Interactions between staphylococci, osteoblasts and osteoclasts: What do we know in 2016?

Pr Frédéric LAURENT

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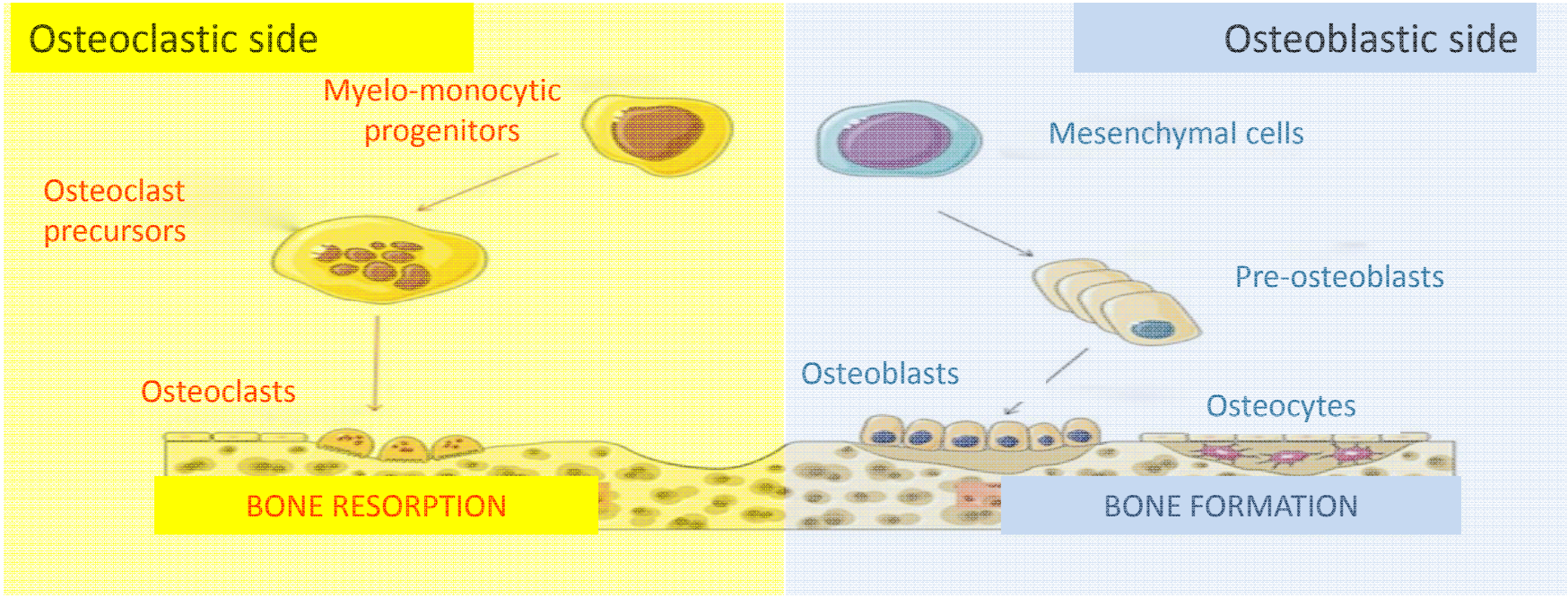
Bone tissue and infection



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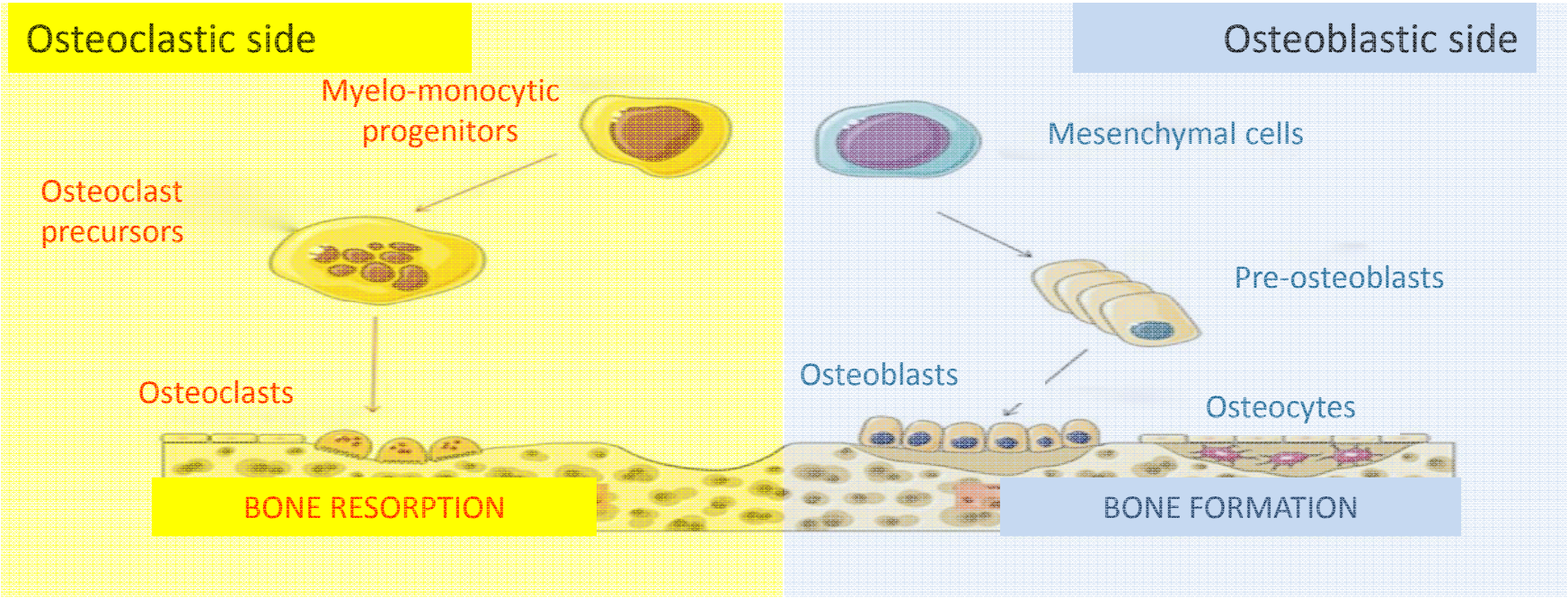
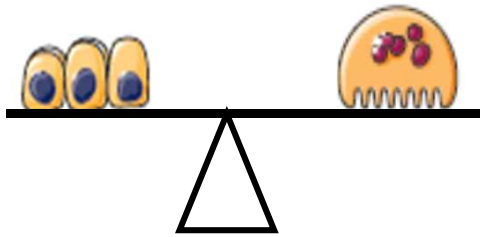


Physiological context

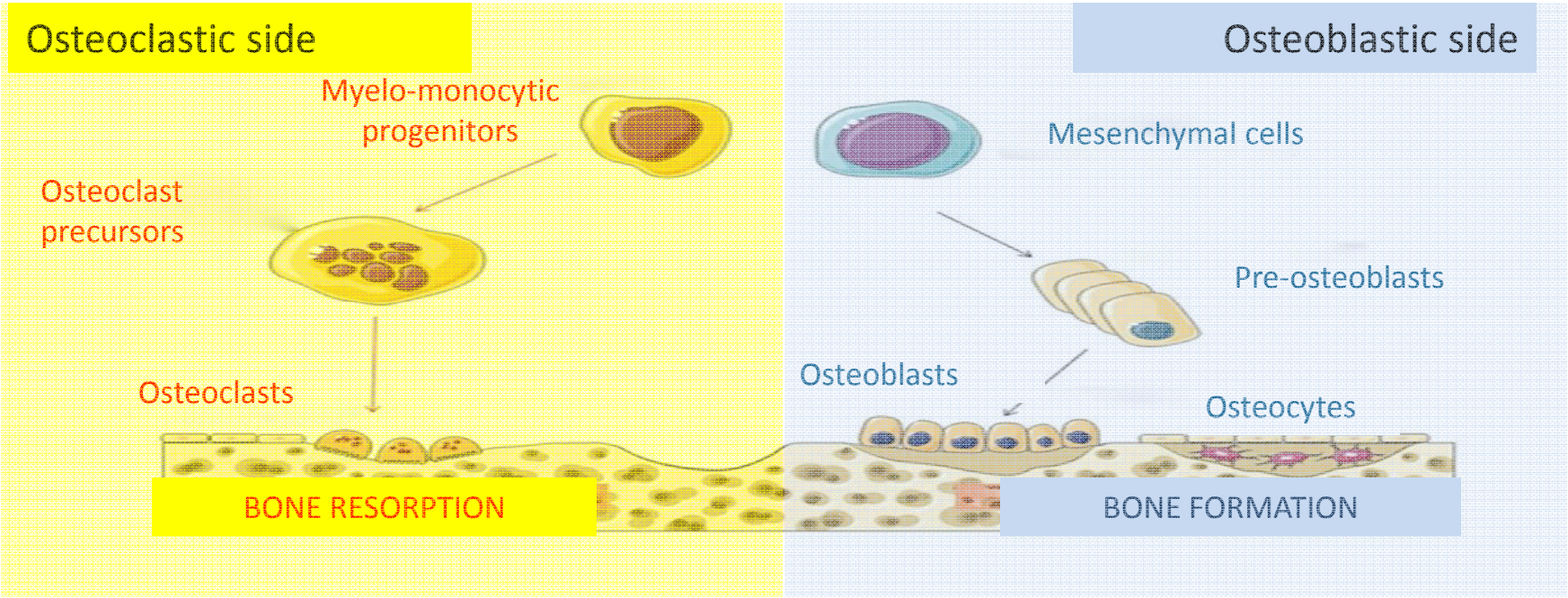
Healthy bone



Healthy bone

Balance osteoblast/osteoclast

Bone tissue and infection



Physiological context

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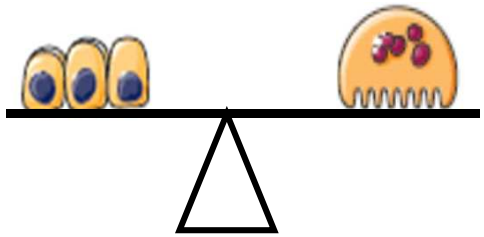

Healthy bone

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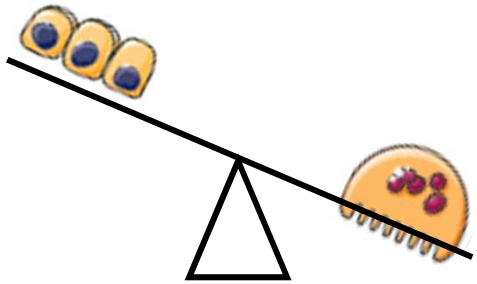
Infectious context



Infected bone

Progressive pain/destruction/bone loss
. prothesis loosening
. loss of function

Infected bone

Pathophysiopathology of staphylococcal BJI

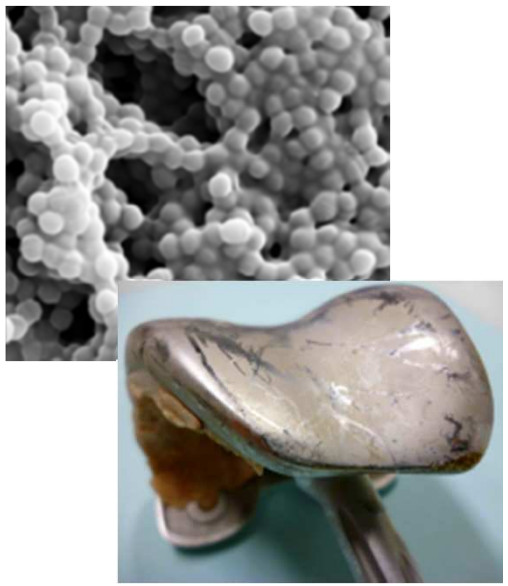
Staphylococcus > 50% of BJI

Classical acute virulence

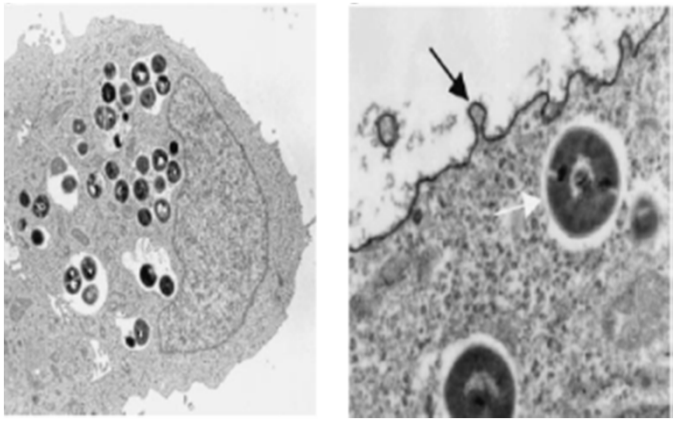
+

Recurrence et chronicity ++

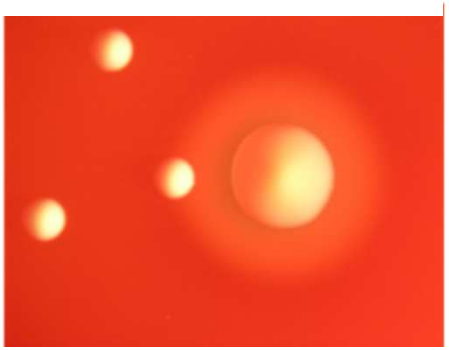
Biofilm



Internalization



Small Colony Variant (SCV)



Pathophysiopathology of staphylococcal BJI

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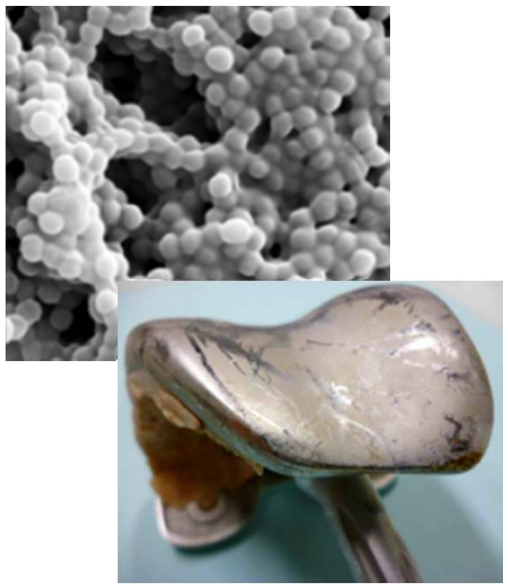
Biofilm



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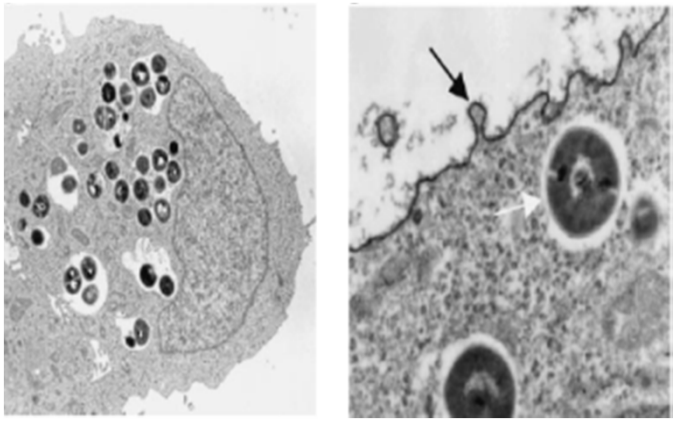

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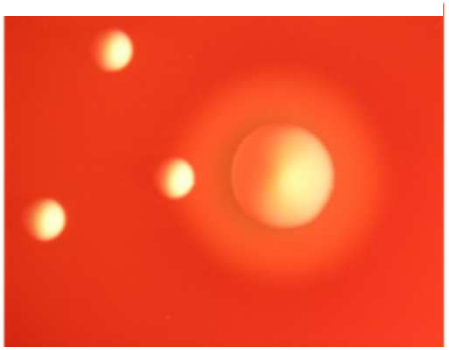
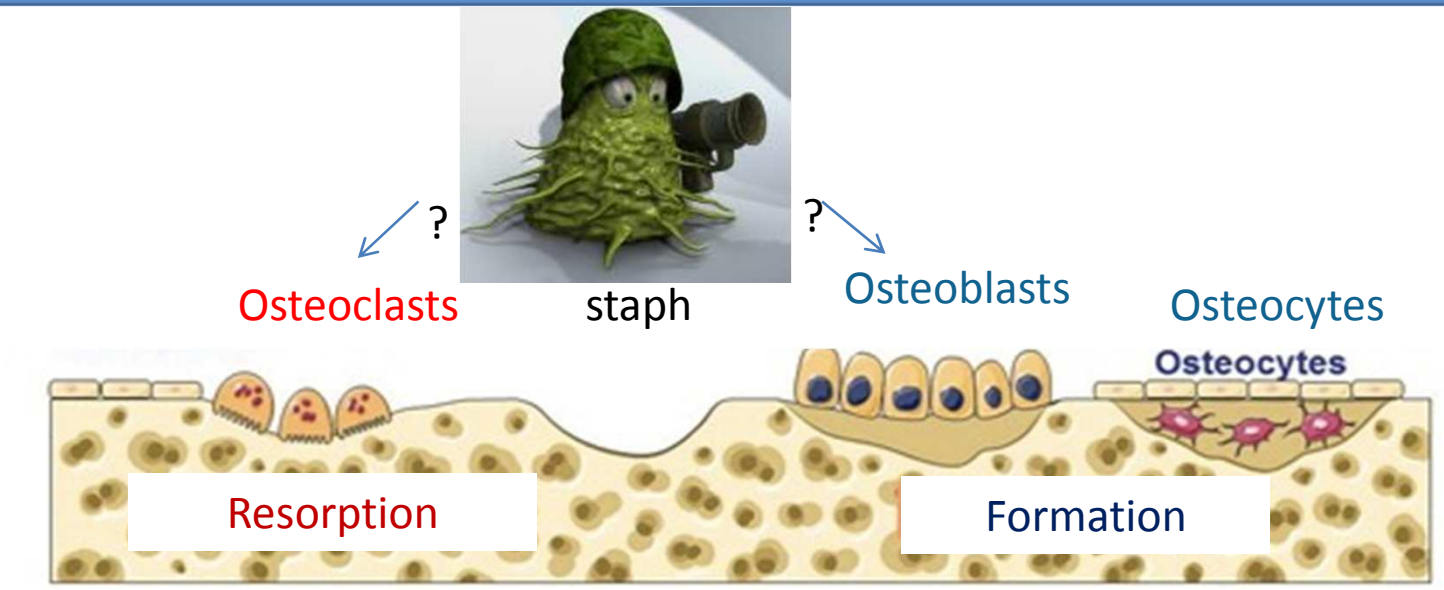


BJI and *Staphylococcus aureus*



Loss of homeostasy due to

Loss of osteoblast function (destruction, reduction of mineralisation) ?

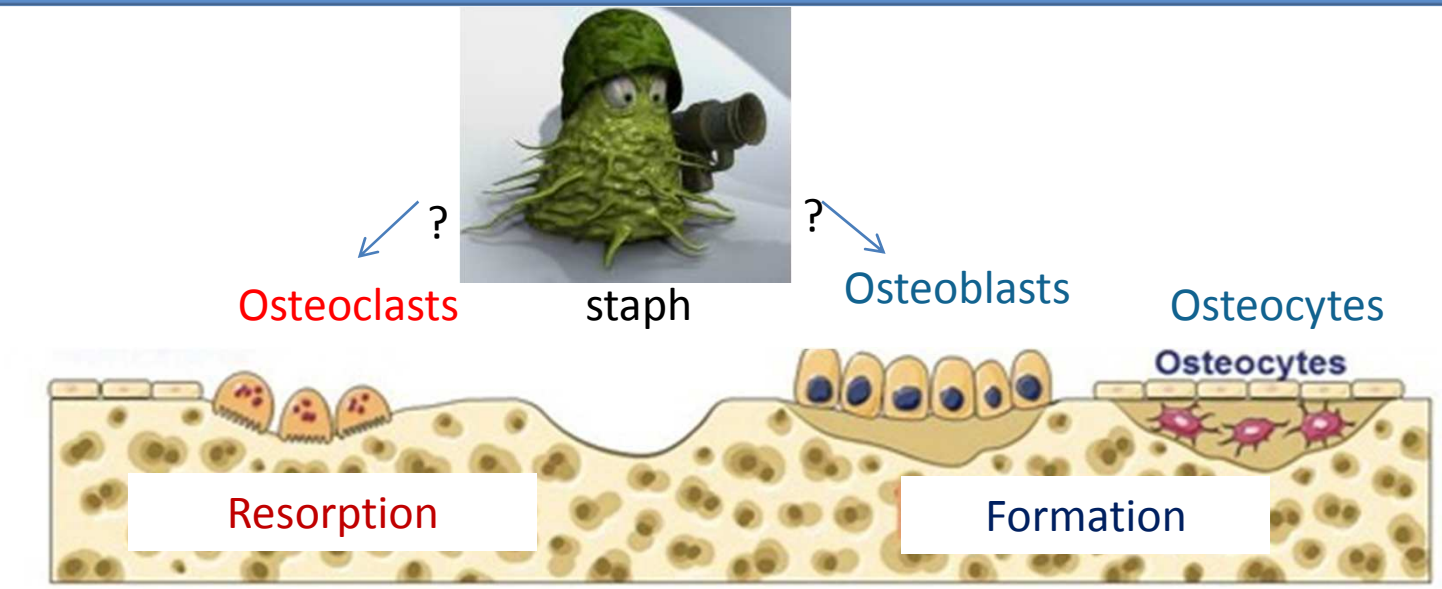
and/or gain of osteoclast function (increase resorption) ?

Consequences of intracellular interaction *in vitro*

S. aureus / osteo**b**lasts ?

S. aureus / osteo**c**lasts ?

Part I: osteoclasts



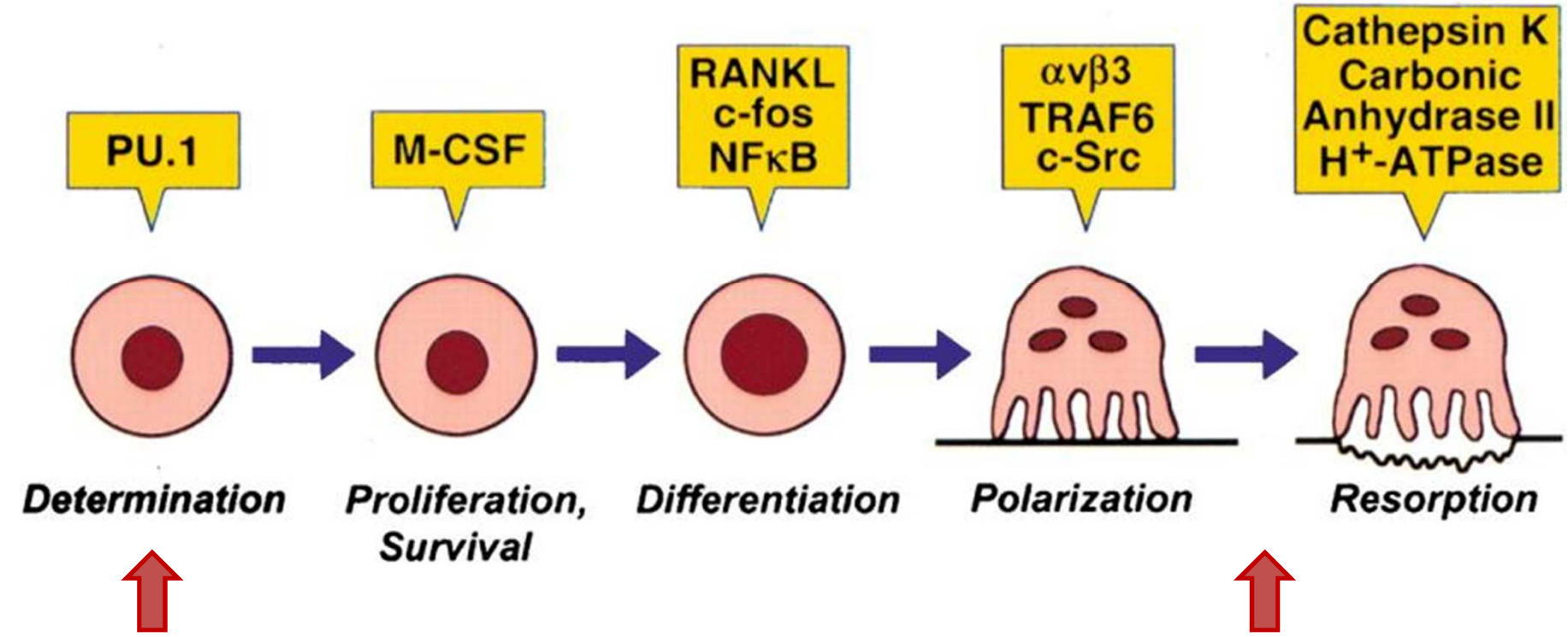
Aim :

To understand the direct impact of live intracellular *S. aureus* on **osteoclasts**:

- Phenotypic aspect: osteoclastogenesis/cytotoxicity
- Functional aspect: bone resorption

Impact of live intracellular *S. aureus* on osteoclasts

To test the *S. aureus* impact on osteoblastic cells
at different steps of differentiation

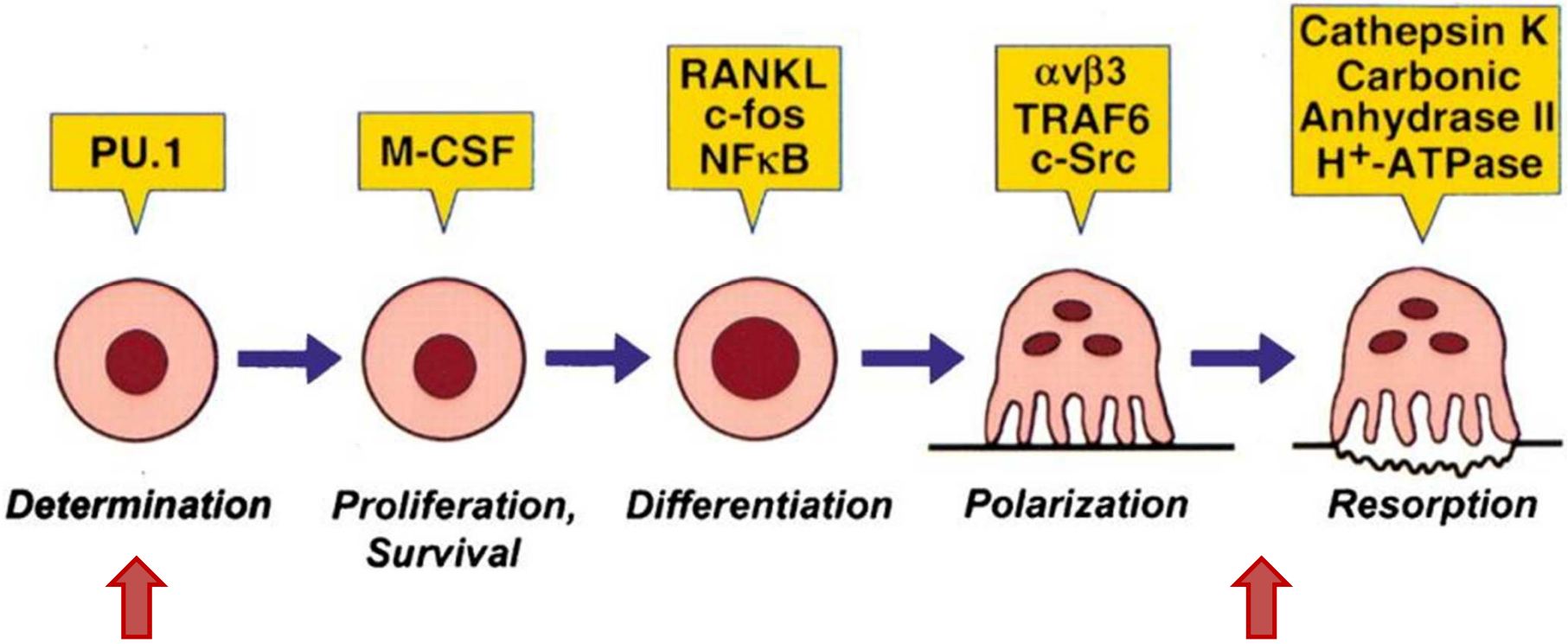


Myelo-monocytic
precursors

Fusion
Large and multinucleated cells

Impact of live intracellular *S. aureus* on osteoclasts

To test the *S. aureus* impact on osteoblastic cells
at different steps of differentiation



Myelo-monocytic
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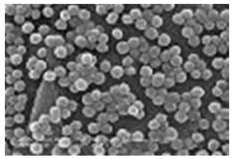
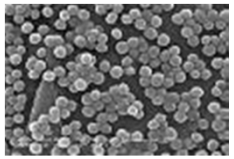


To investigate the effect
on osteoclstogenesis

Fusion
Large and multinucleated cells



To investigate the effect
on bone resorption

Impact of live intracellular *S. aureus* on osteoclasts

Blood

Osteoclast precursors

INVASION

2h

Lysostaphin
200 µg/mL

infected OC precursors

Différenciation in media with M-CSF and RANK-L

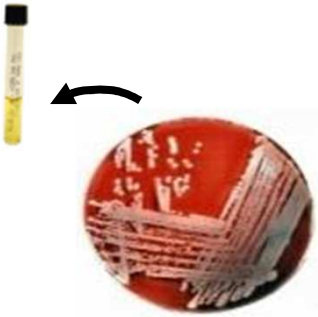
Différenciation in media with M-CSF and RANK-L

Lysostaphin (40µg/ml)

Osteoclastogenesis ?

INVASION

2h

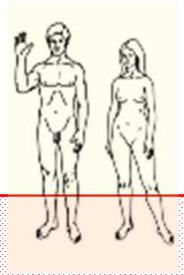


Lysostaphin
200 µg/mL

Infected mature OC

Evaluation of bone resorption on bone matrix

Impact of live intracellular *S. aureus* on osteoclasts

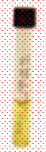


Blood

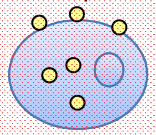
Osteoclast precursors

INVASION

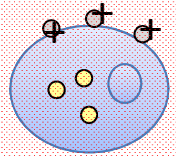
2h



Lysostaphin
200 µg/mL



infected OC precursors



Différenciation in media with M-CSF and RANK-L

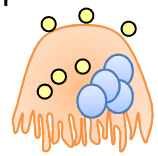
Lysostaphin (40µg/ml)

Osteoclastogenesis ?

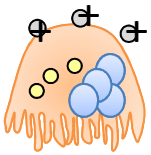
Différenciation in media with M-CSF and RANK-L

INVASION

2h



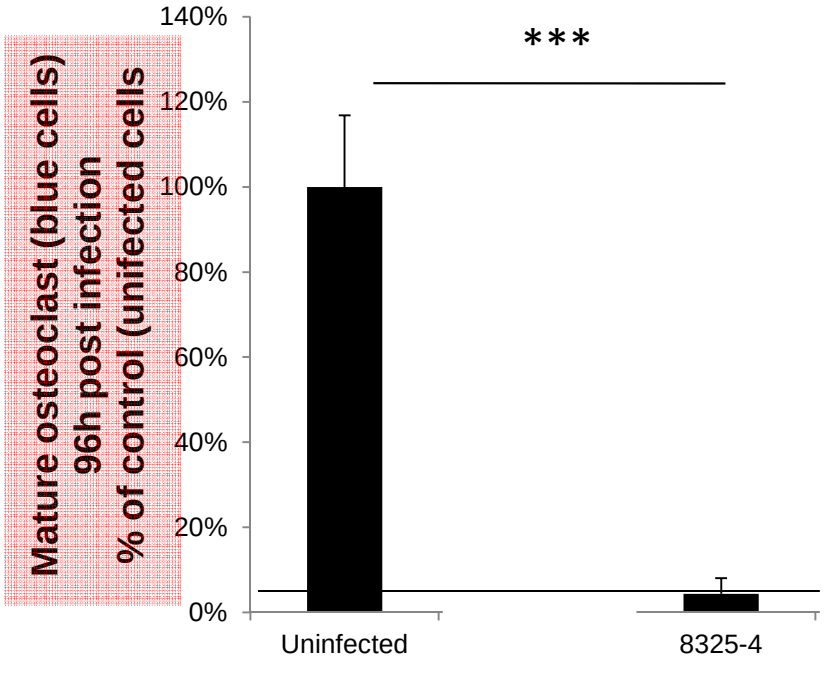
Lysostaphin
200 µg/mL



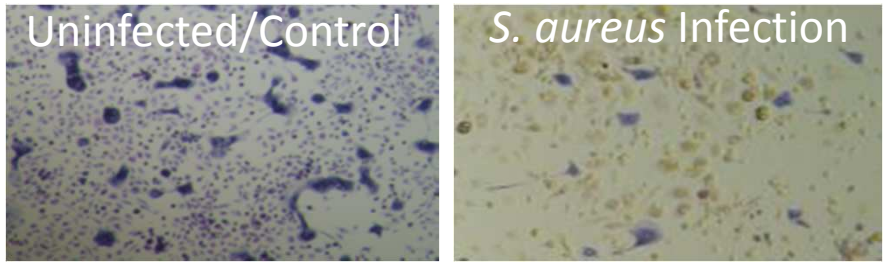
Infected mature OC

Evaluation of bone resorption on bone matrix

Impact of live intracellular *S. aureus* on osteoclasts



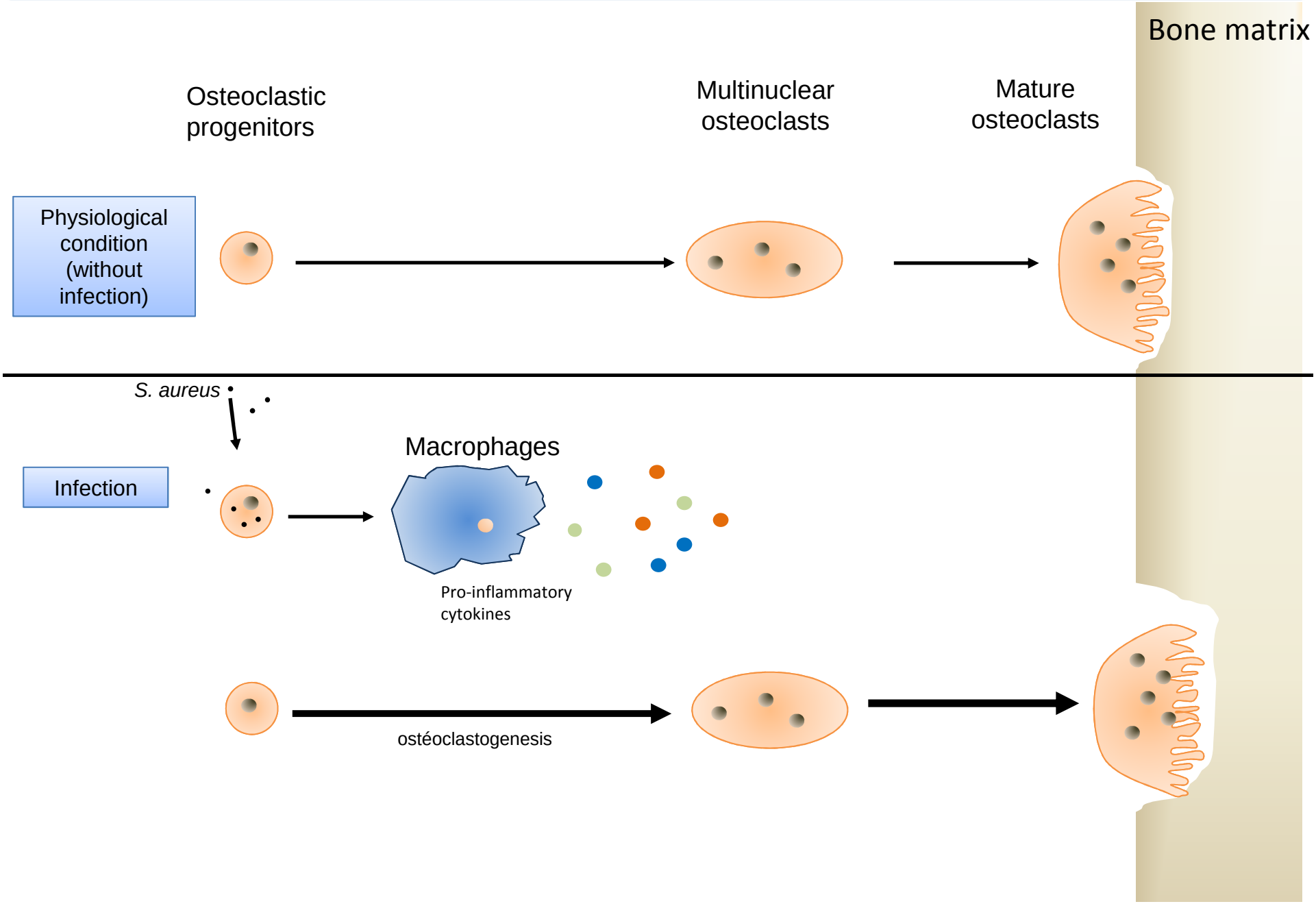
Inhibition of osteoclastogenesis after live *S. aureus* internalization



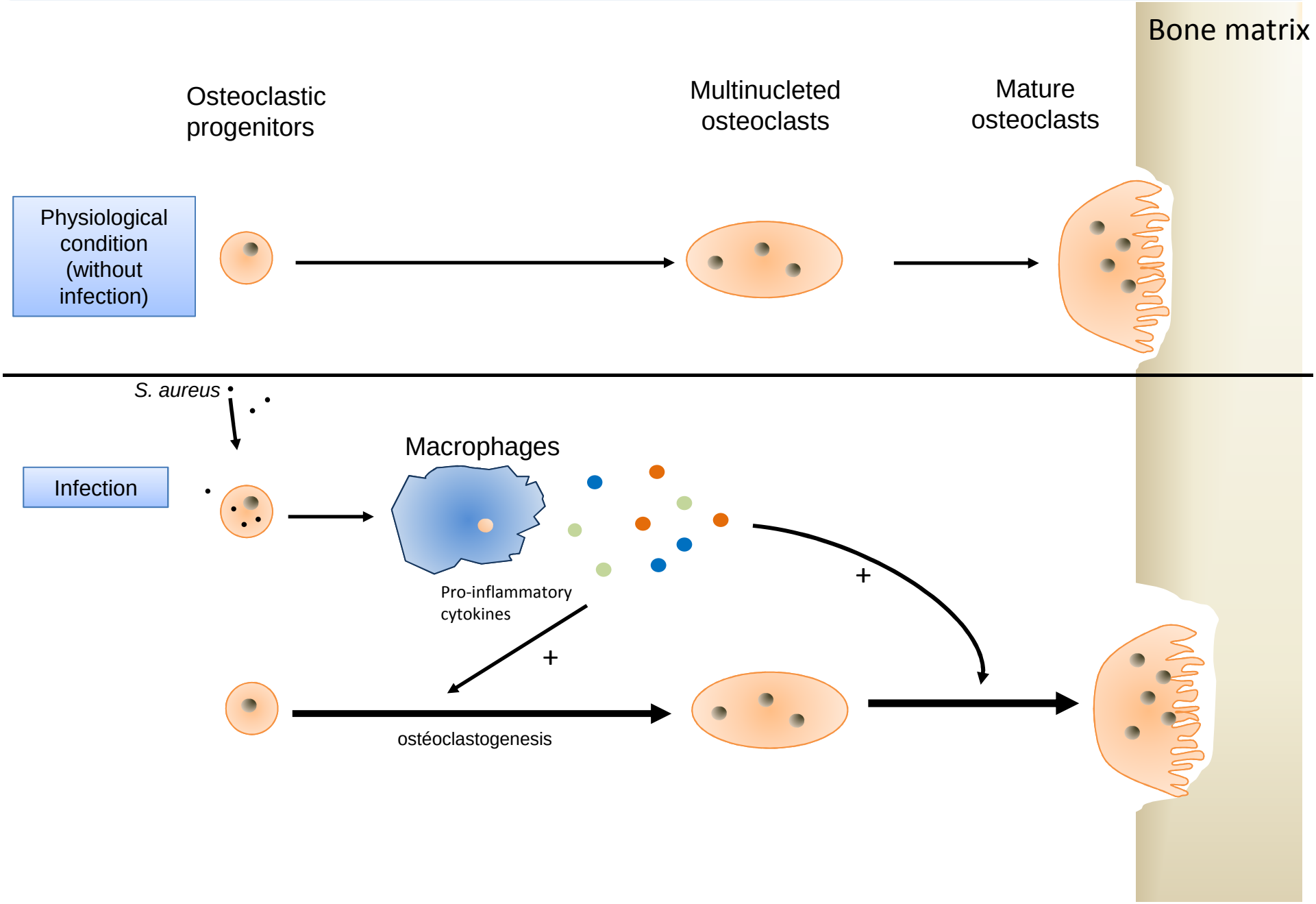
- Cytometry typing = **macrophage**
- Pro-inflammatory chemokine profile**=** **macrophage**

- Supernatant = **. boost resorption by mature uninfected OC**
  . increase differenciation of uninfected OC precursors

Impact of live intracellular *S. aureus* on osteoclastic precursors



Impact of live intracellular *S. aureus* on osteoclastic precursors



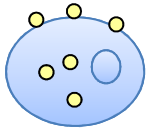
Impact of live intracellular *S. aureus* on osteoclastic precursors

Blood

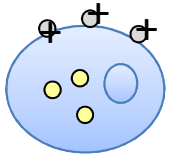
Osteoclast precursors

INVASION

2h



Lysostaphin
200 µg/mL



infected OC precursors

Différenciation in media with M-CSF and RANK-L

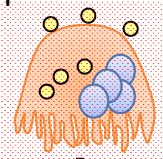
Lysostaphin (40µg/ml)

Osteoclastogenesis ?

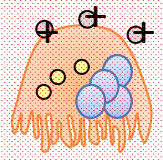
Différenciation in media with M-CSF and RANK-L

INVASION

2h



Lysistaphin
200 µg/mL

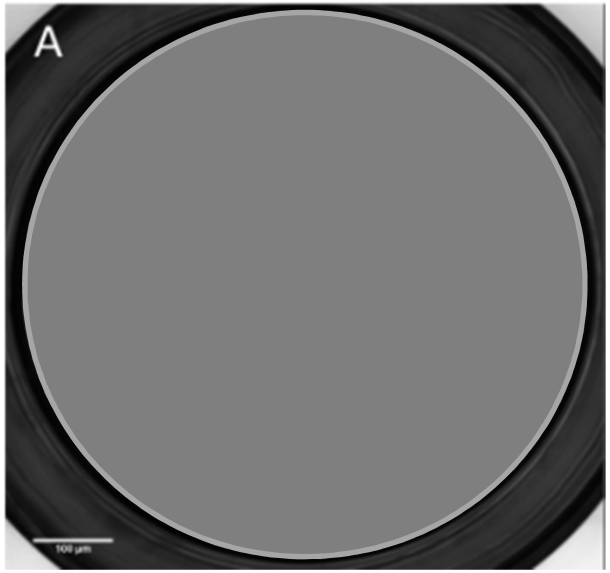


Infected mature OC

Evaluation of bone resorption on bone matrix

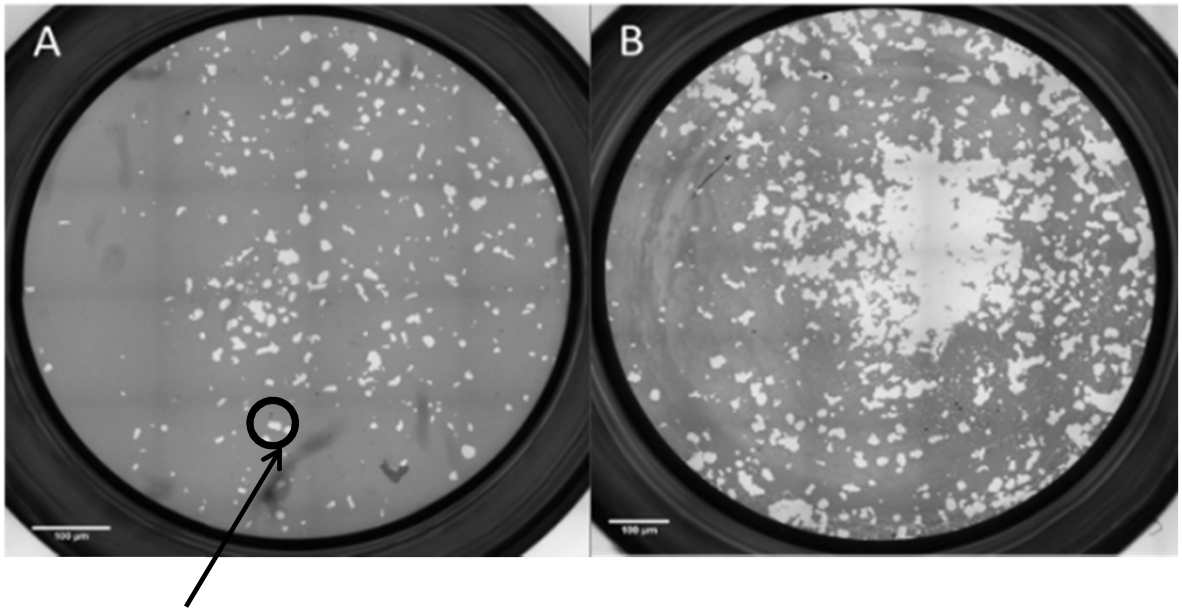
Impact of live intracellular *S. aureus* on mature osteoclasts

Mineral matrix mimicking bone at the bottom of wells

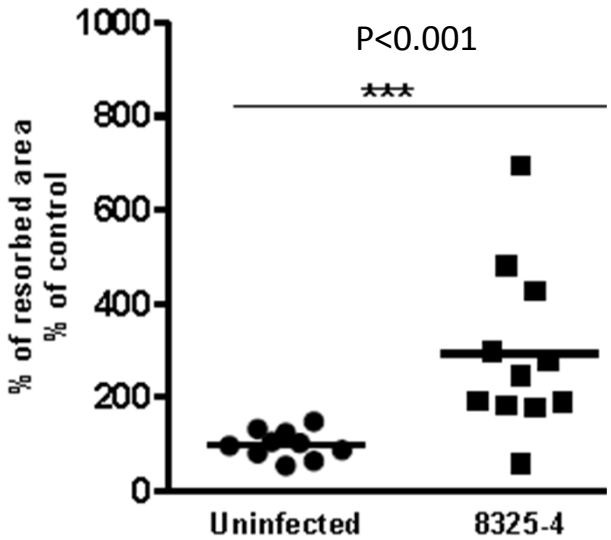


unfected mature osteoclast *S. aureus*-infected osteoclast

24h of incubation

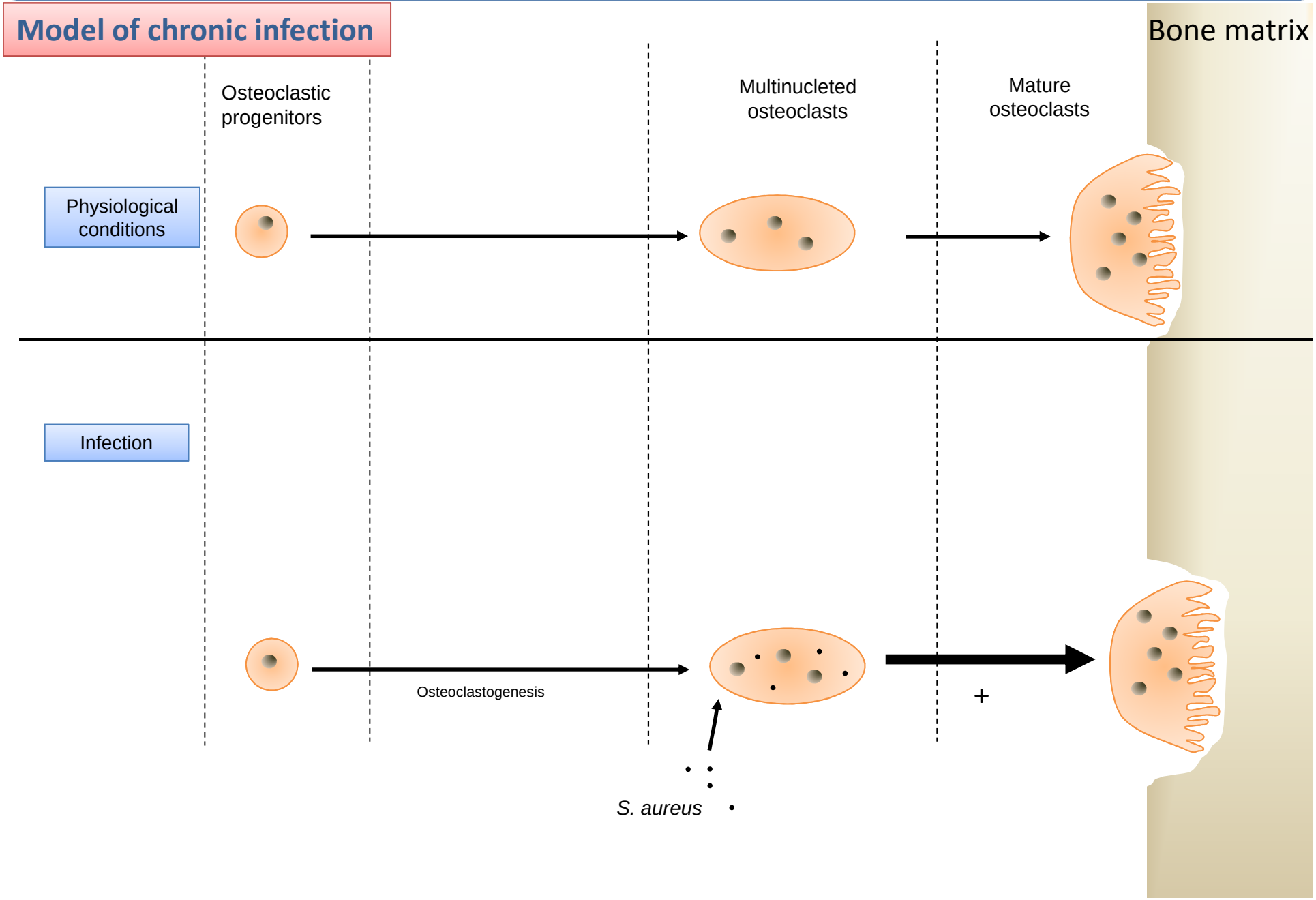


area with bone resorption (in white)

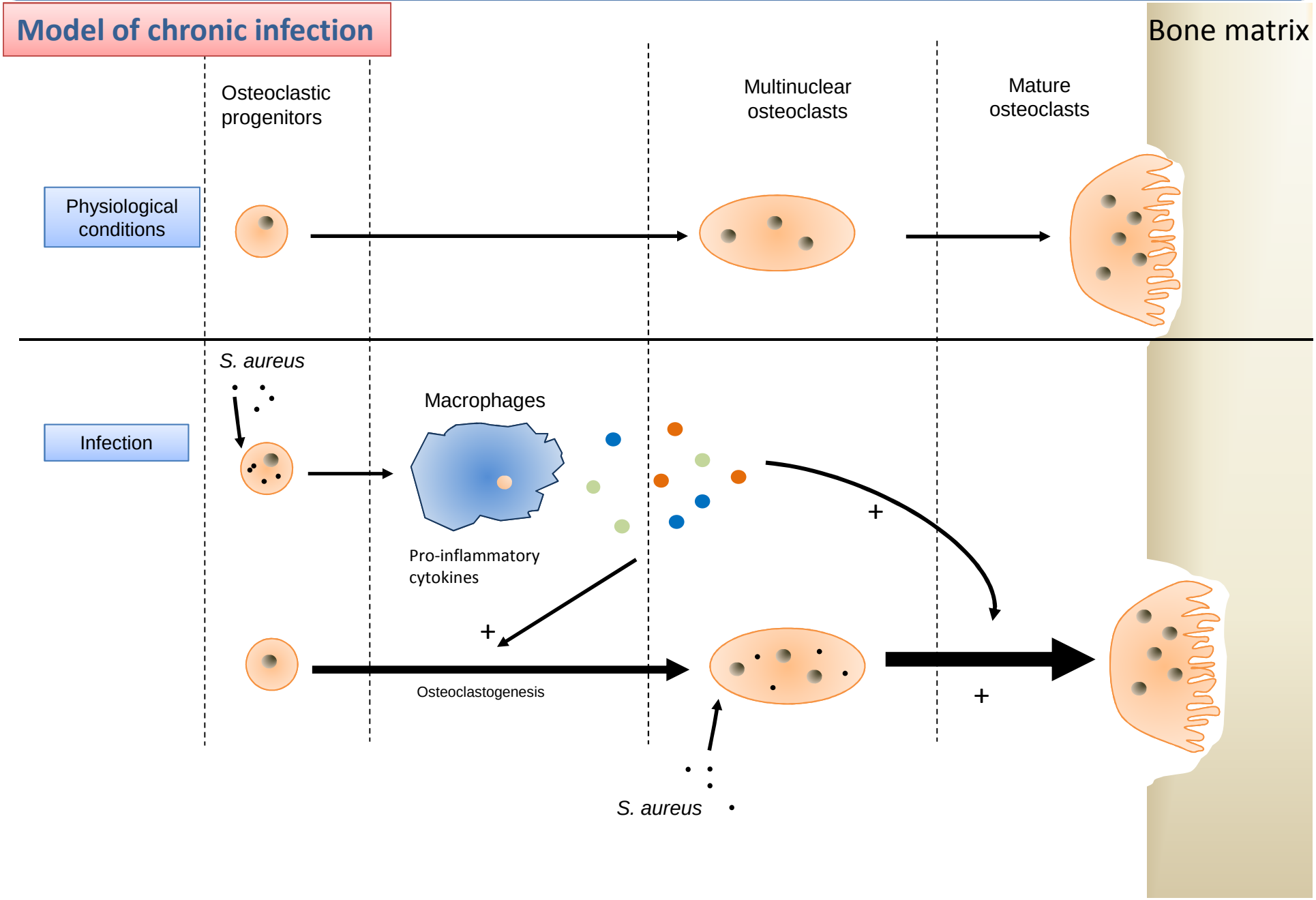


Intracellular *S. aureus* increases the capacity of resorption of mature osteoclasts

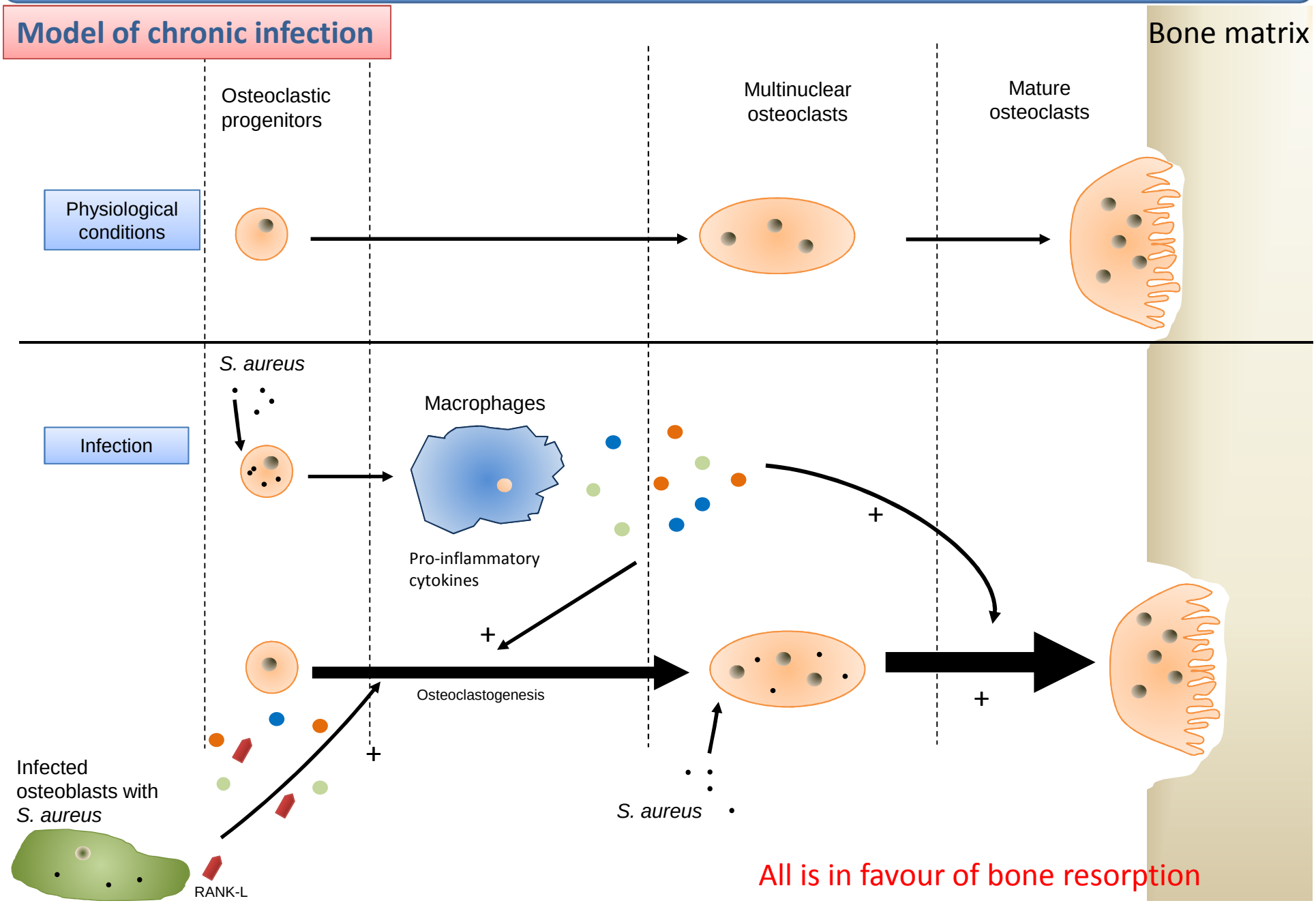
Impact of live intracellular *S. aureus* on osteoclast



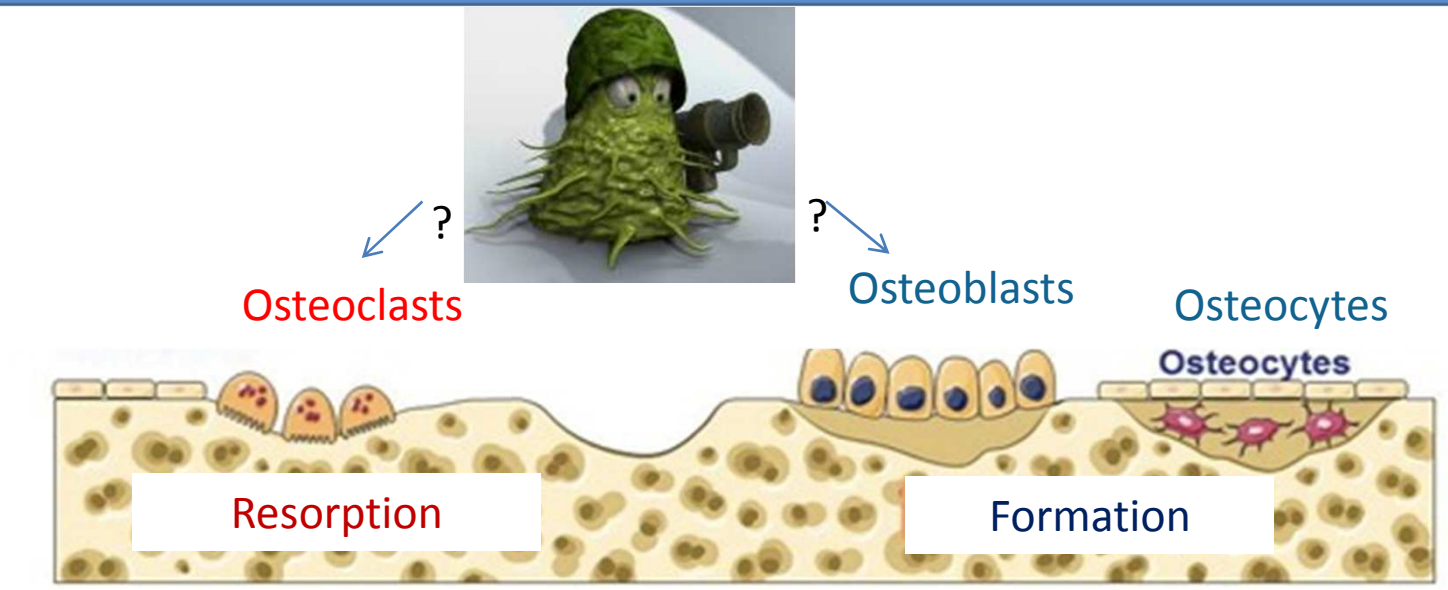
Impact of live intracellular *S. aureus* on osteoclast



Impact of live intracellular *S. aureus* on osteoclast



Part II: osteoblasts



Large bibliography about *S. aureus*-osteoblast interactions

Recent data:

- Investigation of the direct impact of the interaction between live intracellular *S. aureus* and **osteoblasts** in the switch from acute BJI to chronic BJI

Osteoblast and *S. aureus*: the switch from acute to chronic infection

To date, **no study** comparing isolates recovered from **the same patient** at time of acute BJI and at time of chronicization have been performed

Aim of the study

To determine wether bacterial features involved in persisting BJIs are **already present at the acute infection** or are the consequence of ***in vivo*** **adaptations**

Osteoblast and *S. aureus*: the switch from acute to chronic infection

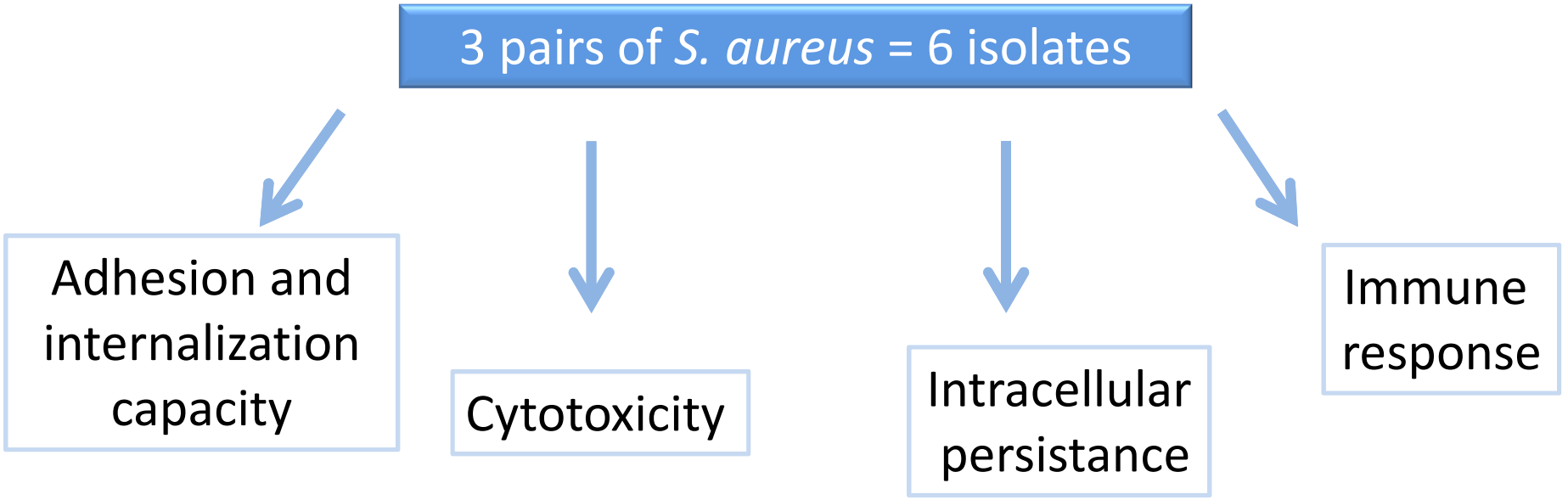
3 pairs of MSSA strains isolated from patients suffering from recurrent/persisting prosthetic joint infection (PJI) at ***initial diagnosis*** of PJI (isolate 1) and at the ***time of relapse*** (Isolate 2)

Clinical data

Patient no	Sexe, age (year)	Site of infection	Duration of symptoms (days)	Surgical treatment	Duration of antibiotherapy (days)	Time to failure or relapse (days)
1	H,26	Tibia osteosynthesis material	12	Material Removed	82	82
2	H,80	Total knee arthroplasty	3	Irrigation and debridment	191	201
3	F,82	Total hip arthroplasty	3	Irrigation and debridment	98	134

Same antibiotic susceptibility profile
Same clonal complex and same WGS

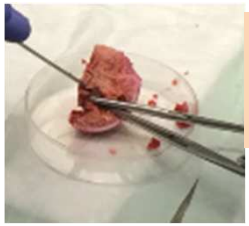
Osteoblast and *S. aureus*: the switch from acute to chronic infection



***in vitro* infection model**

in vitro model of osteoblast infection

Osteoblasts



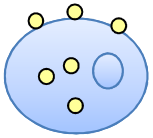
Human primary osteoblasts
MOI:250

Bacteria culture

24h 37 C

Infection

Invasion (2h)

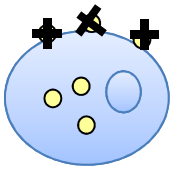


Cellular lysis

H2

Adhesion
Agar plate counting of extracellular bacteria

Lysostaphin 1h



H3

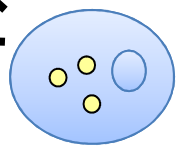
Internalization
Agar plate counting of intracellular bacteria

Cytotoxicity
Quantification of LDH release

H24-H48

Immune response
Quantification of cytokine concentrations in cellular supernatant (Luminex)

H48



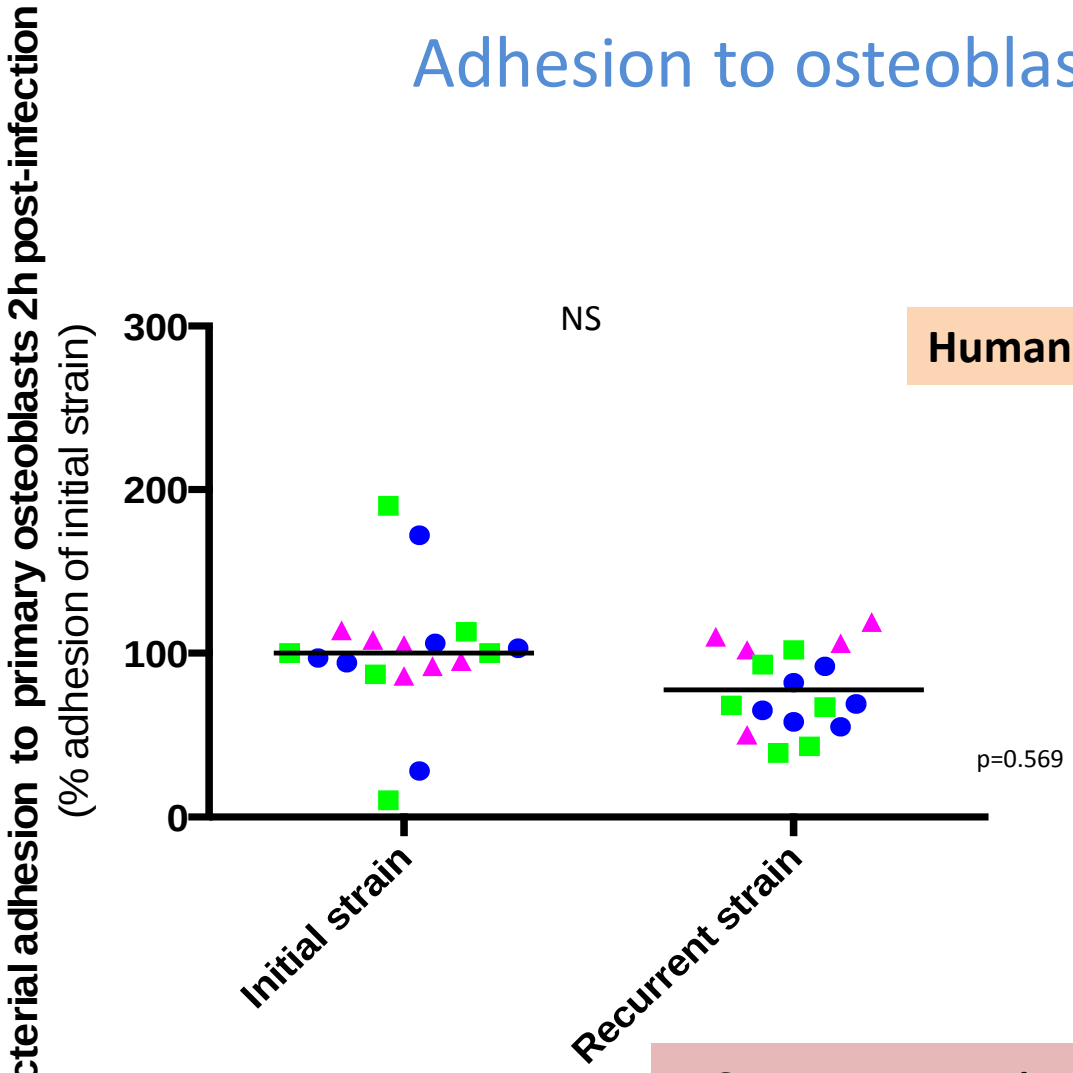
Cellular lysis

After H24-H48-H72

Persistance
Agar plate counting of intracellular bacteria

Osteoblast and *S. aureus*: the switch from acute to chronic infection

Adhesion to osteoblasts



Human primary osteoblasts

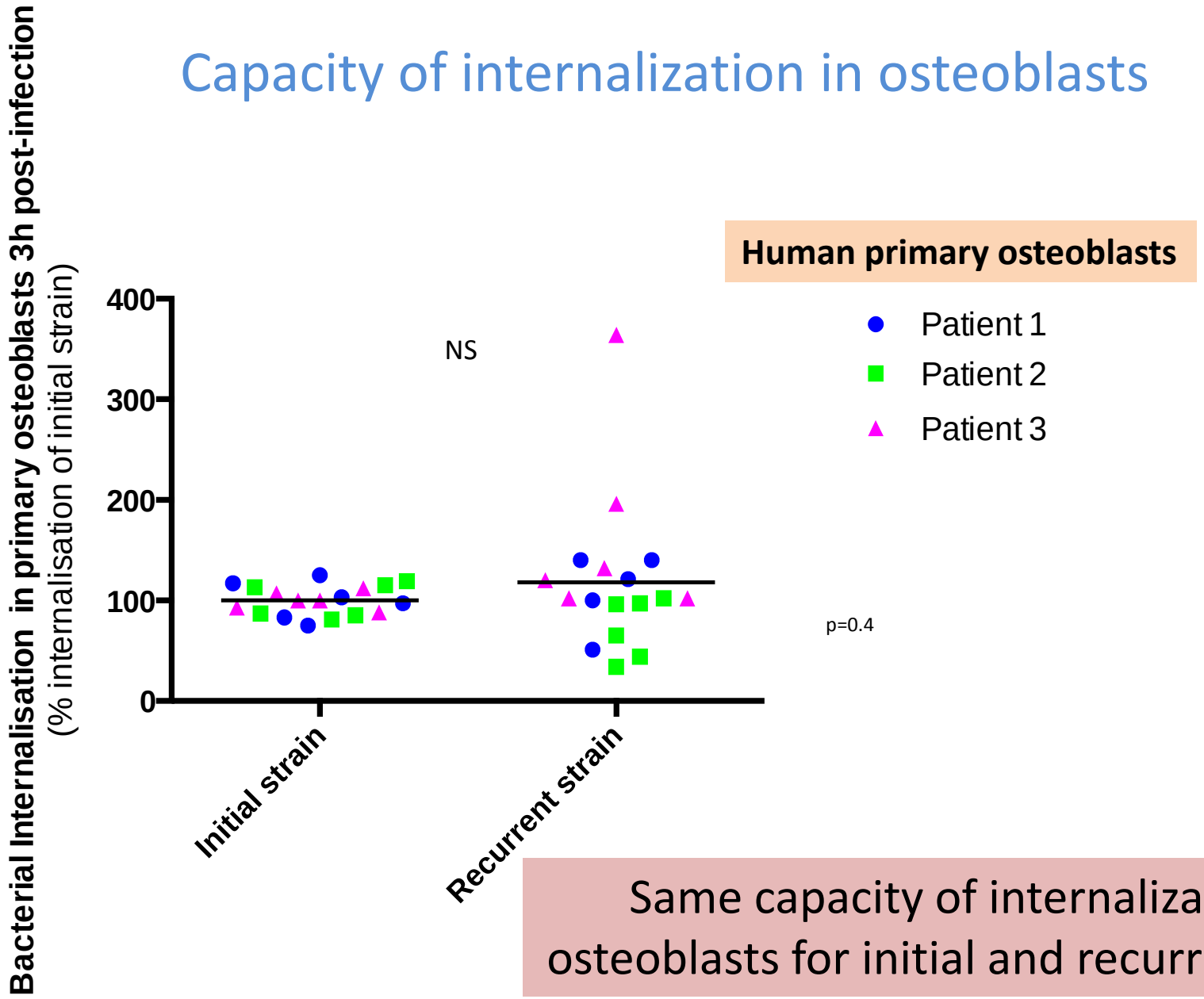
- Patient 1
- Patient 2
- Patient 3

Same capacity of adhesion to osteoblasts for initial and recurrent strains

3 experiments in duplicate. Statistical analysis: Mann- Whitney test (*) confirmed by multivariate analysis controling with patients (Ŧ)

Osteoblast and *S. aureus*: the switch from acute to chronic infection

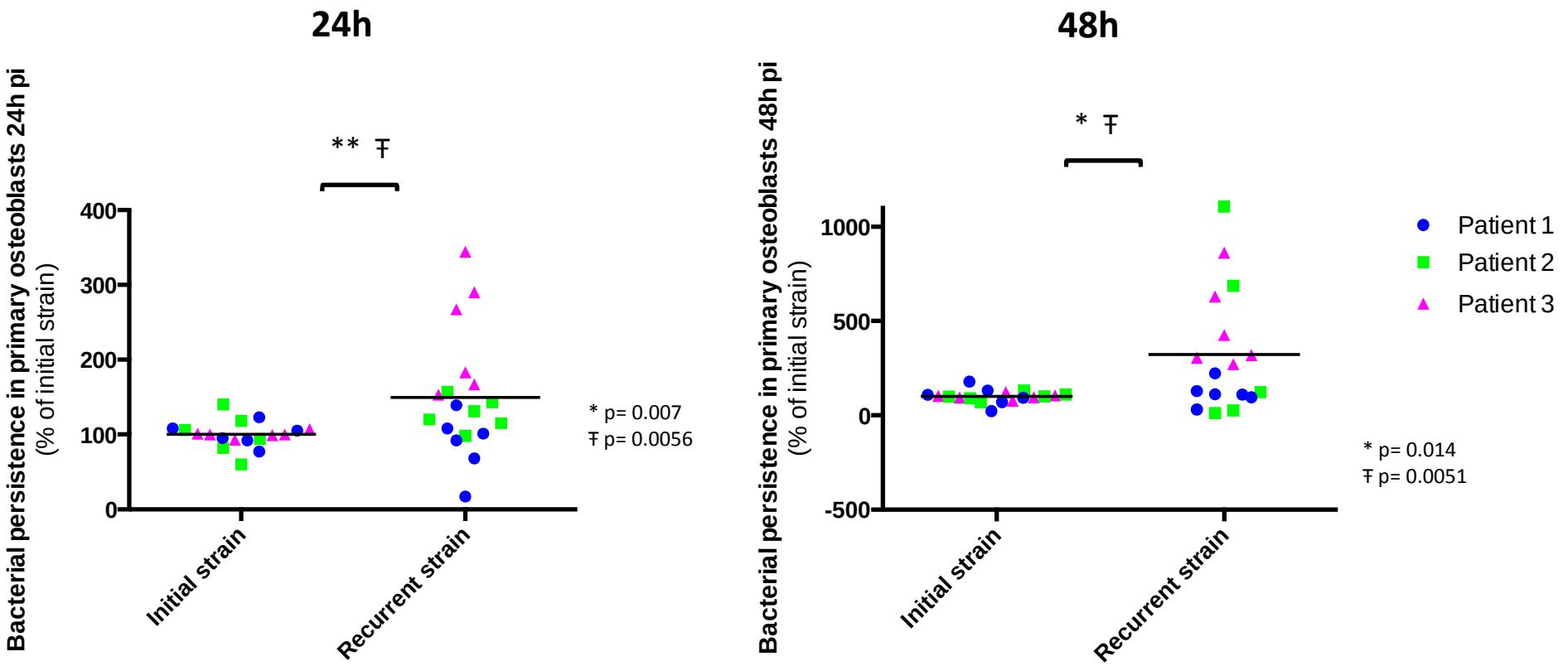
Capacity of internalization in osteoblasts



Same capacity of internalization in osteoblasts for initial and recurrent strains

Osteoblast and *S. aureus*: the switch from acute to chronic infection

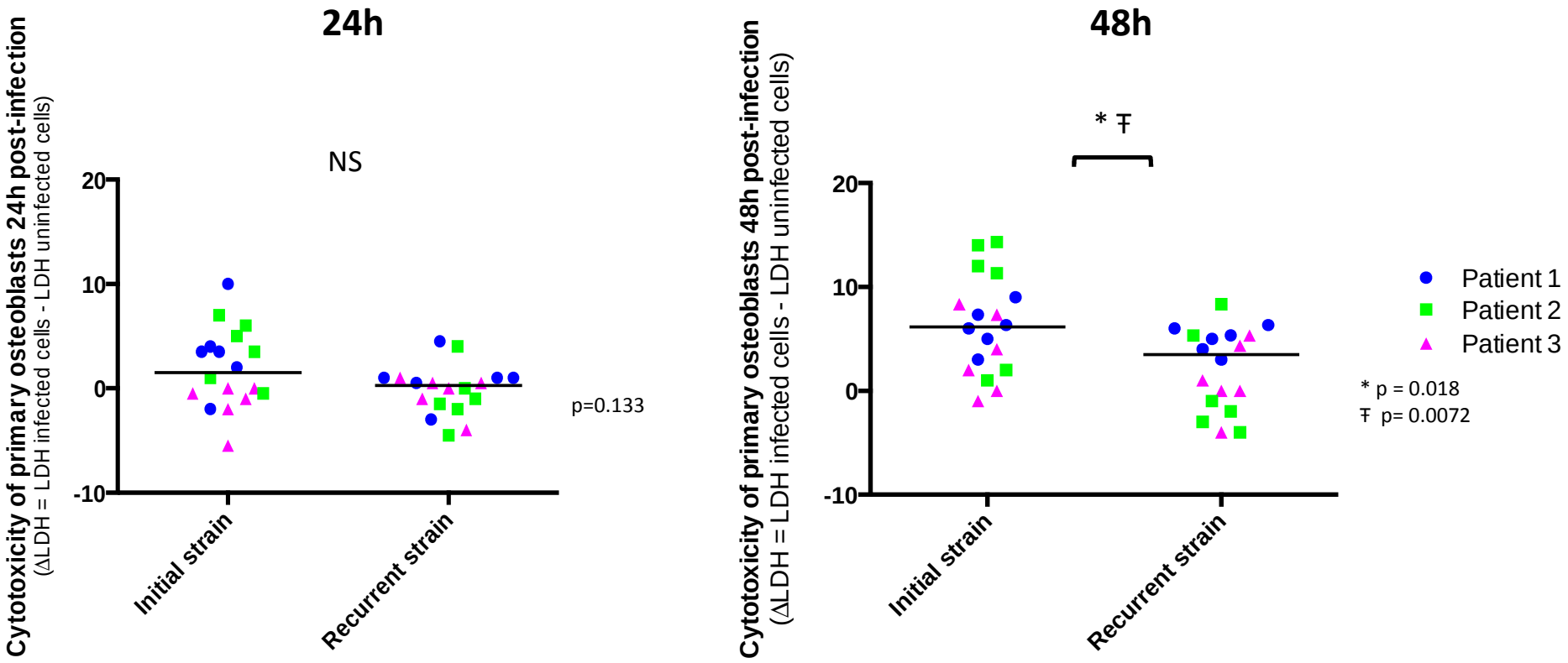
Persistence in human primary osteoblasts



Recurrent strains have a higher capacity of persistence in primary osteoblasts than initial strains at 24h and 48h

Osteoblast and *S. aureus*: the switch from acute to chronic infection

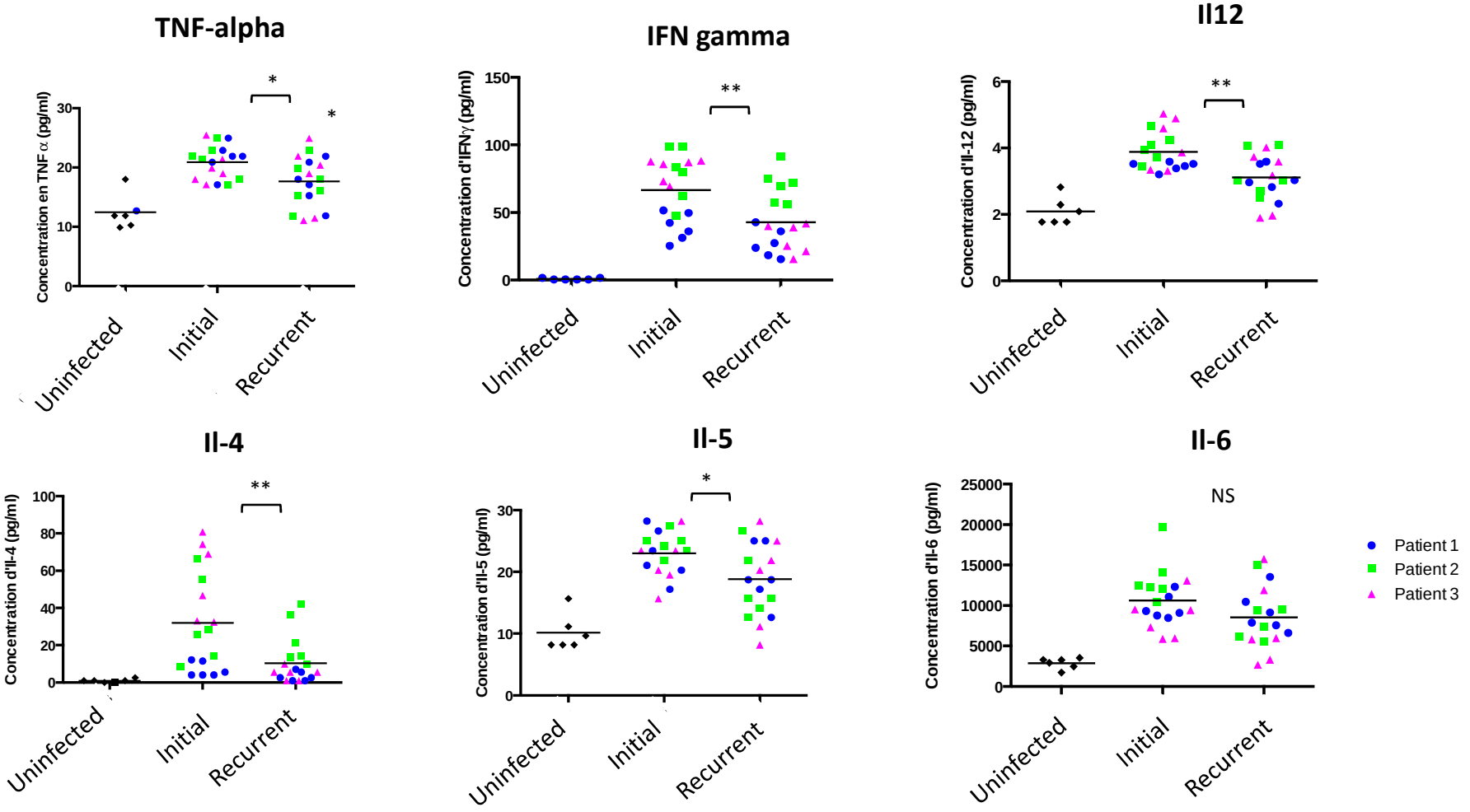
Cytotoxicity: primary human osteoblasts



Recurrent strains are less cytotoxic than initial strains in human primary osteoblasts

Osteoblast and *S. aureus*: the switch from acute to chronic infection

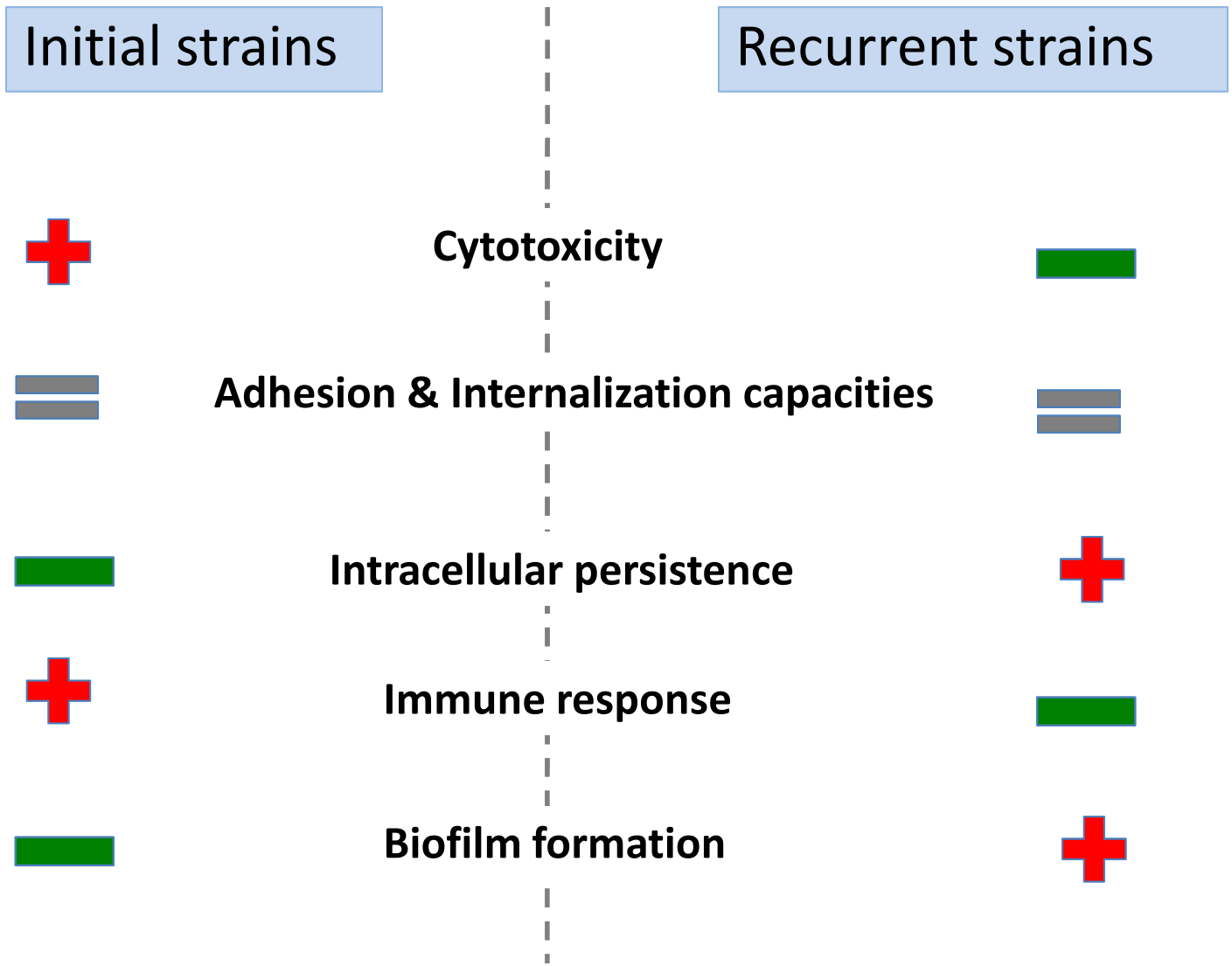
Quantification of cytokines concentrations in cell culture supernatant of human primary osteoblasts 48h post infection



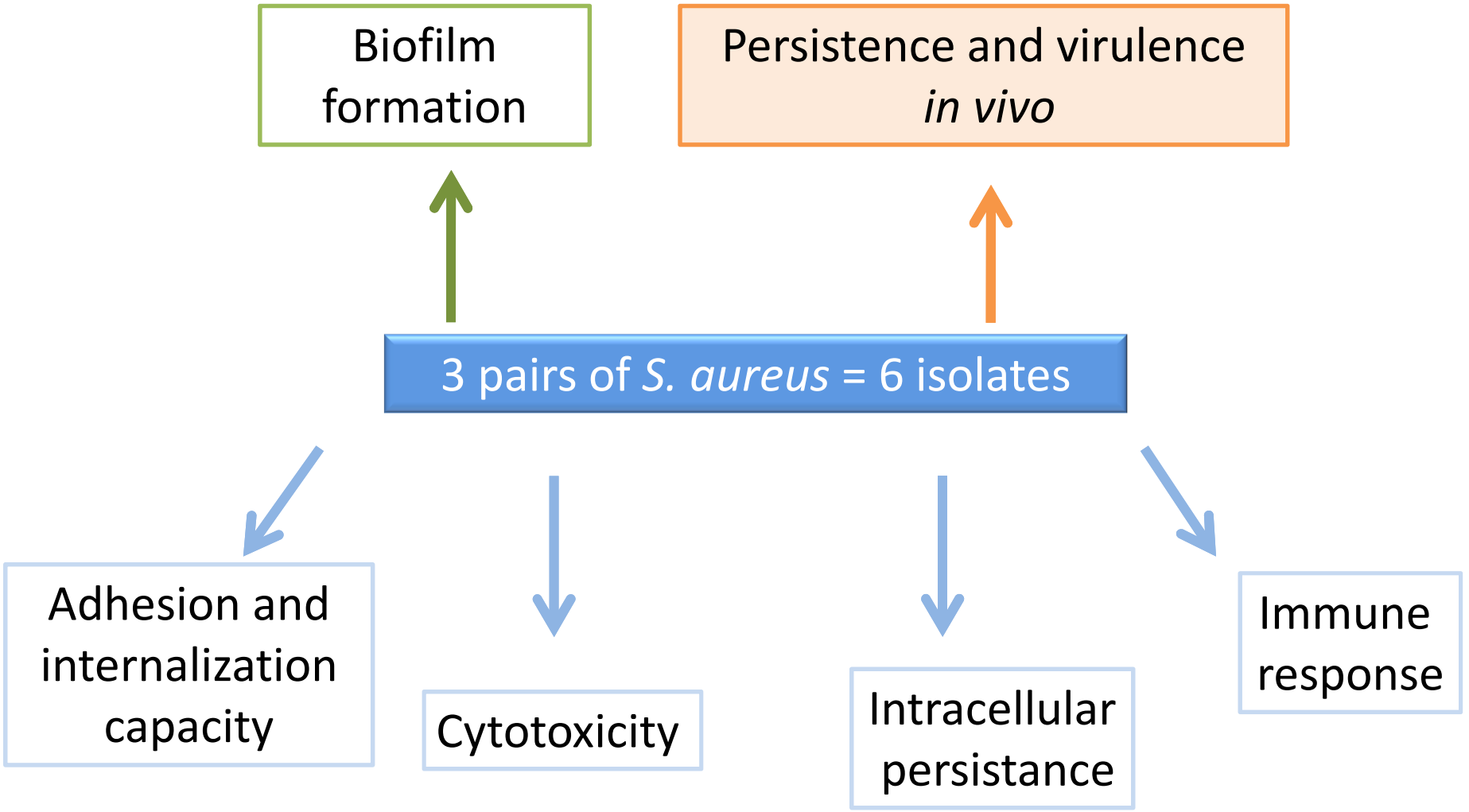
Osteoblasts infected by recurrent isolates secrete **less inflammatory cytokines** that those infected by initial ones

Osteoblast and *S. aureus*: the switch from acute to chronic infection

To sum up



Osteoblast and *S. aureus*: the switch from acute to chronic infection



Osteoblast and *S. aureus*: the switch from acute to chronic infection

In vivo: intra-peritoneal infection model

Evaluation of bacterial dissemination and persistance

48 CD-1 swiss mice

Injection IP
5.10^6 *S. aureus*

24 mice infected with initial isolates

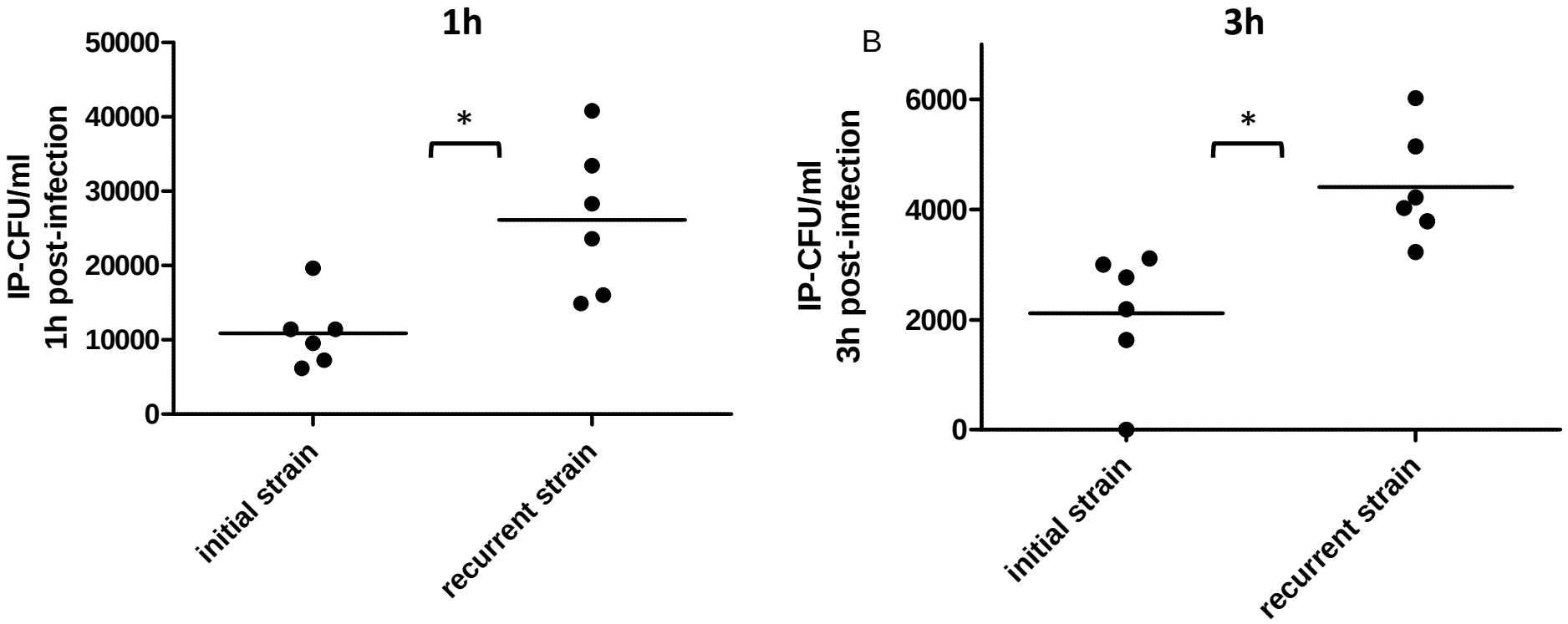
24 mice infected with recurrent isolates

Collection of bacteria by intra-cardiac punction and intra-peritoneal punction
H1, H3, H24 et H48

Agar plate counting

Osteoblast and *S. aureus*: the switch from acute to chronic infection

In vivo: intra-peritoneal infection model



Recurrent isolates **persist longer in peritoneal cavity** than initial ones
-> less recognized by immune cells (because less virulent)

Test de Mann- Whitney

Osteoblast and *S. aureus*: the switch from acute to chronic infection

in vivo: lung infection model

Evaluation of mortality and dissemination

48 CD-1 swiss mice

Nasal Injection
5.10^8 *S. aureus*

24 mice infected with initial isolates

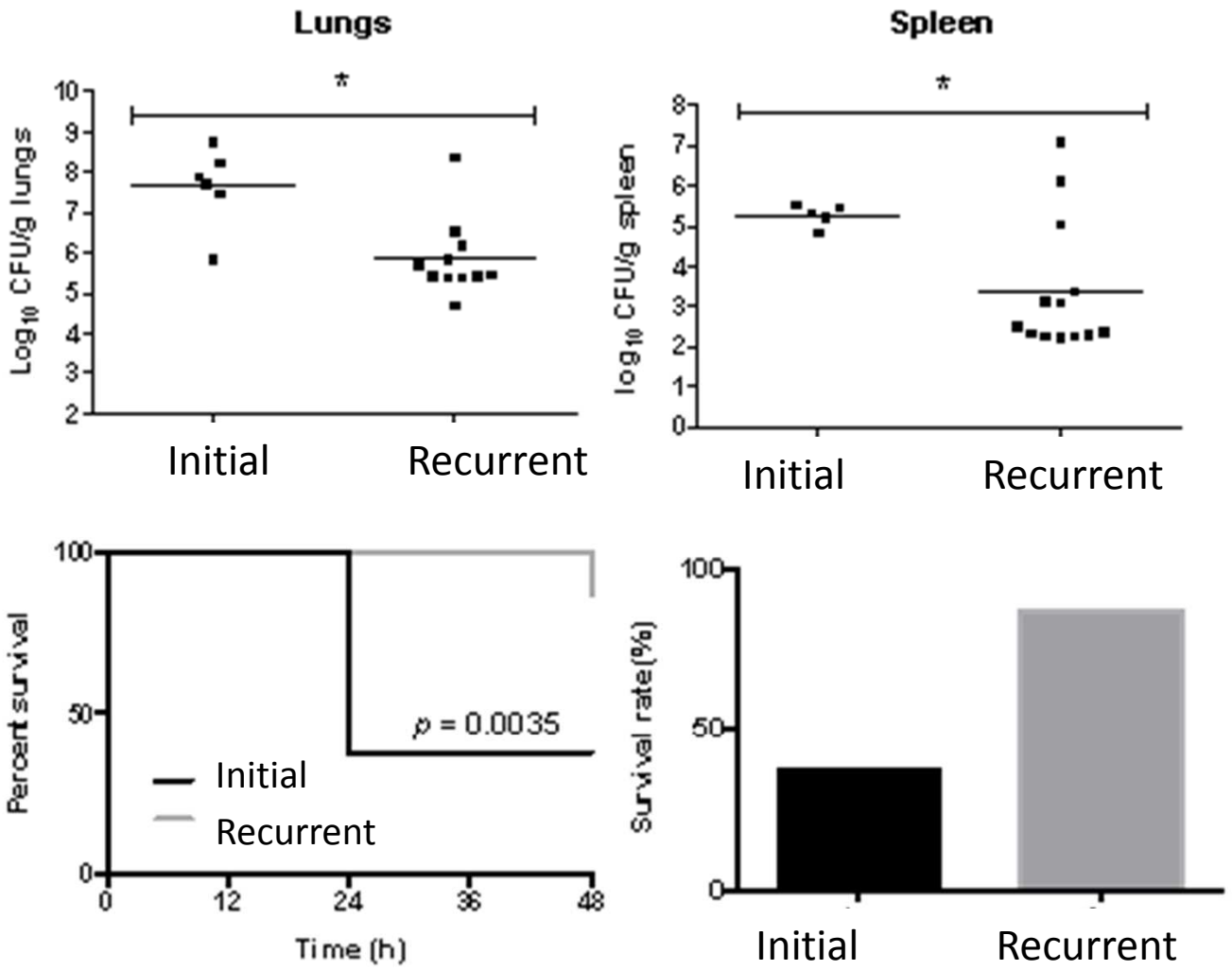
24 mice infected with recurrent isolates

Quantification of bacteria in lung and spleen **H48 post infection**

Agar plate counting

Osteoblast and *S. aureus*: the switch from acute to chronic infection

in vivo: lung infection model



Lower mortality observed with recurrent isolates

Osteoblast and *S. aureus*: the switch from acute to chronic infection

Conclusion

Demonstration of adaptive processes of *Staphylococcus aureus* Isolates during the progression from acute to chronic bone and joint infections in patients

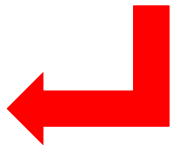
If we understand ”how” and ”what” is happening
on the bacterial side **and** on the cell side
we will be able to adapt/optimize/design drugs and ways
to avoid chronicization

Osteoblast and *S. aureus*: the switch from acute to chronic infection

Genomic data

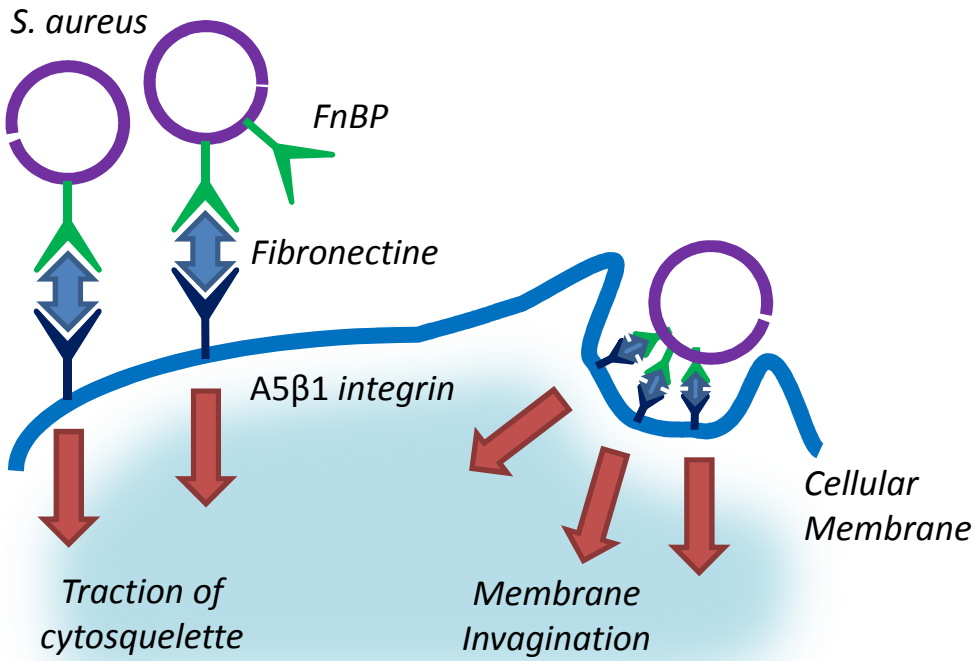
Patient	Isolate	Chromosome size (bp)	Plasmid size (bp)	% GC content chromosome	% GC content plasmid	Overall alignment rate (initial genome covered by recurrent isolate reads)	Total nb of SNPs (recurrent vs initial)	Nb of SNPS in coding regions
1	initial	2726238	20632	32,89	28,38	98.88	5	0
	recurrent	2726193	20633	32,89	28,38			
2	initial	2749852	17307	32,82	28,38	99,07	5	0
	recurrent	2749888	17307	32,82	28,38			
3	initial	2679642	20720	32,86	28,37	99,14	4	3
	recurrent	2678500	no plasmid	32,86	-			

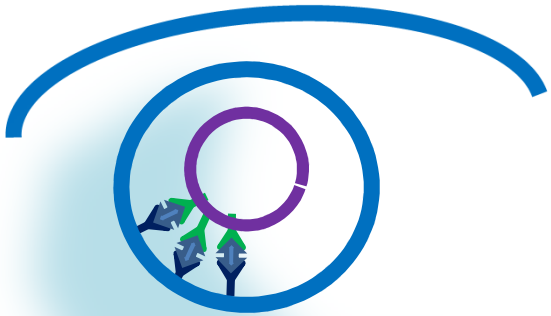
No genetic convergenceentr when comapring SNP in the three pairs
No gene with specific function involved

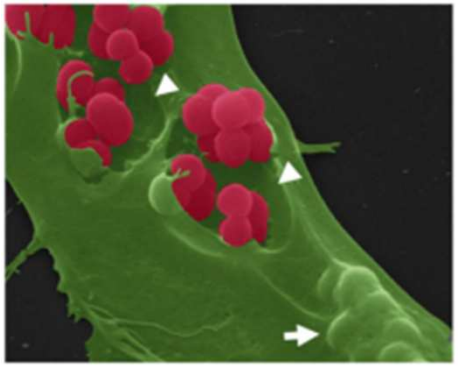


If it is not DNA ….. Could it be RNA ? RNAseq underway …
If its not RNA … Could it be epigenetic modifications ? …

Osteoblast invasion by *S. aureus*







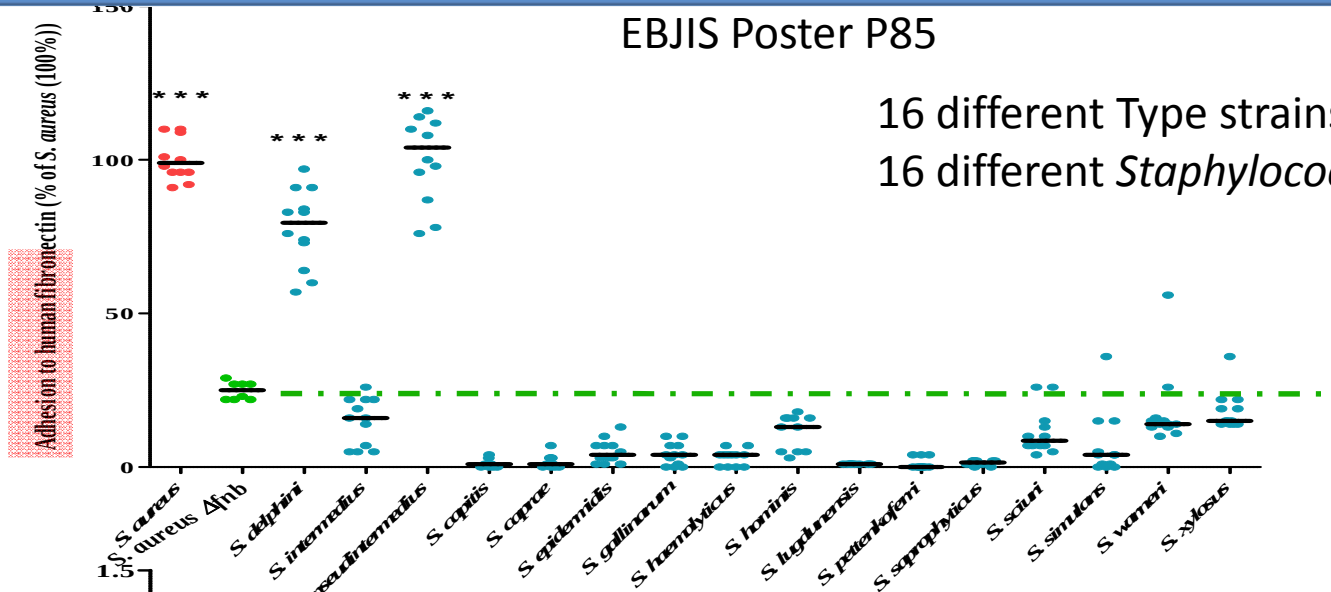
Electronic microscopy
Hoffmann *et al.*, Eur J Cell Biol 2011

Invasion of host cells is thought to result in a **bacterial sanctuary**

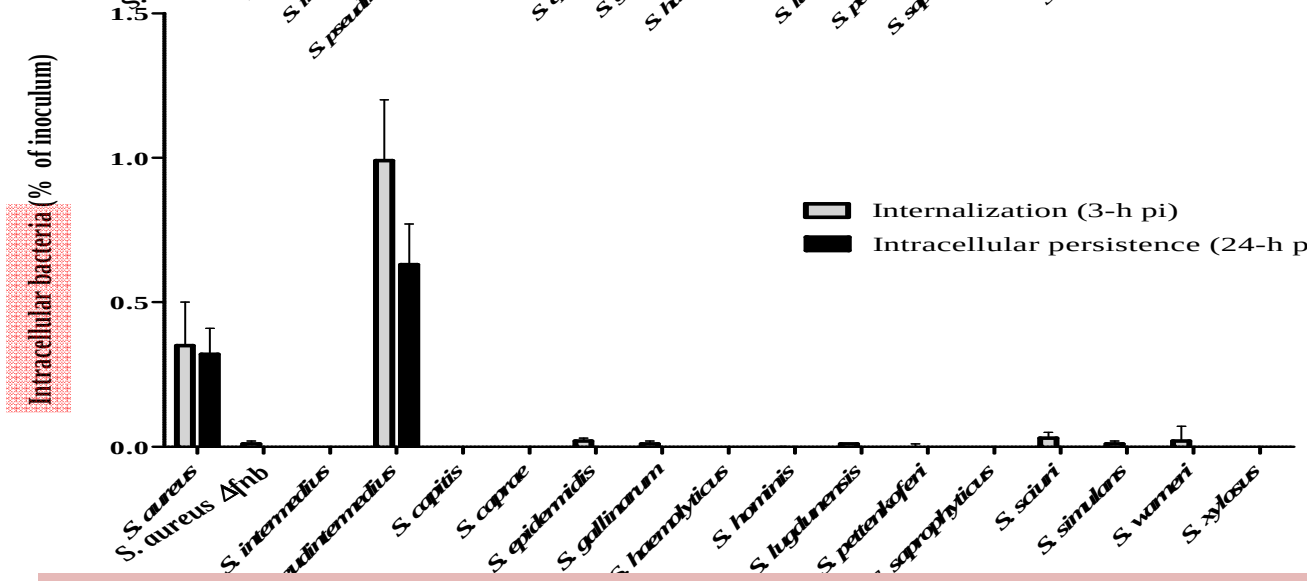
Osteoblast invasion by *S.* non-*aureus*

EBJIS Poster P85

16 different Type strains belonging to
16 different *Staphylococcus* non-*aureus* species



Adhesion

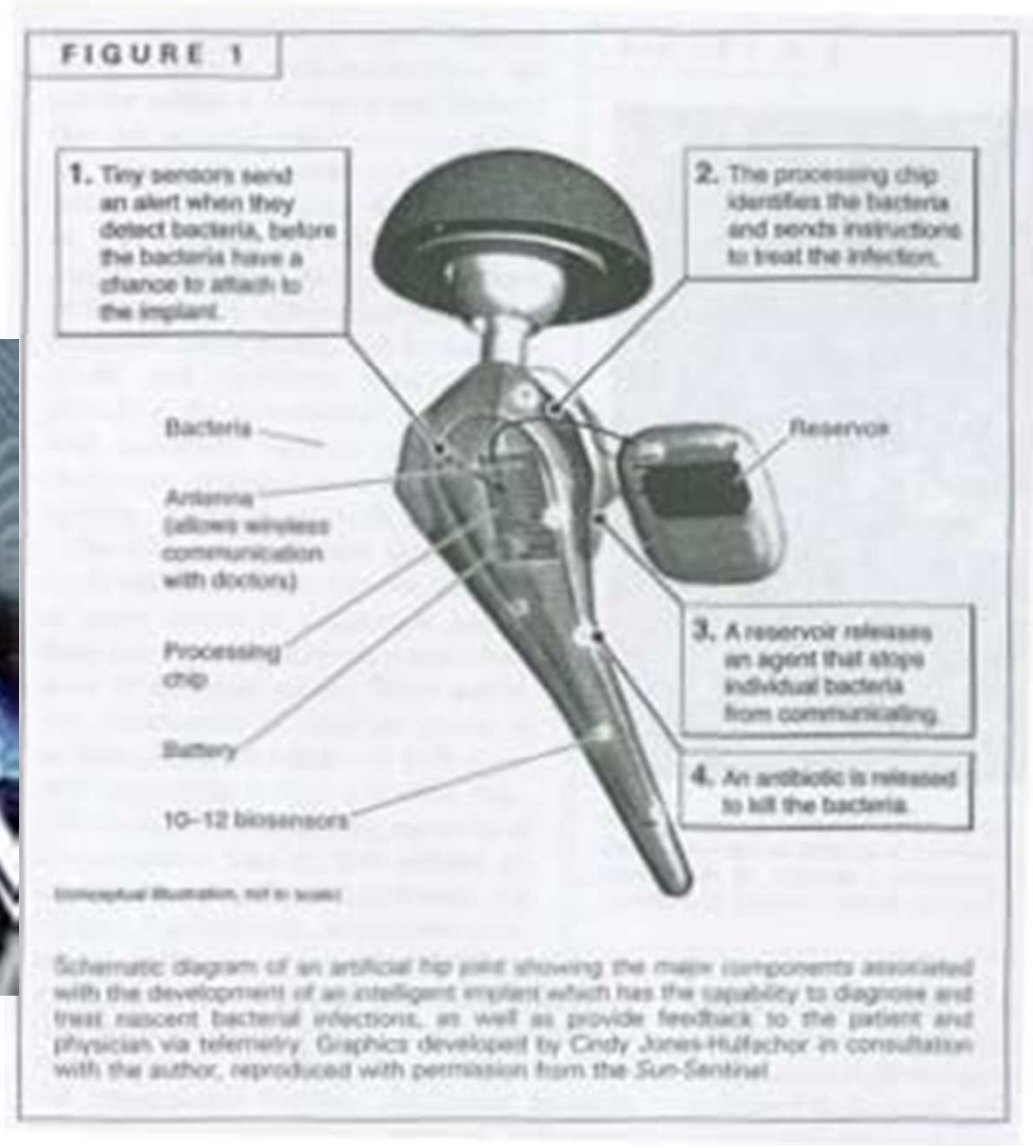


Intracellular bacteria

Specific behaviour confirmed on ap anel of coagulase negative clinical isolates :
Internalization is not a main virulence mechanisms in coagulase negative staphylococci except *S. pseudintermedius*

It is crucial to investigate physiopathology of BJI …

it is and it will be a hot topic of interest in the future



It is crucial to investigate pathophysiology of BJI …

it is and it will be a hot topic of interest in the future

2016

It is crucial to investigate pathophysiology of BJI …

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2016

2036

+ 2 hip prosthesis

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toutes les techniciennes du Nord !!!