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Intracellular Infection of THP-1 Human Macrophages by Staphylococcus aureus and Role of Haemolysins

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ABSTRACT

Background: *S. aureus* is an opportunistic pathogen that produces a wide range of virulence factors among which haemolysins alpha, beta and gamma are known to display a lytic action on cellular membranes. Infections due to *S. aureus* are often persistent and recurrent, which has been ascribed to the ability of *S. aureus* to penetrate, survive and proliferate in various cell types including professional phagocytes. The aim of the present work was to determine whether *S. aureus* haemolysins are involved in the development of intracellular infection.

Methods: The study was performed with the reference strains ATCC 25923 and 8325-4 (parental strain) and mutant strains with specific gene disruption(s): DU1090 (alpha-haemolysin disruptant), DU5719 (beta-haemolysin disruptant), DU5942 (gamma-haemolysin disruptant), and DU5938 (alpha-, beta- and gamma-haemolysins disruptant). Genotypes were checked by PCR. Intracellular growth was measured in THP-1 cells as previously described (Antimicrob. Agents Chemother. 50(3):841-851, 2006). Effective intracellular multiplication was checked by electron microscopy with strains ATCC 25923 and DU5942.

Results: (The table shows the bacterial growth recorded for each strain at 24 h.)

Strain	ATCC 25923	8325-4	DU1090	DU5719	DU5942	DU5938
Intracellular growth [a]	0.4 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	1.9 ± 0.1 *	0.3 ± 0.1

[a] log [CFU/mg at 24h / CFU at 0h] (post-phagocytosis) with n=3; * p < 0.001 (ANOVA)

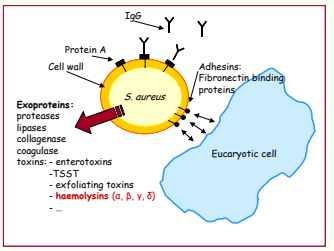
Electron microscopy showed that ATCC 25923 and DU5942 were located in vacuoles and divided actively.

Conclusion: The comparison of the different strains used suggests that specific disruption of the gamma-haemolysin favours the intracellular multiplication of *S. aureus* in professional phagocytes.

INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen that can infect various tissues and induce a large range of pathologies, most of which present a persistent or recurrent character. This recurrent character is probably related to its capacity to penetrate, survive and proliferate in various cell types [2, 3, 4, 5] including professional phagocytes [4]. *S. aureus* also produces a wide range of virulence factors among which, haemolysins alpha, beta and gamma are known to display a lytic action on cellular membranes. The role of these toxins in the intracellular fate of *S. aureus* is however still unknown.

Aim of the project : to study the role of haemolysins in the development of intracellular infection by *S. aureus* in a phagocytic cell type.



S. aureus produces a large range of virulence factors to evade host defence and to invade tissus.

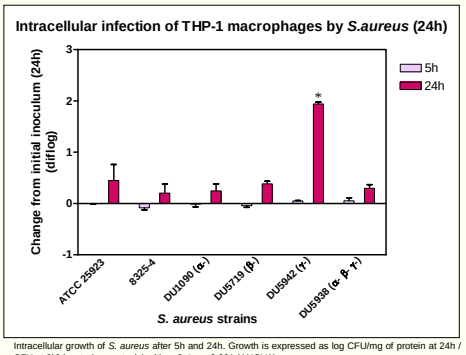
METHODS

Bacterial strains: The study was performed with the reference strain ATCC 25923, strain 8325-4 (parental strain), and 4 mutant strains with specific gene disruption(s): DU1090 (*hla*-), DU5719 (*hlb*-), DU5942 (*hlg*-), and DU5938 (*hla*-, *hlb*- and *hlg*-).

Infection: THP-1 cells were incubated with opsonised *S. aureus* for 1h to allow phagocytosis. Extracellular bacteria were eliminated by incubating cells for 45 min with gentamicin (100 x MIC). Macrophages were then incubated up to 24h with gentamicin (1 x MIC) to prevent extracellular growth. Cells were harvested at 24h post infection by centrifugation and washed with PBS. Bacterial growth was assessed by the number of CFU on TSA per mg of proteins [1].

Electron microscopy: Infected macrophages were washed with PBS, fixed with glutaraldehyde and osmium tetraoxyde, and stained "en bloc" with uranyl acetate.

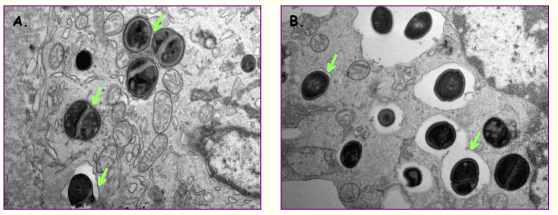
RESULTS



Intracellular growth of *S. aureus* after 5h and 24h. Growth is expressed as log CFU/mg of protein at 24h / CFU at 0h] (post-phagocytosis) with n=3; * p < 0.001 (ANOVA).

After 5h of infection, there was no apparent modification of intracellular inoculum (left). At 24h post infection, the intracellular growth was not significantly different for the ATCC strain, the parental strain 8325-4, the alpha-haemolysin mutant, and the beta-haemolysin mutant. In contrast, the gamma-haemolysin mutant showed a much more important growth (2 log).

Electron microscopy (right): after 24h of infection, *S. aureus* was still enclosed in vacuoles where it is able to divide. These vacuoles seem more spacious around the gamma-haemolysin mutant than around the ATCC 25923 reference strain.



Electron microscopy of infected THP-1 macrophages at 24h post infection. **A.** W.T. strain *S. aureus* ATCC 25923. **B.** gamma-haemolysin mutant *S. aureus* 5942. *S. aureus* are surrounded by a membrane (green arrows).

CONCLUSION

The intracellular infection model developed in THP-1 macrophages allows us to demonstrate the ability of *S. aureus* to survive and multiply in professional phagocytes, to study the kinetics of its multiplication, and to observe the intracellular localization of *S. aureus*. Comparison of strains disrupted for haemolysins suggests that the specific disruption of the gamma-haemolysin favours the intracellular multiplication of *S. aureus*.

REFERENCES

1. Barcia-Macay, M., Seral, C., Mingeot-Leclercq, M-P., Tulkens, P.M. and Van Bambeke, F. (2006) Pharmacodynamic evaluation of the intracellular activity of antibiotics against Staphylococcus aureus in a model of THP-1 macrophages. *Antimicrobial Agents and Chemotherapy* **50**(3):841-851
2. Brouillette, E., Grondin, G., Shkreta, L., Lacasse, P. and Talbot, B.G. (2003) *In vivo* and *in vitro* demonstration that *Staphylococcus aureus* is an intracellular pathogen in presence or absence of fibronecting-binding protein. *Microbial Pathogenesis* **35**: 159-168
3. Gresham, H.D., Lowrance, J.H., Caver, T.E., Wilson, B.S., Cheung, A.L. and Lindberg, F.P. (2000) Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. *Journal of Immunology* **164**: 3713-3722
4. Hébert, A., Sayasith, K., Sénéchal, S., Dubreuil, P.and Lagacé, J. (2000) Demonstration of intracellular *Staphyloccocus aureus* in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk. *FEMS Microbiology Letters* **193**: 57-62
5. Qazi, S.N.A, Harrison, S.E., Self, T., Williams, P. and Hill, P.J. (2004) Real-time monitoring of intracellular *Staphylococcus aureus* replication. *Journal of Bacteriology* **186**: 1065-1077

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