PS9: 20

Staphylococcus epidermidis biofilms with higher proportions of dormant bacteria induce a lower activation of murine macrophages

Filipe Cerca[1], Filipa Andrade[1], Ângela França[2], Elva Bonifácio[1], Adília Ribeiro[1], Agostinho Almeida[3], Nuno Cerca[2], Gerald Pier[4], Joana Azeredo[2], Manuel Vilanova[1]

[1] ICBAS, University of Porto, Portugal, [2] CEB-IBB, University of Minho, Portugal, [3] Farmacy School, University of Porto, Portugal, [4] Harvard Medical School, Boston, USA,

Staphylococcus epidermidis 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 to the host immune response. We observed that *S. epidermidis* biofilms grown in excess glucose accumulated high amounts of viable but non-culturable (VBNC) bacteria, as assessed by their low ratio of culturable bacteria over the number of viable bacteria. This effect, which was a consequence of the accumulation of acidic compounds due to glucose metabolism, was counteracted by high extracellular levels of calcium and magnesium added to the culture medium allowing modulation of the proportions of VBNC bacteria within *S. epidermidis* biofilms. Using bacterial inocula obtained from biofilms with high and low proportions of VBNC bacteria, their stimulatory effect on murine macrophages was evaluated *in vitro* and *in vivo.* The inoculum enriched in VBNC bacteria induced *in vitro* a lower production of TNF-α, interleukin-1 and interleukin-6 by bone-marrow-derived murine macrophages and, *in vivo,* a lower stimulatory effect on peritoneal macrophages, assessed by increased surface expression of Gr1 and MHC class II molecules. Overall, these results show that environmental conditions, such as pH and extracellular levels of calcium and magnesium, can account to induce dormancy in *S. epidermidis* biofilms. Moreover, they show that bacterial suspensions enriched in dormant cells are less inflammatory suggesting that dormancy can contribute to the immune evasion of biofilms.

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) PTDC/BIA-MIC/113450/2009 and FEDER FCOMP-01-0124-FEDER-014679. FC, AF and EBA were respectively supported by FCT fellowships SFRH/BD/27638/ 2006, SFRH/BD/62359/2009 and SFRH/BD/38380/2007