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QUESTION 3: Is the biofilm on orthopaedic implant surfaces permeable to neutrophils and macrophages in vivo? Are these innate immune cells (meaning any macrophages or neutrophils) capable of engulfing and killing bacteria?

RESPONSE: A mature bacterial biofilm has limited permeability to neutrophils and macrophages. Those that get through are clinically ineffective at eradicating biofilm bacteria. While neutrophils and macrophages are capable of engulfing and killing planktonic bacteria, they are not innately capable of effectively engulfing and killing sessile bacteria in biofilm.

LEVEL OF EVIDENCE: Strong

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

PRE-MEETING RATIONALE

The most important pathogenic mechanism involved in implant-related infections is the ability of the microorganisms to form a biofilm [1], which leads to protection against environmental stress, host immune defense and antimicrobials [2]. The first cells arriving at the infection site are the neutrophils and macrophages [3]. The permeability and the phagocytosis ability of these immune cells have mainly been evaluated in two types of infection: cystic fibrosis [4–8] and device related infection, mainly catheter-related infection [9–17] and periprosthetic infection [18].

Neutrophils are innate immune cells capable of secreting an arsenal of toxic oxygen species, degrading enzymes, defensins and lipid inflammatory mediators to fight off infection [6]. These cells have shown the ability of sticking but not penetrating into a mature biofilm and phagocytizing biofilm encased microorganisms [4–8,10,11,14,19–23]. The exopolymeric substances of the biofilm matrix seem to be involved in the formation of neutrophil extracellular traps in biofilm of *Streptococcus suis* [21], *Candida albicans* [10] and *Candida glabrata* [11]. Data shows that neutrophils can destroy a two to six day old *Staphylococcus aureus* (*S. aureus*) biofilm, but a mature biofilm is capable of resisting penetration by these cells [24].

Guenther et al. studied the different behavior of polymorphonuclear neutrophils (PMNs) towards the biofilm formed by either *S. aureus* or *Staphylococcus epidermidis* (*S. epidermidis*). In the case of biofilm formed by *S. aureus*, the PMNs were observed to move across and scavenge bacteria along their path. Conversely, PMNs in contact with *S. epidermidis* biofilm were nearly immobile and phagocytized only bacteria in close proximity. Why biofilms of *S. aureus* appear more sensitive to a PMN attack compared to those produced by *S. epidermidis* is not well understood [19]. Insights on the behavior of biofilm formed by *S. epidermidis* have been offered by the in vitro and in vivo studies of Kristian et al. These authors found that *S. epidermidis* biofilms triggered higher levels of complement activation in terms of C3a formation than planktonic wild-type bacteria and isogenic ica-negative bacteria. On the other hand, a decreased deposition of immunoglobulin G (IgG) and C3b was observed in biofilm-embedded bacteria. This could possibly explain the evasion of PMNs killing [25].

Alhede et al. evaluated the role of immune system against biofilm formed by *Pseudomonas aeruginosa*. They demonstrated that both in vitro and in vivo biofilms of *Pseudomonas aeruginosa* produce

a shield of excreted rhamnolipids, which offers protection from the bactericidal activity of PMNs [26].

Arciola et al. did an extensive study of biofilm formed by Staphylococcus on an implant surface. Based on their work, PMNs were found to surround biofilm and become activated, but PMNs were not able to migrate into the biofilm, probably because of a lack of a chemotactic signal as well as by hindrance of migration into the "slimy" material. Thus, the inability of PMNs to penetrate biofilm results in progression of implant related infections. The activation of PMNs and their attempt to kill bacteria results in secretion of numerous cytotoxic and proteolytic enzymes that cannot act against bacteria but results in damaging and destroying the surrounding host tissues [27].

Macrophages become the prevailing cells and remain at the infection site a high concentration for several weeks and they are related to recognition, phagocytosis, secretion of enzymes, cytokines, chemokines and growth factors, to destroy and digest the phagocytized pathogens [3]. These cells can penetrate into a mature biofilm in a similar way as neutrophils, and phagocytize biofilm encased microorganisms, but not destroying them [9,12,13,18]. Moreover, these sessile phagocytized bacteria can even persist into peri-implant tissue inside macrophagic cells not only in experimental models, but also in the tissues of patients with intravenous catheters colonized by different bacteria [16,17]. *S. aureus* prosthetic infection in vivo model showed that limited bacterial macrophage uptake is due to inflammatory attenuation by *S. aureus* biofilm [13], which favor the transformation from M1 macrophages presents a high antimicrobial activity to M2 type inherently possesses less antimicrobial activity [13], and the cell death induction though leukocidin A/B [28] and human leukocyte antigen production [18]. At the site of staphylococcus biofilm infection, macrophages exhibit: down-regulation of interleukin (IL)-1β, tumor necrosis factor, CXCL2 and CCL2 expression, reduced bacterial uptake, minimal iNOS expression and consequent low efficiency in killing phagocytized bacteria and reduced induction of lymphocyte production of interferon-γ. These scavenging cells appear able to migrate into the biofilm but cannot clear the site from the pathogen causing the infection as their bactericidal activity appears compromised [27].

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