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REVIEW ARTICLE

Interactions between the foreign body reaction and *Staphylococcus aureus* biomaterial-associated infection. Winning strategies in the derby on biomaterial implant surfaces

Colin W. K. Rosman[a], Jan Maarten van Dijl[b] [ID] and Jelmer Sjollema[a] [ID]

[a]Department of Biomedical Engineering, University of Groningen, University Medical Center Groningen, Groningen, Netherlands; [b]Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, Netherlands

ABSTRACT

Biomaterial-associated infections (BAIs) are an increasing problem where antibiotic therapies are often ineffective. The design of novel strategies to prevent or combat infection requires a better understanding of how an implanted foreign body prevents the immune system from eradicating surface-colonizing pathogens. The objective of this review is to chart factors resulting in sub-optimal clearance of *Staphylococcus aureus* bacteria involved in BAIs. To this end, we first describe three categories of bacterial mechanisms to counter the host immune system around foreign bodies: direct interaction with host cells, modulation of intercellular communication, and evasion of the immune system. These mechanisms take place in a time frame that differentiates sterile foreign body reactions, BAIs, and soft tissue infections. In addition, we identify experimental interventions in *S. aureus* BAI that may impact infectious mechanisms. Most experimental treatments modulate the host response to infection or alter the course of BAI through implant surface modulation. In conclusion, the first week after implantation and infection is crucial for the establishment of an *S. aureus* biofilm that resists the local immune reaction and antibiotic treatment. Although established and chronic *S. aureus* BAI is still treatable and manageable, the focus of interventions should lie on this first period.

1. Introduction

As the global human population continuously expands and reaches higher ages, steadily increasing numbers of biomaterial implants are being utilized to treat age-related diseases, such as osteoarthritis and cardiac disease, and to sustain the quality of life (Voigt et al. 2006; Kurtz et al. 2007). The number of individuals who carry some sort of man-made material inside their body are further increased by cosmetic and temporary implants (e.g. catheters) (Lalani 2018). All of these foreign materials activate the human immune system, starting the so-called foreign body reaction (FBR), which elicits a cascade of immunological and cytological processes. Simultaneously, the implanted materials provide a foothold to pathogenic bacteria that colonize the respective surfaces to form hard-to-eradicate biofilms and lead to biomaterial-associated infections (BAIs), which can cause major tissue damage and loss of implant function (Wagner and Hansch 2017; Josse et al. 2019). For

example, the current infection rate of total knee and hip arthroplasties in the US ranges from 2 to 2.4%, with a recurrence of infection after treatment in 15% of the patients (Garvin and Konigsberg 2011). Due to the growing number of biomaterial implants, combined with the increased prevalence of age- and welfare-related diseases (e.g. diabetes and obesity), which impede the human body's defense systems, the incidence of BAI is steadily increasing as well (Voigt et al. 2006; Greenspon et al. 2011; O'Toole et al. 2016). Thus, the annual costs of treating periprosthetic joint infections (i.e. the most common type of prosthesis infections) in the US were estimated to amount to $566 million in 2009, and they are projected to exceed $1.62 billion by 2020 (Kurtz et al. 2012). A major contributor to BAI is the Gram-positive bacterium *Staphylococcus aureus*, on which we will focus in this review (Arciola et al. 2005; Polyzos et al. 2015). Ordinarily, *S. aureus* presents itself as a harmless commensal, but it is also

CONTACT Jelmer Sjollema ✉ j.sjollema@umcg.nl 🖅 Department of Biomedical Engineering, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, 9713 AV, Groningen, Netherlands

known for its tenacity in BAI due to the ability to form biofilms, the rapid acquisition of resistances to antibiotics, resilience to native immune system functions, and adaptations that allow it to effectively colonize bone tissue (Savage et al. 2013; Lindsay 2014; O'Gara 2017; Muthukrishnan et al. 2019; de Vor et al. 2020).

In everyday clinical practice, precautions are taken to prevent BAI, including antibiotic prophylaxis, the use of drug-releasing bone cement and coatings, and perioperative air filtration (Jones et al. 2016; Harnoss et al. 2017; Oliveira et al. 2017). However, this does not entirely eliminate the risk of infection. Once a serious BAI does occur, the standard treatment is the removal of the infected implant and treatment with a high dosage of antibiotics. Implant removal is often necessary because, where soft tissue infections can be treated relatively effectively with antibiotics, BAIs are much harder to treat due to the drug-impermeable nature of the biofilm and the nutritional depletion deeper in the biofilm. The latter keeps the metabolic activity of bacteria in a biofilm low, which renders them poorly susceptible to antibiotics. Only after confirmation of the causative pathogen's eradication a new device can be implanted, though with an increased risk of secondary infection due to S. aureus persisting in the surrounding tissue (Conlon 2014). This type of treatment is further complicated by multi-drug resistant S. aureus strains that circulate in hospitals and the community. Unfortunately, the emergence of antibiotic-resistant infections is projected to increase more rapidly in the coming years due to inappropriate administration and usage of antibiotics, and due to the selection of microorganisms that have acquired drug resistance following mutation and horizontal gene transfer (Chambers and Deleo 2009; Lindsay 2014).

To prevent the increasing use of antibiotics new strategies are needed to prevent BAIs. The design of such strategies requires not only a deeper understanding of the pathogenesis of BAI but also the biofilm mode of bacterial growth and the FBR itself. This view is supported by observations that the presence of an implanted foreign body prevents the immune system from properly eradicating pathogens that manage to colonize its surface (Zimmerli et al. 1984; Arciola 2010; Wright and Nair 2010; Hanke and Kielian 2012; Scherr et al. 2014; Paharik and Horswill 2016; Arciola et al. 2018; Ricciardi et al. 2018; Campoccia et al. 2019; Seebach and Kubatzky 2019; Amin Yavari et al. 2020). The current knowledge about the combined FBR and S. aureus BAI is extensive, with more details being continuously uncovered through the usage of modern analytical techniques. Research into this clinical challenge

covers various therapeutic approaches, including the killing of the causative pathogen with drugs or disinfectants, prevention of biofilm formation by employing non-adhesive coatings, dispersion of biofilms, or stimulation of the immune system (Lauderdale et al. 2010; Lister and Horswill 2014; Romano et al. 2015; Jones et al. 2016; Masters et al. 2019; de Vor et al. 2020; Yan et al. 2020). The overarching objective of this review is to chart the factors that initially result in sub-optimal clearance of bacteria involved in BAIs, either with or without therapeutic interventions. Furthermore, we evaluate the recently published insights concerning the ways the S. aureus biofilm and host-immune processes related to the FBR influence each other, and how these mechanisms may be helpful in preventing or treating BAI. In doing so, we pinpoint the relevant factors that result in sub-optimal clearance of bacteria involved in BAIs and possible novel targets for treatment that do not suffer from the progressive prevalence of antibiotic resistance.

2. The main players in the derby on biomaterial implant surfaces

2.1. Foreign body reaction

When a foreign material is implanted into the human body, it triggers an immune cell-mediated response that allows the body to react to the implant and, eventually, to seal it off in a fibrous capsule (Anderson et al. 2008). From the moment of implantation, serum proteins adhere to the implant surface, which functions as an anchor and source of markers for the foreign body reaction. At the forefront of this reaction are polymorphonuclear cells (PMNs) and macrophages. PMNs are present during any inflammatory process in the human body (both sterile and infectious) and are responsible for the first line of defense of the innate immune system. They are initially attracted to the surgical site due to the trauma caused by the implantation procedure. PMNs create an inflammatory environment through the secretion of interleukins and the formation of neutrophil extracellular traps (NETs) (Jhunjhunwala et al. 2015; Vitkov et al. 2015). Macrophages are phagocytic cells of monocytic origin, which can polarize into two sub-types: pro-inflammatory, search-and-destroy, M1 macrophages, and anti-inflammatory, fix-and-repair, M2 macrophages. Although these polarized macrophages have different characteristics, there usually is a continuum of polarization states in vivo (Yamada and Kielian 2019). While macrophages shift to a predominantly M1 profile in response to infection, M2 macrophages are formed as a response to sterile inflammation, as occurs upon internal injury (bruising/

fracture), after surgery, and around implants or tumours (Bronte and Murray 2015; Mariani et al. 2019). During the FBR macrophages shift to the anti-inflammatory M2 state within 24 h and secrete a set of interleukins that inhibit chemotaxis of neutrophils and promote wound healing (Garg et al. 2013). *In vitro* this response is prioritized above shifting to the pro-inflammatory M1 profile as macrophages confronted with both bacteria and a foreign body exhibit an M2 phenotype, though it is not clear if this behaviour is induced by the bacteria or a result of intrinsic macrophage behaviour (Yamada and Kielian 2019; Luan et al. 2020). M2 macrophages stimulate fibroblasts to differentiate into myo-fibroblasts that are the main cell type to deposit the collagen that constitutes a fibrotic capsule around a foreign body (Lupher and Gallatin 2006). After ∼7 days the macrophages on the implant surface fuse to form foreign body giant cells (FBGCs) in a CCL2-dependent process (Anderson et al. 2008; Shiels et al. 2020). Finally the implant is covered by layers of collagen (Anderson et al. 2008).

The foreign body also primes PMNs to release reactive oxygen species (ROS), reactive nitrogen species (RNS), and specific granules. These granules contain myeloperoxidase and B12-binding proteins, components of NETs, and proteases, such as collagenase, which can contribute to implant loosening. Though most extensively described, PMNs and macrophages are not the only cells that participate in the FBR. Lymphocytes (especially Th2 lymphocytes), fibroblasts, osteoblasts, and complement factor activation all help to regulate this process (Anderson et al. 2008; Dapunt et al. 2016; Keselowsky and Lewis 2017).

Implants are susceptible to infection by *S. aureus* at bacterial loads that are 10^5-fold lower than tissues without implants (Zimmerli et al. 1984). Zimmerli et al. proposed in 2011 that this is due to a three-way interaction between the foreign material, the host response system, and the adhering pathogen (Zimmerli and Sendi 2011). In this interaction, phagocytes exhaust themselves trying to take up the foreign body or the wear particles created by friction, resulting in "frustrated" phagocytosis, where the phagocytes become less active (Zimmerli and Sendi 2011; Amin Yavari et al. 2020). This impaired phagocytosis creates a window of opportunity for infecting microorganisms, like *S. aureus*.
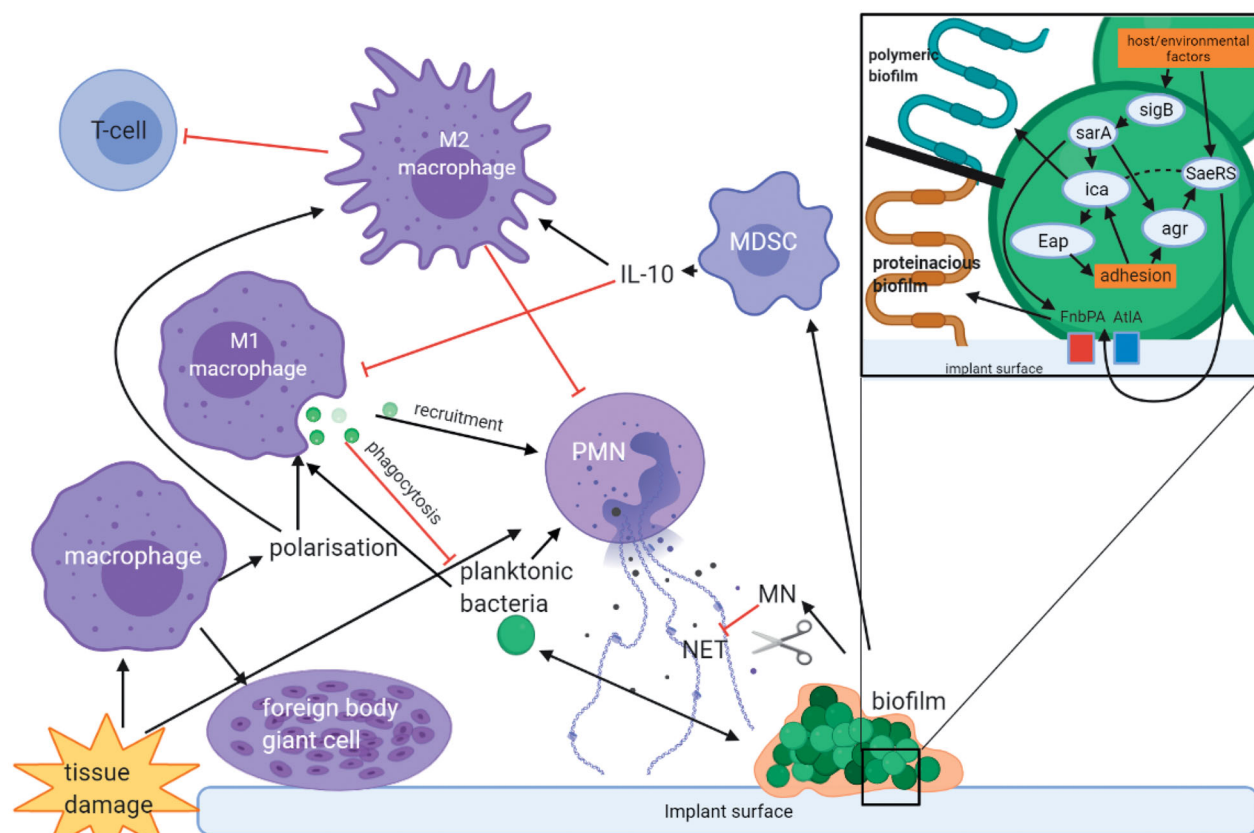
2.2. S. aureus biofilms

S. aureus is a Gram-positive bacterium commonly found on the skin, nasopharynx, and gut (Raineri et al. 2021). While generally a harmless commensal, it can cause opportunistic infections by colonizing open wounds, skin lesions, airways of cystic fibrosis patients, and foreign materials inside the human body (Tong et al. 2015; Moormeier and Bayles 2017). In the latter case, the bacteria may be introduced to the implant during surgery, or at a later moment in time through bacteraemia. *S. aureus* is a dreaded biofilm former, whose biofilm-forming capacities have been extensively studied. These biofilms consist of bacteria embedded in a protective matrix of polysaccharide intercellular adhesin (PIA), extracellular DNA, and proteins, which are collectively called extracellular polymeric substances (EPS) (Arciola et al. 2015; Nguyen et al. 2020). In 2017, Moormeier et al. proposed a 5-stage model for *S. aureus* biofilm formation, which differed from the classical biofilm formation pattern where it was assumed that the bacteria form thick aggregates early in the maturation process, before spreading out over the surface and producing the final, mature biofilm (Moormeier and Bayles 2017). The alternative 5-stage model was based on observations of biofilm formation in a microfluidic flow-cell device mounted into a fluorescence microscope. The five proposed stages entail the attachment, multiplication, exodus, maturation, and dispersal of the biofilm-forming bacteria.

During the attachment stage, *S. aureus* makes use of the matrix of serum proteins deposited on the implant surface (Zimmerli and Sendi 2011). Upon adhesion, *S. aureus* starts to multiply and forms big aggregates. For this to happen efficiently, *S. aureus* employs several gene regulatory systems that orchestrate the biofilm formation (Vergara-Irigaray et al. 2009). In Figure 1, we present a schematic overview of the most important ones, as well as particular niche systems that play a role in the interaction with the host immune system.

Studies with an *in vitro* *S. aureus*-PMN co-culture model showed that bacteria become neutrophil-resistant after 3 h of adhesion, suggesting that this period of time is needed to develop biofilm aggregates that are too big for phagocytosis (Ghimire et al. 2019). After about 6 h, the exodus stage starts, where *S. aureus* will spread in order to cover as much surface as possible (Moormeier and Bayles 2017). This is a nuclease-mediated, *agr* or quorum-sensing (QS)-independent process (Moormeier et al. 2014). In the maturation phase, the biofilm grows thicker, and the typical tower-like structures start to form (Moormeier et al. 2014). The exact structure, density, and roughness of the biofilm seem to play a role in immune recognition, as researchers observed that biofilms with less density, and more open surface as a result of gene deletion or inhibition, triggered much more pro-inflammatory reactions and

Figure 1. Schematic overview of interactions between host immune cells and *S. aureus* biofilm. Tissue damage as a result of implant surgery and the presence of bacteria activate the immune system, leading to both anti- and pro-inflammatory responses depending on the situation. IL-10, a product of MDSCs is anti-inflammatory cytokine that prevents T-cell proliferation and monocyte/macrophage recruitment. In *S. aureus*, the *agr* genes encode the main quorum-sensing system, which has varying effects on biofilm formation, depending on the conditions (O'Gara 2007). The SaeRS gene regulatory system controls the expression of ∼40 virulence factors, including adhesins like Eap (Palma et al. 1999; Hussain et al. 2001; Lee et al. 2004), various toxins, the micrococcal nuclease MN, and immune evasion proteins (Liu et al. 2016). SaeRS does not affect other regulatory systems, but it is affected by environmental factors related to phagocytosis by human neutrophils, such as human neutrophil peptide 1–3 (HNP1–3), calprotectin, and hydrogen peroxide. The *ica* genes play an important role in biofilm formation, through synthesis of PIA, which is a major constituent of the EPS. *ica* expression is positively influenced by SarA, and indirectly and strain-dependent positively or negatively influenced by SigB (Cue et al. 2012). While *ica* regulates the classical polymeric biofilm formation, *S. aureus* is also capable of proteinaceous biofilm formation, which is referred to as *ica*-independent or PIA-independent biofilm formation (Vergara-Irigaray et al. 2009). This alternative biofilm formation is associated with FnbPA and AtlA, and it is the primary form of biofilm formation *in vivo* (Vergara-Irigaray et al. 2009; Khalil et al. 2011; Laverty et al. 2013; Gries et al. 2020). Both are heavily influenced by SarA, which also stimulates *agr* functionality (Tsang et al. 2008; Vergara-Irigaray et al. 2009; Cue et al. 2012). In addition, FnbPA is stimulated by SaeRS (Gries et al. 2020). Black arrows indicate a causative or positive relation, red blunt-headed arrows indicate an inhibitory relation. *agr*: accessory gene regulator; AtlA: Autolysin aureus; Eap: extracellular adherence protein; EPS: extracellular polymeric substance; FnbPA: fibronectin binding protein A; MDSC: myeloid-derived suppressor cell; MN: micrococcal nuclease; NET: neutrophil extracellular trap; PMN: polymorphonuclear cell; PIA: polysaccharide intercellular adhesin; *ica*: intercellular adhesion operon; SaeRS: *Staphylococcus aureus* exoprotein expression responder sensor (a two-component gene regulatory system); SarA: *Staphylococcus* accessory regulator A; SigB: sigma factor B.

were cleared more rapidly (Bosch et al. 2020). Likewise, immature biofilms are more susceptible to phagocytosis (Gunther et al. 2009). The last stage involves the dispersal of the biofilm. This is a process heavily influenced by the *agr* QS mechanism (Moormeier et al. 2014). It also coincides with the expression of phenol-soluble modulins (PSMs), though PSMs do not seem to initiate the biofilm dispersal (Moormeier and Bayles

2017). The dispersal occurs in smaller aggregates of the mature biofilm, which ensures protection of the dispersed aggregates, and is especially effective when bacteria can reach the bloodstream (Gronnemose et al. 2017). As bacteraemia can lead to BAI, where bacteria in the bloodstream will adhere to an implant and subsequently start forming a new biofilm, the reverse process occurs as well. It has been shown that dispersal of
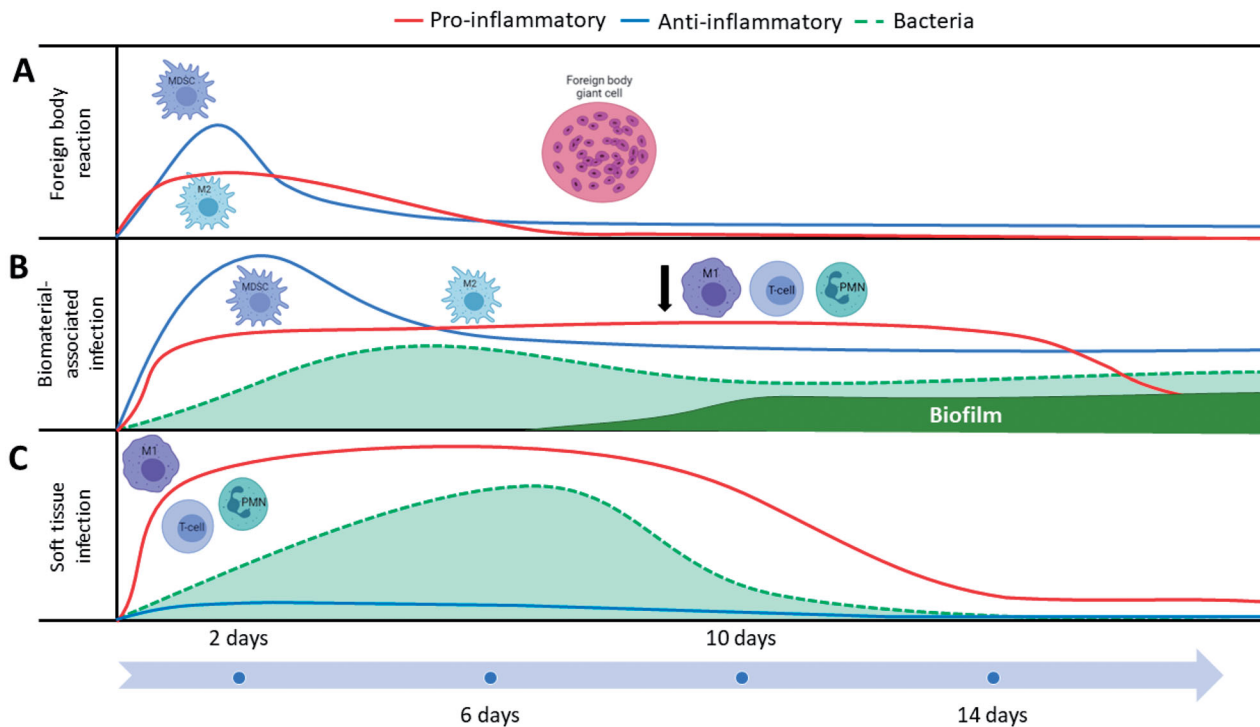
Figure 2. Timeline with pro- and anti-inflammatory processes during the exposure of a foreign body, *S. aureus* bacteria or both to the immune system. (A) Within 24 h after a sterile device implantation the number of MDSCs and M2 macrophages increase significantly before decreasing again at day two or three after implantation, generating an anti-inflammatory response (Peng et al. 2017). An influx of neutrophils is observed after implantation of biomaterials, which decreases over the course of 7–10 days (Snowden et al. 2012). Around this time FBGCs have finished fusing and a mature population of monocytes and macrophages surrounds the implant (Shiels et al. 2020). After this milestone, implants are significantly more resilient to infection (Shiels et al. 2020). (B) When an implant is infected during implantation and *S. aureus* is recognized by the immune system, it manages to elicit a proliferation of MDSCs at the infection site creating an immune suppressive regime. Active MDSCs are found inside the biofilm up to 28 days after infection (Heim et al. 2014, 2015, 2018). M2 macrophages are found in higher numbers than in sterile infections until at least day 7 post-infection (Peng et al. 2017). Although active recruitment of anti-inflammatory cells takes place, pro-inflammatory responses are still active as detectable by increased TNF-α and IL-1β levels, though more moderate than in a soft tissue infection (indicated by the arrow) (Prabhakara et al. 2011; Rochford et al. 2016). *S. aureus* establishes most of its immune-skewing and immune-resisting (biofilm) capabilities 3 days after infection, when the host ends its own immunosuppressive regime. Bacterial load decreases between day 3 and 5, and a biofilm matrix can be observed from day 7 (Gries et al. 2020). Decreased T-cell populations have been observed until 4 weeks after infection, while relative absence of macrophages has been described at least 8 weeks after infection (Vantucci et al. 2021). (C) Soft tissue infection or sepsis scenarios. In both scenarios no implant is present and only minor anti-inflammatory immune-skewing can be observed, for example through superantigens (Pozzi et al. 2015). *S. aureus* can still persist in such scenarios due to non-biofilm specific immune evasion strategies, but the host environment will be exceedingly pro-inflammatory. In both biomaterial-associated infection and soft tissue infection the peak CFU is around 7 days after infection (Snowden et al. 2012).

the biofilm can lead to sepsis and severe metastatic infections, such as endocarditis, osteomyelitis, and pneumonia (Gronnemose et al. 2017; Fleming and Rumbaugh 2018).

3. *S. aureus* biofilm–host cell–implant interactions

From research executed over the last 10 years, a picture has emerged showing that *S. aureus* employs a plethora of mechanisms to counteract protective host cell functions, or even make use of them and that many of these mechanisms are influenced by the presence of a substrate (implant). These mechanisms have consequences starting from the moment of infection and can last indefinitely (Figure 2). Present insight in BAI distinguishes three types of interactions between pathogens and the host immune system in the presence of a substrate:

1. Direct interactions with host cells.
2. Impact on (inflammatory) cellular communication signals.
3. Immune system evasion.

Lastly, there are certain environmental stimuli that have the potential to influence these interactions.

3.1. Direct interactions with host cells

Similar to many other pathogens, *S. aureus* can kill eukaryotic cells. Without an active immune system, any infection would cause major damage, as a BAI model showed that *S. aureus* caused significant destruction of host cells and tissue necrosis after bone marrow suppression (Makino et al. 2015). Within the *S. aureus* biofilm, toxic leukocidins (e.g. LukAB) and α-toxin are QS-dependently expressed and their diffusion into the surrounding tissue causes macrophage dysfunction and death (Scherr et al. 2015; Lei et al. 2017). These toxins are found during chronic, but not during an acute infection. Because leukocidin expression is regulated by the QS-system and activated in mature biofilms, deletion of QS-related genes was shown to lead to restored macrophage function in an animal BAI model (He et al. 2019). *S. aureus* can also cause degradation of collagen, deployed by myofibroblasts as a fibrous capsule, by stimulating the production of matrix metalloproteases (MMPs) by macrophages or by producing MMPs themselves, enabling the bacterium to spread through bone (Lei et al. 2017). When implant infection is further complicated through a mixed *Candida albicans—S. aureus* biofilm, the cytotoxic capabilities increase (de Carvalho Dias et al. 2017). Nonetheless, co-culturing of macrophages with *S. aureus* biofilms showed that some macrophages may invade the biofilm, although displaying reduced phagocytosis once buried beneath the surface layer (Thurlow et al. 2011). Several studies have been performed on biofilm destruction by PMN's. While some studies showed that PMNs can infiltrate and clear *S. aureus* biofilms upon contact (Gunther et al. 2009), other studies showed that the biofilm will overcome PMN infiltration if it has matured sufficiently (Ghimire et al. 2019). After phagocyting *S. aureus* biofilms, the PMNs seem to go into apoptosis, presumably to prevent the spilling of bactericidal and cytotoxic products (Guenther et al. 2009).

Apart from directly killing host immune and non-professional phagocytic cells, *S. aureus* has also been shown to directly influence the polarization of macrophages. Macrophages are polarized towards an M1 or M2 profile as described above, and this polarization is intimately tied to their metabolic state (oxidative phosphorylation or aerobic glycolysis) (Russell et al. 2019). In fact, changes in the metabolic state itself might be a trigger to polarize towards either the M1 or M2 profile. *S. aureus* produces bacterial lactate during its growth and also does so during biofilm formation (though less than when growing planktonically) (Heim et al. 2020). Deletion of the genes encoding for bacterial lactate formation resulted in biofilms that caused significantly lower activation of myeloid-derived suppressor cells (MDSCs) upon contact, which in turn resulted in less IL-10 and less anti-inflammatory signals (Heim et al. 2020). MDSCs constitute a population of bone marrow-borne cells and include progenitor cells, immature macrophages, granulocytes, and dendritic cells (Gabrilovich and Nagaraj 2009). These in turn generate anti-inflammatory signals and suppress T-cell responses (Peng et al. 2017; Yamada et al. 2018). MDSCs proliferate at the infection site and are not recruited from the blood or bone marrow after proliferation (Heim et al. 2018). It has been suggested that the bacterial lactate is transported into the cell through monocarboxylate transporters which transport lactate both intra- and extracellularly. Conversely, when an oxidative phosphorylation inhibitor was used on macrophages in a BAI animal model, a shift towards the anti-inflammatory profile was observed, and animals were able to clear established biofilms in combination with systemic antibiotics, where they were not able before (Yamada et al. 2020).

The pathogen *S. aureus* has several ways of handling host defense peptides. The ability of PMN's to produce NETs, in response to implants and/or *S. aureus* biofilms, and partly as a result of apoptosis, has long been regarded as an adequate antimicrobial defense mechanism (Meyle et al. 2010; Vitkov et al. 2015). However, *S. aureus* produces a nuclease that allows it to escape from these NETs (Berends et al. 2010). Recently, it was suggested that, after breaking down these NETs, *S. aureus* is even harder to eradicate (Gutierrez Jauregui et al. 2019). This could be caused by the ability of *S. aureus* to convert components of the NETs into cytotoxic compounds (Thammavongsa et al. 2013).

Phagocytes are also capable of killing bacteria through the production of nitric oxide (NO). *S. aureus* can counter this in various ways, including the detoxification of NO by the Hmp flavohemoprotein, modulation of the cellular redox state and oxidative phosphorylation, and the production of endogenous NO by nitric oxide synthase (Nos) (Buchan et al. 2019). The endogenously produced NO protects the bacteria against oxidative damage and promotes aerobic and nitrate-based respiration (Favazzo et al. 2019). A less common defense against NO is provided by the NO reductase (Nor) (Favazzo et al. 2019). These NO-protective mechanisms allow the bacteria to spread to nearby soft tissue, and other organs *via* the bloodstream.

Another consequence of interaction with the immune system is the selection of bacteria with specific traits. A low-grade, or long-lasting, infection by *S. aureus* means that the bacteria will be exposed to host defense peptides for long periods of time, thereby undergoing a selective pressure for resistance against membrane-acting antimicrobials, such as daptomycin (Mishra et al. 2013).

During BAI a shift towards QS dysfunction has been observed, which relates to a so-called "quorum cheating" bacterial sub-population (He et al. 2019). In a planktonic state, the QS-deficient bacteria do not survive very long, but within a biofilm, they will thrive by saving energy as long as there are some QS-proficient bacteria in the biofilm. The compact biofilms formed by mostly QS-deficient bacteria are in fact harder to penetrate for PMNs than biofilms formed by QS-capable bacteria.

The presence of bacteria (or bacterial proteins) can prevent osteoblasts and fibroblast-like cells from adhering to the surface of implanted biomaterials and from proliferating as they would normally do in a sterile FBR. They thereby prevent the proper integration of biomaterials into the body (Yue et al. 2015). In particular, mesenchymal stem cell adhesion to a titanium alloy was found to be impeded by the presence of *S. aureus*. How the bacteria influence the stem cells is not known, other than that it does not involve toll-like receptors.

3.2. Impact on (inflammatory) cellular communication signals

A phenomenon observed during BAI and FBR interactions is that pathogen activities compel the host immune system to adopt an anti-inflammatory behaviour, interfering with the innate anti-inflammatory environment of the FBR itself. Specifically, the *S. aureus* biofilm is known to skew the immune system towards an anti-inflammatory state, more so than the planktonic bacteria (Thurlow et al. 2011; Gries and Kielian 2017; Yamada and Kielian 2019). We have listed the following items according to their impact on the innate or adaptive immune systems.

3.2.1. Impact on the innate immune system
A relevant example of *S. aureus* biofilms impacting the innate immune defences concerns their stimulating activity on the development of MDSCs. Both M2 macrophages and MDSCs express arginase-1 (Arg-1), a protein that reduces the levels of free arginine that are needed for NO synthesis. The local depletion of arginine and generation of its metabolites stimulates cell proliferation, fibrosis, and suppresses T-cell development (Thurlow et al. 2011; Arlauckas et al. 2018). Not only are these cells actively recruited to *S. aureus* BAIs, but the expression of Arg-1 is also increased as well (Hanke et al. 2013; Heim et al. 2015). Arginine can also be used by *S. aureus* to stimulate growth, however, infection models of Arg-1 knockout mice indicate that *S. aureus* biofilm growth is not restricted by the absence of arginine, as the growth is identical in Arg-1 KO mice and naïve mice (Yamada et al. 2018). This means that although *S. aureus* can make use of this process, it is not dependent on it. While *S. aureus* implant infections are typified by a robust MDSC infiltrate, it has been shown that IL-12-deficient mice do not recruit MDSCs and, as a result, have an increased resistance to *S. aureus* implant infections (Heim et al. 2015). This lack of MDSCs was accompanied by elevated levels of cytokines (IL-1β, TNF-α, and G-CSF) and chemokines (CXCL1 and CCL5) and increased monocyte recruitment, which are all hallmarks of active inflammation. Depletion of MDSCs had a similar effect (Heim et al. 2014). The susceptibility to implant infection was restored when MDSCs were administered systemically. Further, the *S. aureus* biofilm is capable of stimulating the conversion of MDSCs into M2 macrophages (Peng et al. 2017). Recruitment of MDSCs is stimulated by a low initial bacterial burden. When confronted with an initial high dose of bacteria (e.g. 10^5 bacteria), a pro-inflammatory environment is created with a low number of MDSCs, whereas a low dose (10^3 bacteria) stimulates the activation of MDSCs and an anti-inflammatory environment (Vidlak and Kielian 2016). That the immune response is skewed towards an anti-inflammatory state only in the case of low doses of bacteria is remarkable. Low initial doses are typically found in surgical situations where the implant area is exposed to around 50 bacteria per cm^2, which potentially grows into the range that MDSCs are actively recruited (Harnoss et al. 2017). It should be noted that in many murine BAI models much higher inocula are applied. In particular, when using bioluminescent bacteria in an established *in vivo* imaging assay, detection of amounts lower than 10^5–10^7 bacteria per site of bioluminescent bacteria is challenging and easily cleared by the immune system of the mouse, rendering the experiment void (Busscher et al. 2020), which complicates the interpretation of results obtained from implant infection models in terms of chronic infections and infections in general.

The *S. aureus* biofilm has more strategies to promote the polarization of macrophages towards the anti-inflammatory M2 state. In particular, the biofilm inhibits the macrophage MyD88 pathway which results in

decreased cytokine production, *S. aureus* clearance, and increased fibrosis (Hanke et al. 2012). The *S. aureus* biofilm further skews macrophages to an anti-inflammatory state by secreting soluble molecules that attenuate the bactericidal and proinflammatory responses, and inhibit the NF-κB pathway (Alboslemy et al. 2019). This is exclusive to the biofilm mode of growth, as planktonic *S. aureus* is not capable of this behaviour. Furthermore, the biofilm blunts chemotactic cytokine expression, slowing phagocytotic cell recruitment, or making them move in erratic patterns (Brady et al. 2018; Gries et al. 2020).

Deletion of *sarA*, a regulatory gene of *S. aureus* affecting a cascade of bacterial gene expression (Figure 1) was shown to lead to an elevated pro-inflammatory response in an animal infection model and decreased bacterial survival (Snowden et al. 2013). Since SarA has a pivotal role in biofilm formation, deletion of the *sarA* gene also led to reduced protection from the biofilm, and a lowered skewing of the immune system to an anti-inflammatory response, making the immune response more effective.

Implant materials can influence the complement system and cytokine secretion by immune cells. However, these biomaterial-dependent differences are all over-ruled and become indistinguishably complicated when confronted by a bacterial infection (Rochford et al. 2019). This was demonstrated in an *in vitro* implant infection model, using commercially available polyether–ether–ketone or titanium implants. Each of these materials displayed particular effects in absence of an infection, but implantation of both materials led to comparable levels of C3a activation and increased NF-κB activation when an *S. aureus* BAI was introduced.

3.2.2. Impact on the adaptive immune system

Apart from the strong effects on myeloid cells, the *S. aureus* biofilm also affects lymphocytes. Murine implant infection models have shown that the *S. aureus* biofilm can promote an early pro-inflammatory Th1 and/or Th17 response that is, however, unable to clear infections at an early stage during BAI (Prabhakara et al. 2011; Rochford et al. 2016; Brady et al. 2018). The shift to a Th1/Th17 dominant profile leaves the host unable to clear the infection and allows the biofilm to persist chronically (Prabhakara et al. 2011). Mice with a genetic disposition to the Th2 response were able to clear implant infections. In contrast, mice with a predisposition to the Th1/Th17 response, or where the T cell proliferation was forced to this profile, were not able to clear implant infections. The Th1 and Th17 responses with a downregulated Th2 and regulatory T cells

response were observed in chronic biofilm animal models with low initial doses of bacteria (Prabhakara et al. 2011). This again shows the relationship between low-grade infection and a reduced inflammatory response, leading to BAI persistence.

Another example of *S. aureus* biofilm actively skewing the immune system was obtained with a multiple sclerosis model. During BAI, increased levels of INF-gamma, IL-6, and B- and T-cells were observed, which exerted a degree of neuroprotection (Kumar et al. 2015). The Extracellular adherence protein (Eap) of *S. aureus* has strong anti-inflammatory properties and was shown to prevent neutrophil recruitment, which may explain these findings (Chavakis et al. 2002). Indeed, deletion of the *eap* gene prevented an increase in the aforementioned immune parameters, resulting in decreased neuroprotection (Kumar et al. 2015).

Altogether, it can be concluded that the bacteria colonizing an implant can create a less hostile environment for themselves than bacteria that remain in the soft tissues. As a consequence, BAI is characterized by a strongly prolonged persistence compared to soft tissue infection. Moreover, the sterile FBR as described by Anderson et al. (2008), is effectively modulating the immune system, thereby allowing bacteria to get a strong initial foothold in the case of BAI. This is schematically represented in Figure 2, which depicts the timeline of a BAI in relation to the timelines of an FBR and soft tissue infection.

3.3. Immune system evasion

Any active clearance of foreign cells from the host starts with the immune system recognizing those cells as out of place. The *S. aureus* biofilm can evade pattern recognition by macrophages, which *S. aureus* cannot do in a planktonic state (Thurlow et al. 2011). Another form of evasion of immune recognition involves the expression of the surface protein SdrE and its allelic variant Bbp. These proteins bind the complement regulator C4BP, increasing bacterial survival of classical complement pathway-mediated neutrophil killing (Hair et al. 2013). Deletion of the *rsaA* gene, a non-coding RNA, was shown to increase capsule formation and, in turn, to decrease opsonophagocytic killing by PMNs (Romilly et al. 2014). However, deletion of this gene also diminished biofilm formation and decreased *S. aureus* implant infections *in vivo* (Romilly et al. 2014; Crosby et al. 2016).

Furthermore, it has been proposed that the thick EPS layer of the biofilm can hide antigens from recognition by the immune system. Apart from hiding certain

surface compounds to avoid opsonization, *S. aureus* can enter most eukaryotic cells (Arciola et al. 2012; Foster 2019; Zhu et al. 2020). Though infiltration of cells is a well-known form of immune evasion, this process is not benign and may cause cell death. A review by Foster et al. describes the importance of Fibronectin-Binding Protein A (FnBPA) during this process (Foster 2016). After treatment of BAI in a rat implant infection model, infection was shown to re-occur in those animals where *S. aureus* had managed to infiltrate immune cells (Gao et al. 2020). The significant role that these intracellular persister cells play in secondary infection was confirmed by successfully treating secondary BAI with cell wall-penetrating antimicrobials. Though intracellular *S. aureus* often originates from bacteria entering immune cells at the site of primary infection, the bacteria causing the secondary infection can come from any site where *S. aureus* comes into contact with the immune system, like the gut (Zhu et al. 2020; Raineri et al. 2021). Direct killing of osteoblasts after invasion by surface-adhering *S. aureus* has been observed *in vitro* (Subbiahdoss et al. 2011). A co-culture with macrophages reduced this effect but did not prevent it. Internalization in osteoblasts leads to an increased expression of IL-6 and IL-8 (Jauregui et al. 2013). The osteoblast in turn can kill the bacteria through ROS production after they activate TLR9 (Mohamed et al. 2016). It was further shown that the internalization of *S. aureus* in eukaryotic cells is also promoted by co-infection with *Candida albicans* (Carolus et al. 2019).

Lastly, *S. aureus* involved in an implant infection can influence the immune system by releasing so-called superantigens, which are exotoxins that help the bacteria to evade the host immune system (Maina et al. 2018). These superantigens cause non-specific T-cell activation and massive cytokine release, eventually exhausting the T-cells (Chung et al. 2015). This event is, for instance, observed during the so-called toxic shock syndrome. Afterwards, the bacteria can recuperate faster than the immune system, giving them a competitive edge. Besides superantigens activating T-cells, *S. aureus* also produces the virulence factor staphylococcal protein A (SpA) (Pozzi et al. 2015; Muthukrishnan et al. 2019). This protein has five binding sites for immunoglobulins and can cause an overwhelming activation of B-cells. This leads to polyclonal proliferation, activation, migration, or supraclonal deletion due to activation-induced cell death (Pozzi et al. 2015). Besides clonal B-cell deletion, the superantigenic activity of SpA also causes immunodominance of SpA-attuned B-cells rendering the humoral defense less sensitive to all other *S. aureus* antigens accounting for impaired

protection and memory formation (Pauli et al. 2014; Pozzi et al. 2015). In addition, Spa interferes with Fc-Fc domain interactions, thereby inhibiting the hexamerization of IgGs bound to an *S. aureus* target cell, which is required for effective IgG-dependent complement activation and subsequent bacterial killing (Cruz et al. 2021). Lastly, SpA can bind to free immunoglobulins, blocking their binding site and preventing them from binding to *S. aureus* and opsonophagocytosis.

3.4. Reaction to environmental factors

The implant surface properties can substantially influence the interactions between the *S. aureus* biofilm, the FBR, and the immune system. For instance, the type of surgical meshes used for soft tissue repair (macroporous or microporous) may determine the degree of abscess formation and fibrous capsule thickness. A microporous-monofilament mesh resulted in less fibrosis and abscess formation in an infection model, though an exact mechanism has not been proposed (Stoikes et al. 2017).

Obesity and the associated high fibrin concentration may lead to an upregulated expression of the *S. aureus* clumping factor A (Farnsworth et al. 2017). This will increase staphylococcal virulence, as evidenced by increased abscess formation and bone destruction. Diabetes was also shown to lead to increased implant infection propensity, even though the white blood cell count and neutrophil infiltration were increased (Lovati et al. 2013). Though not classically seen as a systemic affliction, disruption of the native gut microbiome was shown to render mice more susceptible to BAI (Hernandez et al. 2019).

Lastly, wear particles from an implant can attribute greatly to the FBR, since they have a proportionally large surface due to their small size. In particular, small polyethylene particles were shown to stimulate macrophages, increase TNF-α release, reduce macrophage viability, and increase osteolysis (Chen et al. 2017). In turn, this resulted in an increased bacterial burden. This observation can be explained by an increased level of impaired phagocytosis where phagocyte activity declines after a period of excessive activation.

4. Intervention strategies in implant infection

The majority of studies selected for this review were conducted with the purpose of better understanding the interaction between *S. aureus* BAI and the FBR as described above. However, several studies were also aimed at treating BAI and addressed the effects of

interventions on bacterial survival. In the following sections, we review and analyze several studies specifically aimed at possible methods to prevent or treat *S. aureus* BAI. Because all interventions using bacterial cells or bacterial products have already been described above to give insight into *S. aureus* BAI pathogenesis, and generally do not pertain to realistic treatment options like genomic alteration of the bacterium, those experiments are not repeated in the following section.

4.1. Interventions that pertain to the host cells and their products

It has been shown that mesenchymal stromal cells can work synergistically with the immune system and stimulate wound healing. They actively travel to the infection site and secrete antimicrobial peptides, increase phagocytosis by PMNs, and create a pro-inflammatory environment (Johnson et al. 2017). Implantation of these cells stimulates healing in soft-tissue infections but aggravates implant infections *in vivo* (Seebach et al. 2015; Johnson et al. 2017). Furthermore, it was shown that, when added as a coating in an implant infection model, these cells cannot survive without the co-integration of antibiotics (Guerra et al. 2015).

Administration of M1 macrophages has been successfully used to treat *S. aureus* implant infection in mice (Hanke et al. 2013). To this end, M1 macrophages were harvested from MyD88 knockout mice, or M1 macrophages were created by blocking the MyD88 pathway, as both of these interventions force the transition of macrophages into the M1 phenotype. The success of this approach underpinned the important effect of immune skewing to an anti-inflammatory environment on *S. aureus* survival. In the same trial, the administration of PMNs, even in high doses, was shown to have no effect. This was unexpected due to the generally high bactericidal capacity of the latter cells. Possible explanations were sought in the rapid PMN degranulation following *in vivo* transfer, poor vascularization at the site of the BAI, or protection provided by the implanted device in the form of hard-to-reach niches, like a catheter lumen.

Several ways of using host cell-derived products, like interleukins, have been studied to eradicate *S. aureus* biofilms. Systemic supplementation of IL-12 in an animal model stimulated Th1 development and increased macrophage activity but did not significantly reduce implant infection (Lindsey et al. 2010). This is not surprising as IL-12 has been linked to MDSC recruitment and persistence of the *S. aureus* implant infection (Heim

et al. 2015). Accordingly, the application of IL-12 as a nanocoating induced better-wound healing and decreased *S. aureus* persistence (Li et al. 2010). This did not affect the white blood cell count and cytokines and, instead, increased the lymphocyte count (Li et al. 2010). The monocyte chemoattractant protein 1 (MCP-1) was also used as a nanocoating, thereby decreasing the bacterial burden while increasing the number of local neutrophils, decreasing the number of lymphocytes, and ultimately creating a pro-inflammatory environment (Li et al. 2010).

Production of the pro-inflammatory IL-1β, IL-4, and IL-6 is increased at the site of BAI, when compared to sterile implants, for up to 3 weeks after infection (Prince et al. 2021). Especially IL-1β is important for a "normal" immune response to BAI, as IL-1β knockout mice showed an increased biofilm load upon infection and decreased neutrophil recruitment (Bernthal et al. 2011; Wang et al. 2020). Accordingly, systemic suppletion with high doses of IL-1β has been investigated as a means to enhance *S. aureus* biofilm clearance, but this only enhanced the biofilm and increased spread to other organs (Gutierrez Jauregui et al. 2019). Conclusively, a lack of IL-1β gives way for bacteria to spread, while too much strengthens the position of the biofilm. Supplementation with other cytokines usually invoked in BAI (IL-6, IL-10, IL-12, IL-17, IL23, IFNγ, and TNFα) did not stimulate *S. aureus* biofilm propagation.

Contrary to bacteria hiding from the immune system, the possibility to enhance the efficacy of the immune system against *S. aureus* through passive or active vaccination approaches has been investigated in recent years. Zymosan, a toll-like receptor-2 agonist, was used to non-specifically activate the immune system to increase resilience to BAI (Zhu et al. 2020). This would be analogous to the non-specific protection humans develop upon injection with *Bacillus* Calmette-Guérin, smallpox, and measles vaccines. Indeed, treatment with zymosan did increase pro-inflammatory markers before implantation and decreased the bacterial load during infection. However, it did not lead to any spontaneous clearance of infection, and this non-specific immune activation could theoretically even trigger an auto-immune response. An example of a more specific treatment is the development of monoclonal antibodies targeting the housekeeping protein IsaA, a soluble lytic transglycosylase of *S. aureus* involved in cell wall biogenesis. The IsaA-specific monoclonal antibody 1D9 can be effectively used to detect *S. aureus* biofilms on spinal and shoulder implants *in vivo*, demonstrating that the protein is accessible to the immune system (Zoller et al. 2019; Sheppard et al. 2020). Further, the IsaA-specific

antibody UK-66P showed great efficacy in a sepsis model and limited haematogenous spread from an infected implant, although it did not manage to ultimately cure the BAI when tested in an implant infection model (Lorenz et al. 2011).

4.2. Implant functionalization

S. aureus cannot form a biofilm when it is not attached to a surface. Certain measures have been proposed to prevent the attachment of the bacteria. These usually involve changing surface qualities like roughness, chemistry, charge, or hydrophobicity (Song et al. 2015). The balance between non-adhesive properties to prevent bacteria from adhering, and allowing host cells to adhere is dubbed "the race to the surface." This reflects the idea that the cells that settle first (bacterial or host cells), will gain the upper hand (Busscher et al. 2012). Instead of preventing bacteria from adhering, a different strategy has been tried where an increase of cellular adhesion has decreased the number of adhering bacteria (da Silva Domingues et al. 2015). Here it was shown that a more hydrophobic surface allowed macrophages to adhere to the implant surface, which is required to search and engulf any surface-adhering pathogens.

Tantalum is a material that has been used in prosthetic joint implants with a high degree of success, which is attributed to its biocompatibility. During the exploration of its antibacterial capabilities, a tantalum nanofilm was shown to have no direct antibacterial activity, but it did increase the clearing of an *S. aureus* implant infection (Yang et al. 2019). This is presumably achieved by aiding the host cells in the race to the surface. Follow-up experiments showed that the nanofilm increased the efficacy of PMN phagocytosis, TNF-α, and IL-6 release, but had no effect on the complement system. In addition, selenium nanotubes have proven to increase ROS secretion by macrophages, which results in bacterial killing (Liu et al. 2016).

To diminish "frustrated" phagocytosis by wear particles, polyethylene has been cross-linked with vitamin E (Chen et al. 2017). This method succeeded in negating the immune inhibition caused by nanoparticles. Other materials, like poly(trimethylene carbonate) (PTMC) and poly(D,L-lactic acid) (PDLLA) have been shown to modify the expression of the pro-inflammatory cytokines TNF-α, IL-6, and IL-23 by dendritic cells in response to *S. aureus*, but this did not change the Th1/Th2 cell response (Balraadjsing et al. 2018). It is a recurring observation that immunomodulatory coatings do not function as intended once confronted with an infection, lending credit to the resilience of either the natural immune cell behaviour or the power of bacterial immune profile skewing. Lastly, antimicrobial coatings are widely researched. Even though the applied antibiotics are usually sufficient to kill the bacteria, in practice they never reach 100% prevention of infection. This is at least in part due to the fact that bacteria adhering to antimicrobial surfaces downregulate their metabolism, and are therefore less susceptible to antibiotics (Alves et al. 2018). However, despite this downregulation, their uptake by macrophages is not inhibited.

4.3. Environmental modulation

While *S. aureus* can thrive in anaerobic conditions, as encountered in the deep layers of a biofilm, a lowered oxygen concentration of <2% is enough to impair the antibacterial functions of neutrophils (Ghimire et al. 2019). Therefore, the lowered oxygen tension inside the biofilm can be regarded as another bacterial defense strategy to evade the immune system and, accordingly, as a therapeutic target. Indeed, the reverse condition, established through hyperbaric oxygen treatment (90% O_2 at 2 atmosphere), has been successfully used to treat chronically infected wounds. However, in BAI it was found to be associated with delayed bone healing and increased non-union, even though bacterial survival did not differ (Buren et al. 2018).

5. Conclusion

The persistence of *S. aureus* BAI is a major clinical problem that proved hard to prevent and treat while affecting the health and well-being of many people around the globe. Once such a BAI has established itself, the host's immune system has trouble eliminating the pathogen. This is initially due to the anti-inflammatory processes of the host in response to the implantation of a biomaterial, the trauma that accompanies it, and the presence of low numbers of pathogens. By the time this anti-inflammatory response subsides, *S. aureus* has entered the early stages of biofilm formation. This also marks the start of many processes that lead to persistence of the *S. aureus* biofilm, including directly influencing host cells, skewing of cellular communication and environment, and evasion of immune recognition, resulting in a local chronically impaired immune function.

Altogether, the compiled data imply that implant susceptibility to infection is not a result of the exhaustion of one immunological resource. It thus seems that depletion of phagocytotic capability upon "frustrated"

phagocytosis is not the sole cause of BAI resilience. Instead, it is the consequence of a combination of deliberate activities of the bacterium. Due to the large versatility and the intricate interrelationships of cellular processes, it is foreseeable that there will not be a "magic bullet," or single effective treatment to cure BAI. However, based on the data that were compiled and analyzed for this review, it seems critically important to prevent the development of an anti-inflammatory environment that allows bacteria to create a foothold at the implant's surface to prevent BAIs. Achieving this will greatly aid bacterial clearance, as the situation will be more comparable to soft tissue infections where no implant is present and the immune system can adequately clear the infection. We anticipate that such immune-modulating strategies will have to address all three major contributors to the BAI environment: the pathogen, the implant surface, and the host cells.

Disclosure statement

J.M.v.D. reports that he filed a patent application on the use of 1D9, which is owned by his employer University Medical Center Groningen. C.W.K.R. and J.S. report that they have no competing interests relevant to this study.

ORCID

Jan Maarten van Dijl http://orcid.org/0000-0002-5688-8438
Jelmer Sjollema http://orcid.org/0000-0003-0714-3082

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