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RESEARCH TOPIC CLI14

Antibiotic resistance and Staphylococci periprosthetic joint infections: deciphering the interaction between bacterial quorum sensing and immune response

Research area
Surgical area

Clinical Unit name
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Abstract

Background

The looming crisis of increasing antibiotic resistance among microorganisms poses a grave challenge, especially in the realm of modern medical devices. This concern is especially pronounced for implants used in medical procedures. The culprits often responsible for complications are biofilms – dense bacterial colonies adhering to surfaces, frequently interspersed with an intricate self-produced matrix of extracellular polymeric substances. Moreover, these biofilms, along with the host's immune response, can lead to both septic and aseptic failures of the implant. Despite the long-standing effort aimed at controlling biofilm-associated periprosthetic joint infections (PJIs), the crosstalk between the host immune system and the biofilm in this context remains largely uncharted.

The incidence of PJIs is ~2% of all procedures performed (~150.000/year in Italy) and it is projected to rise due to the increasing frequency of arthroplasties [1]. Of particular concern are infections caused by Staphylococcus species, notably S. aureus (comprising 31–43% of cases) and coagulase-negative (CS) staphylococci like S. epidermidis (20–31%) [2]. The pathophysiology of PJIs is inextricably linked with biofilms, which often show high levels of antibiotic resistance. In the biofilm maturation phase, bacteria undergo density-dependent changes once a critical threshold is met (quorum sensing - QS) [3]. The reduced effectiveness of antibiotics and the ability to skew the host immune response toward an anti-inflammatory, pro-fibrotic state, contributes to the chronicity and recurrence of biofilm-related infections [4].

One of the most important regulators of biofilm development and disassembly in Staphylococcus species is the accessory gene regulator (agr) QS system. The expression of the agr locus - which is low during the initiation phase of biofilm development and high in a mature biofilm - activates the histidine kinase AgrC leading to the production of proteases, toxins, and phenol-soluble modulins (PSMs) that can act against host defences [5]; it has been also associated with the skewing of macrophage response towards an anti-inflammatory state [6]. Finally, immature monocytes and granulocytes called myeloid-derived suppressor cells (MDSCs) have recently been shown to play an important role in promoting S. aureus orthopedic biofilm infections [7]. Our preliminary data on synovial fluid samples form hip and knee joints underwent septic or aseptic joint replacement revision surgery confirmed that among the leukocytes subsets, there is a significant difference in circulating monocytes abundance between acute and chronic PJIs. The differences in leukocyte profiles were further supported by the presence of pro- and anti-inflammatory cytokines in the synovial fluid. Both septic and aseptic revisions had higher levels of cytokine release compared to that of synovial fluids obtained during primary replacement surgeries. Specifically, IL-6, which is commonly used as a marker of infection, showed significantly higher levels in septic samples, as expected. However, our analysis also revealed that CXCL10 and IL-10 exhibited a similar trend. [8].

Hypothesis and objectives

S. aureus is a high-virulence pathogen often associated with acute PJIs, whereas CS staphylococci are low-virulence organisms usually associated with chronic PJIs [9]. In this respect, the role played by the agr QS system in acute versus chronic PJIs has not been established. The central hypothesis of this proposal is that agr regulation is associated with the antibiotic resistance and the ability to skew the immune system into an immunosuppressive environment. Therefore, the main goal is to identify the effect of the agr-mediated biofilm formation and gene expression in the modulation of the antibiotic resistance and the host immune response in acute and chronic PJIs. We will dissect and characterize the interaction between the immune system and the bacteria biofilm, to contrast the increase of antibiotic resistance. Shedding light on these processes will provide novel diagnostic and therapeutic strategies to prevent and control orthopedic implant-associated infections.

Research design, specific aims and methods

AIM1: Identifying cell infiltrates, inflammatory mediators and agr expression in acute and chronic PJIs associated with Staphylococcus species.

We will collect samples from 100 patients affected by hip or knee acute and chronic PJIs associated with Staphylococcus species according to the periprosthetic fluid and/or soft tissue positive cultures (plus 100 aseptic revisions as controls). Samples of synovial and sonication fluid will be used to quantify RNAIII (the effector molecule of agr [10]) expression levels by real-time qPCR (Task 1). The same samples will be also analysed through FACS to identify differences in the distribution of the immune subtypes and their activation status (MDSCs, neutrophils, macrophages, CD4 and CD8 cells). Both inflammatory (TNFα, INFγ, CXCL10, IL1b, IL 6) and anti-inflammatory (TGFβ and IL10) mediators will be screened to identify biomarkers differentially expressed in S. aureus and CS staphylococci PJIs. Finally, single-cell RNA sequencing will be used to map the immune composition and to obtain a higher resolution identification of the immune cell subsets (Task 2).

AIM2: Identifying the whole genome of Staphylococcus species isolated in acute and chronic PJIs.

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Shotgun metagenomic DNA libraries from the collected synovial and sonication fluids will be sequenced on the Illumina platform available in the Genomic Facility of the Humanitas Research Hospital. By using this experimental approach and thanks to the available sequencing platforms at ICHthe simultaneous sequencing of up to 10 different libraries in the same RUN will be possible. For each sample sequencing libraries will be prepared with the Nextera XT Illumina kit in order to start with very low amount of input DNA and to improve the reads assembly step. De novo assembly will be performed by using MetaVelvet (Nam12) that deal explicitly with the non-clonality of natural populations, the microbial strain-level population structure will be derived by applying StrainPhlAn (Tru17), a novel metagenomic strain identification approach that relies on per-sample dominant sequence variant reconstruction within species-specific marker genes. The presence of gene clusters for antimicrobial resistances, in the metagenomic datasets will then be quantified by alignment to existing microbial reference gene catalogues (Task 3).

AIM3: Developing an in-vitro platform to investigate the agr QS system in biofilm formation, immune response and antibiotic susceptibility.
We will take advantage of a newly developed platform [8] to assess biofilm development by Staphylococcus species and immune response, mimicking the environment of prosthetic implants. Biofilm formation will be monitored over time using epi-fluorescence microscopy and samples will be collected for downstream analysis (e.g., qPCR). We will use wild-type and agr knockout strains of S. aureus and S. epidermidis [10], mutants carrying a visual reporter of agr activation and mutants in which the activation of agr is controlled by an exogenous signal (such as IPTG, [11]) (Task 1). This platform will also allow to co-culture bacteria and immune cells (same subtypes of AIM1) and evaluate their crosstalk with Staphylococcus biofilms through in-situ imaging and ELLA detection of inflammatory (TNFα, INFγ, CXCL10, IL1b, IL 6) and anti-inflammatory (TGFβ and IL10) mediators (Task 2). Moreover, the resistance to different antibiotics will be evaluated, as the response of bacteria within a biofilm can be very different compared to planktonic cells. The antibiotic efficacy will be assessed both in the absence and in the presence of selected immune cell subsets identified in AIM 1 and 2 (Task 4).

Scientific references

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Type of contract

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