Session : Experimental pathogenesis and treatment

Evidence of adaptative processes of *Staphylococcus aureus* isolates in the course of chronic bone and joint infections in patients

S Trouillet-Assant, L Lelièvre, JP Rasigade, F Valour, P Martins-Simoes, S Flammier, J Tasse, F Vandenesch, T Ferry, F Laurent.

International Center for Infectiology Research CIRI INSERM U1111 CNRS UMR5308, Lyon, France,

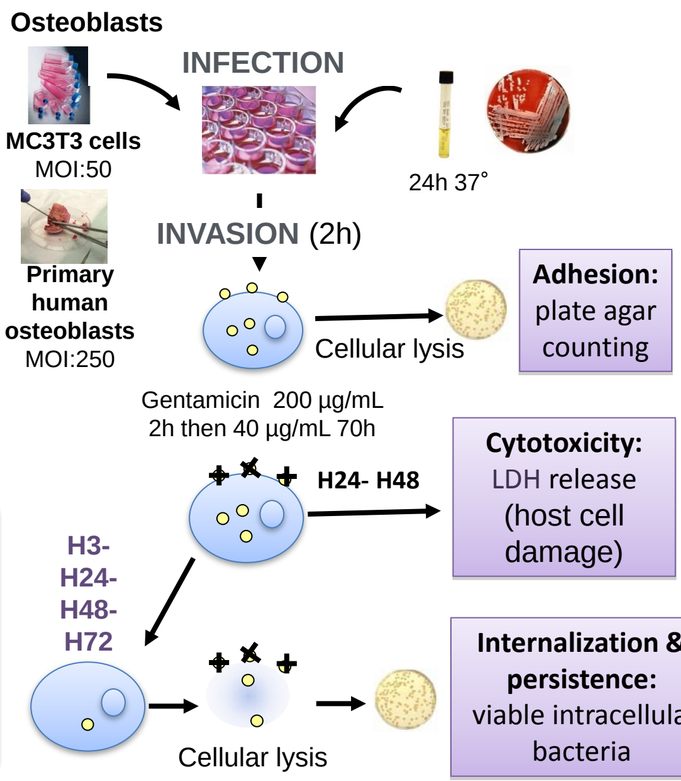
Objectives

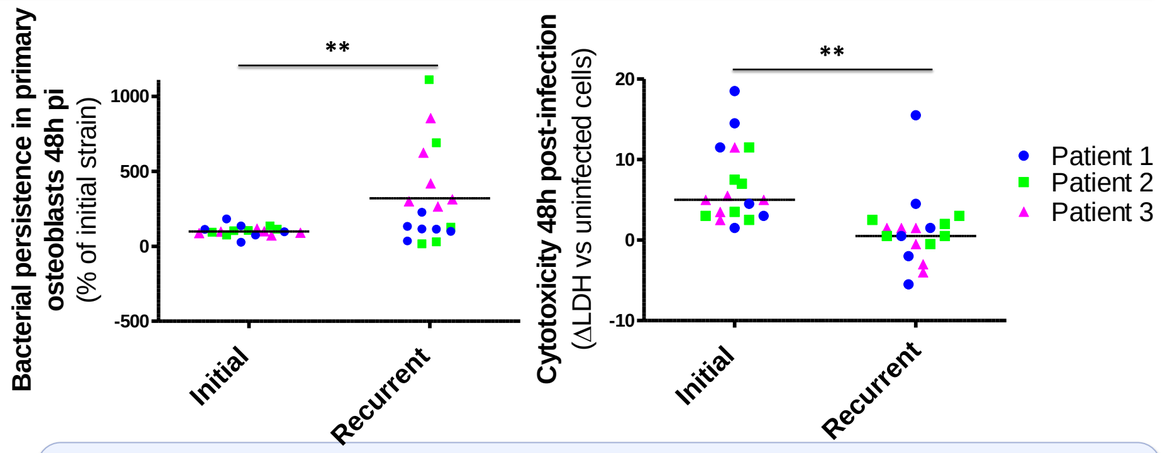
Bone and joint infection (BJI) is associated with significant morbidity and mortality, due to high rates of chronicity and relapse (10-20% of cases). To date, three staphylococcal virulence mechanisms have been associated with BJI chronicization and therapeutic failure, leading to host immune system evasion: i) bacterial internalization in non-phagocytic bone cells such as osteoblasts; ii) biofilm formation; and iii) the phenotype switching to small colony variants, characterized by reduced metabolic and hemolytic activities. The present study aimed to compare isolates recovered from initial and recurrent BJI episode from the same patients toward these bacterial adaptative mechanisms.

Results

The crystal violet staining method revealed that recurrent strains from patient #2 and #3 formed higher levels of mature biofilm (132±23% and 241±67%, respectively) than initial strains (100%) at 48h ($p<0.01$ for both), No difference was observed with strains recovered from patient 1.

In vitro infection model





Results

- **Same capacity of adhesion to and internalization into osteoblasts** for initial and recurrent strains
- **Recurrent strains persist longer in intracellular compartment** of osteoblasts than initial strains
- Osteoblasts infected by **recurrent strains secrete less inflammatory cytokines** than those infected by initial ones

3 couples of MSSA strains isolated from patients suffering from recurrent or persisting prosthetic joint infection (PJI) at initial diagnosis of PJI and at the time of relapse.

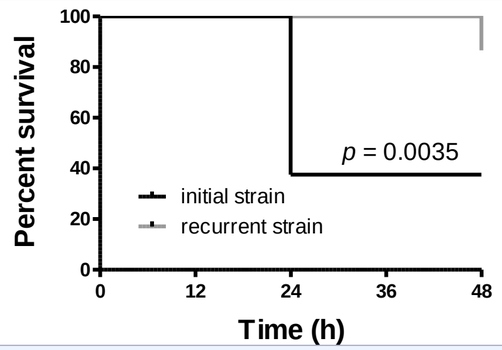
Patient no	Sexe, age (year)	Site of infection	Surgical treatment	antibio-therapy (days)	Time to failure or relapse after initial infection (days)	MLST type	SPA Type
1	H,26	Tibia osteosynthesis material	Material Removed	82	82	ST15 (CC15)	385
2	H,80	Total knee arthroplasty	Irrigation and debridment	191	201	ST25 (CC25)	78
3	F,82	Total hip arthroplasty	Irrigation and debridment	98	134	ST15 (CC15)	84

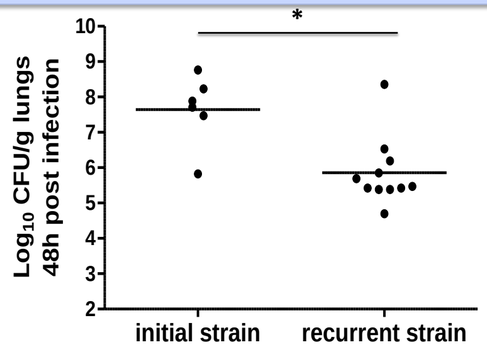
In vivo infection model

Lung infection model
10^8 CFU

Results

In vivo model of lung infection mimicking acute pneumonia revealed that **initial strains caused higher mortality** (62%) as soon as 24h post-infection, than initial strains (0%), $p<0,01$. Furthermore, the bacterial counts in the spleen and the lungs were higher in the initial strain- vs recurrent strain-infected animals (7.65±1.00log10 vs 5.85±3.39log$_{10}$ CFU/lungs ($p<0.05$) and 5.27±0.28log$_{10}$ vs 3.39±1.63log$_{10}$ CFU/spleen ($p<0.05$) respectively, 48h post infection) suggesting that ***S. aureus*** **isolates recovered from recurrent infections harbors lower virulence power than those recovered from initial infection.**





Conclusions

These findings suggests for the first time that *S. aureus* BJI chronicization is associated with an in vivo bacterial adaptation leading to host immune escape, linked with higher intraosteoblastic persistence and biofilm formation.