CHARACTERIZATION OF THE INTERNALIZATION AND INTRACELLULAR PERSISTENCE OF MENADIONE AND HEMIN-DEPENDANT SMALL-COLONY VARIANTS (SCV) OF *STAPHYLOCOCCUS AUREUS* IN A MODEL OF THP-1 CELLS AND A HUMAN AIRWAY EPITHELIAL CELL LINE, CFBE41O-

No. 288

L. Garcia[1], B. C. Kahl[2], K. Becker[2], R.A. Proctor[3], O. Denis[4], S. Lemaire[1], P.M. Tulkens[1], F. Van Bambeke[1]

[1]Université catholique de Louvain, Brussels, Belgium; [2]University Clinics of Muenster, Muenster, Germany; [3]University of Winconsin Med. School, Madison ; [4]Département de Microbiologie & Laboratoire de référence MRSA-Staphylocoques, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium.

ABSTRACT

BACKGROUND: Small-Colony Variants (SCV) of *Staphylococcus aureus* are slow-growing subpopulations that cause persistent and relapsing infections. In this study, we have examined how a MRSA strain and its two stable menadione- and hemin-dependent SCVS were internalized and survived in THP-1 cells and isogenic bronchial cell lines expressing or not a functional CFTR.

METHODS: Activation in macrophages: Incubation of THP-1 monocytes with PMA (200 nM, 48 h). Strains: MRSA COL (K7), *menD* disruptant (K8), *hemB* disruptant (K9) and *hemB* complemented strain (K10). Internalization: Enumeration of cell-associated CFU after 1 or 2 hours phagocytosis in THP-1 or CFBE41o-, respectively. Persistence: Assessed from the change in CFU from post-phagocytosis value after 24 h of incubation.

RESULTS: Internalization: SCVs were less avidly internalized than K7 in all cell types, with differences being more marked in THP-1 macrophages and CFBE41o- WT CFTR. For all strains, internalization was more important in THP-1 monocytes and CFBE41o- KO CFTR than in the other cell lines. Intracellular growth: K8 grew much slower in THP-1 cells, whether differentiated or not, than the other strains, whereas in CFBE41o- KO CFTR, all strains seemed to grow at the same rate. Yet, SCV showed a higher persistence than K7 in CFBE41o- WT CFTR and F508del. K10 displayed a behavior (internalization and growth) similar to K7 in all cell types (not shown).

CONCLUSION: Unexpectedly, differentiation of monocytes in macrophages reduces internalization but does not affect intracellular growth. The fact that K8 grows much slowly than K7 or K9 in THP-1 suggests that the intracellular medium does not contain the nutrients needed for the growth of a menadione-dependent strain. Interestingly, the expression of WT or F508del CFTR seems to reduce *S. aureus* internalization, but SCVs seem to persist much better than a normal strain in CFBE41o-, independently of their CFTR status, as previously described in the literature for others cells types (JID, 2010, 202:1031-40; CID, 2001, 32:1643-7).

Cells	Intracellular counts[a]								
	K7			K8			K9		
	t0	t24	Delta[b]	t0	t24	Delta[b]	t0	t24	Delta[b]
THP-1 monocytes	1.32	4.15	2.83	0.77	2.11	1.34	0.75	3.51	2.76
THP-1 macrophages	0.30	3.02	2.72	-0.76	0.49	1.25	-0.49	2.15	2.64
CFBE41o- KO CFTR	0.98	2.47	1.49	0.12	1.68	1.56	0.14	1.97	1.83
CFBE41o- WT CFTR	0.46	1.15	0.69	-0.56	0.97	1.53	-0.89	0.97	1.86
CFBE41o- F508del	-0.12	0.93	1.05	-0.34	1.17	1.44	-0.54	1.31	1.85

[a] Intracellular counts: Values expressed in $\log 10^6$ bacteria per mg of cell protein
[b] Difference in bacteria counts between 24 h and 0h

INTRODUCTION

Staphylococcus aureus and *Pseudomonas aeruginosa* are often found together in the airways of Cystic Fibrosis patients. It was shown that the *P. aeruginosa* product 4-Hydroxy-2-HeptylQuinoline-*N*-Oxide suppresses the growth of *S. aureus* and provokes the emergence of Small-Colony Variants (SCVs) . The presence of these particular forms has been associated with chronic infections in CF (1).

SCVs are a slow-growing subpopulation displaying different phenotypic characteristics and pathogenic traits such as slow growth, decreased pigment formation, altered expression of virulence genes, auxotrophism for distinct growth factors such as thymidine, hemin and/or menadione, and the ability to revert to the normal phenotype (2). Yet, there is now data so far regarding hemin or menadione-dependent mutants.

Hemin and menadione are two compounds involved in the synthesis of the electron carriers cytochrome and menaquinone. Hemin or menadione-dependent phenotypes are unstable, but stable in vitro mutants can be obtained by inactivation of the *hemB* gene (one of the genes encoding enzymes of the porphyrin biosynthetic pathway) (3) or of the *menD* gene (one of the genes required for menadione biosynthesis) (4).

The aim of this study was to compare the internalization of a phagocytosed *S. aureus* and its SCVs mutants and their capacity to survive in unstimulated & phorbol-ester-differentiated THP-1 cells and in an isogenic bronchial cell lines expressing or not a functional CFTR.

METHODS

Bacterial strains: *S. aureus* COL (K7; MRSA), its respective *menD* (K8) and *hemB* (K9) mutants and *hemB* complemented strain (K10) were used in this study. The *hemB and menD* mutants were constructed by allelic replacement with an *ermB* cassette-inactivated *hemB* gene and an *ermC* cassette-inactivated *menD* gene, respectively (3, 4).

Cells: Experiments were performed with
- Human THP-1 cells (ATCC TIB-202, Manassas, VA), a myelomonocytic cell line displaying macrophage-like activity. Activation of THP-1 was obtained by the addition of 200 ng/mL of PMA during 48 hours;
- Human monocytic THP-1 cells and CFBE41o- isogenic bronchial cell lines expressing or not a functional CFTR (5).

Cell infection (6): Phagocytosis was initiated at a bacteria per macrophage ratio of 10 (1h at 37°C), followed by elimination of non-phagocytosed bacteria by exposing the cells to 50 mg/L gentamicin (45min). Cells were then transferred to fresh medium supplemented with a very low concentration of antibiotics for 24 hours. Results were expressed as the change in the intracellular inoculum at 24 h compared to time 0.

RESULTS

FIGURE 1 A. Growth in broth

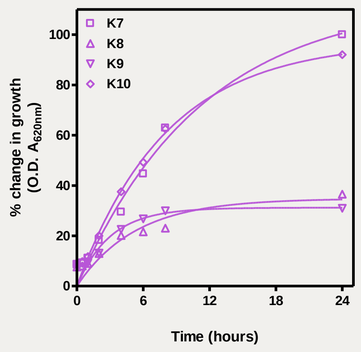


FIGURE 1 B. Persistence in broth

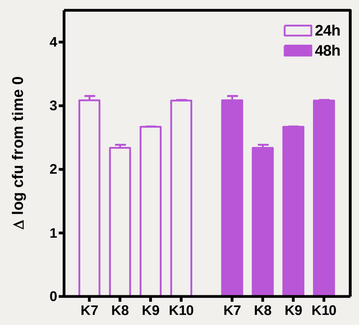


FIGURE 2 A. Uptake by THP-1

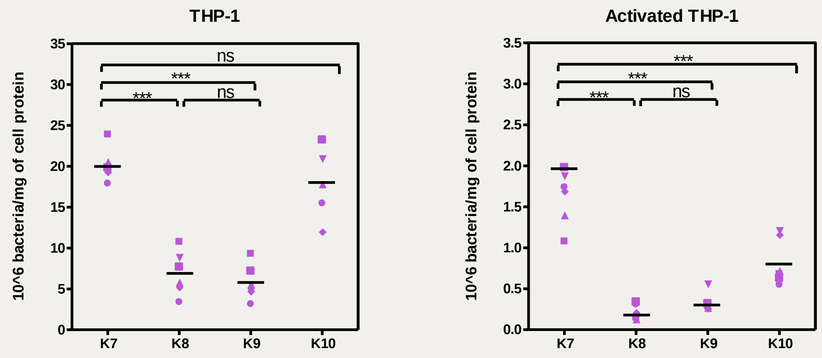


FIGURE 2 B. Persistence in THP-1

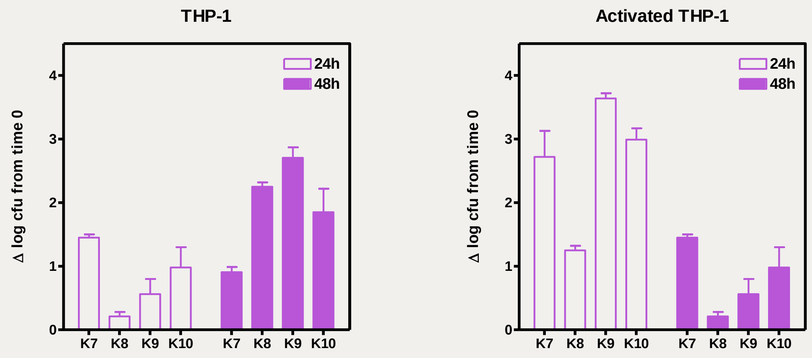


FIGURE 3 A. Uptake by CFBE41o-

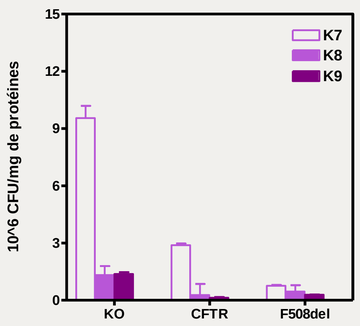
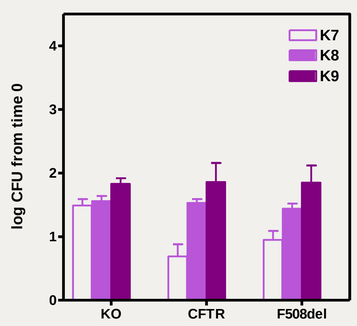


FIGURE 3 B. Persistence in CFBE41o-



CONCLUSION

Global internalization: SCV were less avidly internalized than the normal phenotype strain in all cell types.

In THP-1
- Internalization of each strain is reduced in activated THP-1 in comparison to non-activated cells;
- After 24 hours of infection, intracellular counts were higher in activated cells as compared to non activated cells; yet, the menadione-dependent strain shows a slower growth as compared to the hemin-dependent mutant in activated macrophages;
- After 48 hours, all strains persist to lower levels in activated cells as compared to non activated cells. This can be attributed to a better killing activity of macrophages in comparison to monocytes.

In CFBE41o-,
- The expression of WT or F508del CFTR seems to reduce *S. aureus* internalization;
- SCVs seem to persist much better than a normal strain in CFBE41o-, independently of their CFTR status.

REFERENCES

(1) Hoffman, et al. 2005. *Proc Natl Acad Sci USA*. 103(52):19890-95 **(2)** Proctor et al. 2006. *Nat Rev Microbiol.* 4:295–305; **(3)** Von Eiff et al. 1997. *J Bacteriol.* 179:4706-4712; **(4)** Bates et al. 2003. *J Infect Dis.* 187(10):1654-1661; **(3)** Proctor et al. 1995. *Clin Infect Dis.* 20:95-102; **(4)** Proctor et al. 1994. *Infect Agents Dis.* 3:302-312;(5) Bedok et al. 2005. *J. Physiol.* 569:601-615; **(6)** Barcia-Macay et al. 2006. *Antimicrob Agents Chemother*. 50:841-851.

AKCNOWLEDGMENTS

This research was supported by the Belgian *Fonds National de la Recherche Scientifique,* the *French Association Mucoviscidose-ABCF2* and by the Belgian *Fonds pour la Recherche dans l'Industrie et l'Agriculture.*

This poster will be made available for download after the meeting at : http://www.facm.ucl.ac.be/posters