237 **The proportions of dormant bacteria within *Staphylococcus epidermidis* biofilms affect their inflammatory potential**

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Staphylococcus epidermidis is an opportunistic pathogen due to its ability to establish biofilms on indwelling medical devices. The presence of high amounts of dormant bacteria is a hallmark of biofilms, making them more tolerant to antimicrobials and elusive to the host immune response1. By flow cytometric evaluation using the SYBR Green (SYBR)/Propidium Iodide (PI) (live/dead) staining2, we observed that S. epidermidis biofilms grown in excess glucose presented higher amounts of viable (SYBR+PI-) but non-culturable (VBNC) bacteria, as compared to cells grown in glucose non-supplemented medium (1.45 ± 0.6 × 108 vs. 13.4 ± 1.2 × 108 CFUs,

starting from the same number of viable cells). This effect, which was associated with medium acidification due to glucose metabolism, was counteracted by adding high extracellular levels of magnesium chloride to the culture medium. This allowed the modulation of the proportions of VBNC bacteria within S. epidermidis biofilms. The immunological stimulatory effect of bacteria obtained from biofilms with either high (highVBNC) or low (lowVBNC) proportions of VBNC cells was evaluated. The highVBNC inoculum induced a lower (ten-fold) in vitro production of the pro-inflammatory cytokines TNF-α, interleukin-1 and interleukin-6 by murine macrophages. In vivo, lower proportions of inflammatory (F4/80+Gr-1+) macrophages were observed in peritoneal exudates of highVBNC compared to lowVNBC-infected mice (22.8 ± 4.1% vs. 42.4 ± 5.6%)3. Interestingly, a higher number of bacterial CFU could be recovered from the liver of the highVBNC challenged mice (manuscript in preparation). Overall, these results show that environmental conditions, such as pH and extracellular levels of magnesium, can account to induce dormancy in S. epidermidis biofilms. Moreover, the lower inflammatory potential of bacterial suspensions enriched in VBNC bacteria may explain the higher liver colonization observed, suggesting that dormancy can contribute to the immune evasion of biofilms.

References: 1. Otto M (2009) *Staphylococcus epidermidis* - the _'accidental_' pathogen. Nat Rev Microbiol; 7: 555-67. 2. Cerca F (2011) SYBR green as a fluorescent probe to evaluate the biofilm physiological state of *Staphylococcus epidermidis*, using flow cytometry. Can J Microbiol; 57:850-6. 3. Cerca F (2011) *Staphylococcus epidermidis* biofilms with higher proportions of dormant bacteria induce a lower activation of murine macrophages. J Med Microbiol; 60:1717-24.

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