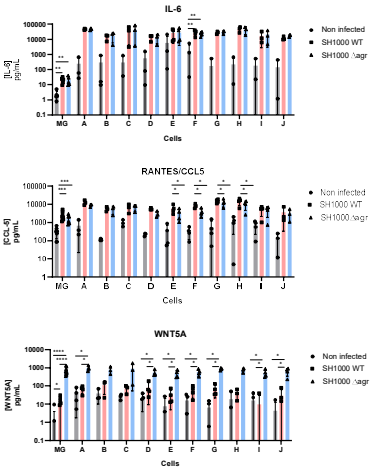
Cell population during infection



Strain-dependent cytotoxicity affects osteoblast proliferation differently in MG-63 and primary cells

- **Proliferation:** MG-63 cells proliferate over 24h regardless of infection, primary osteoblasts do not.
- **Early cytotoxicity (2h):** Both cell types showed reduced cell numbers when infected with WT, Δagr had no significant effect.
- **Late cytotoxicity (24h):** WT infection reduced cell numbers further; Δagr caused a milder but significant reduction in both MG-63 and primary cells.

IL-6, RANTES and WNT5A production



Primary osteoblasts shows a stronger inflammatory response than MG-63 cells :

- **IL-6 & RANTES:** Primary cells produced much higher levels than MG-63.
- **Donor variability:** Significant inter-donor variability in both IL-6 and RANTES production, with some donors (A, G, H) showing much **higher responses** than MG-63.
- **WNT5A:** Both MG-63 and primary cells **upregulated** WNT5A production **upon infection**, with **Δagr** inducing the **highest production**.

Conclusion

This study shows that **primary human osteoblasts** **internalize** and **promote *S. aureus* survival** **better than MG-63 cells**, with **strong inter-donor variability**. Primary cells triggered a **stronger inflammatory** and osteoimmune response compared to MG-63. ***Agr* inactivation favors non-replicative population,** and ***S. aureus* infection induces WNT5A production**.

We highlight the need to use **primary osteoblasts** from multiple donors to capture the **variability of host-pathogen interactions**.

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