Host response against biomaterials: the role in the pathogenesis of biomaterial-associated infections
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The use of biomaterials has been one of the most significant advances in modern medicine. A serious problem, however, associated with the use of all types of biomaterials, is the occurrence of bacterial infections causing considerable morbidity and mortality [1,2]. The pathogenesis of biomaterial-associated infections (BAI), a complex process with various contributing factors, such as bacterial virulence, physico-chemical properties of the biomaterial and alterations in the host defense, is poorly understood. The predominant role and initial step in the pathogenesis is attributed to the adherence of bacteria onto the biomaterial [1-3]. In the prevention of BAI, efforts are focussed on inhibiting the bacterial adherence. However, alterations in the host defense in the vicinity of an implanted biomaterial have been suggested frequently [3-5]. Therefore, the aim of this thesis was to obtain insight into the role of the host response against biomaterials in the pathogenesis of BAI. In this general discussion, the work of the preceding chapters and that of others, regarding (i) the role of bacterial adherence and the supposed virulence factors in BAI, (ii) the host response to various materials and their influence on leukocytes, and (iii) the role of cytokines in modulating these responses, is reviewed and discussed.

The adherence of *S. epidermidis* to biomaterials and biomaterial-associated infection.
The essential process in the pathogenesis in BAI is assumed to be bacterial adherence, followed by the production of extracellular substances (slime, glycocalix) by adherent bacteria. This slime production, in conjunction with adherent host proteins and cells leads to a thick biofilm on the biomaterial surface. The biofilm is believed to facilitate bacterial persistence by impairing the host defense and impeding antibiotic penetration [6-9].

In vitro studies have demonstrated an inhibitory effect of staphylococcal slime on leukocyte function [10,11], suggesting that the ability to produce slime is a virulence factor for *Staphylococcus epidermidis*. However, slime is produced in vitro by only 30 - 60 % of the clinical *S. epidermidis* isolates [10-14]. Conflicting data regarding the importance of biofilm in the pathogenesis of BAI arose from experimental, in vitro [15] and in vivo studies; slime production did not correlate with the ability of *S. epidermidis* to cause experimental endocarditis [16,17]. Other possible virulence factors of *S. epidermidis* described are polysaccharide-adhesin (PS/A) and polysaccharide intercellular adhesin/hemagglutinin (PIA/HA), which are assumed to have a role in the initial adherence of *S. epidermidis* and the adherence of *S. epidermidis* to each other, respectively [18-24]. In animal models it was
found that PS/A or PIA/HA negative \textit{S. epidermidis} mutants were less capable of causing infection than wild type strains [22,24-26]. However, PS/A or PIA/HA negative mutants were cultured from the implanted catheter segments, but in lower numbers than PS/A or PIA/HA producing strain. This indicates that although the implants contaminated with the PS/A or PIA/HA negative mutants did not show sign of a clinical infection, at least, equilibrium existed between pathogen and host defense. Furthermore, results from studies investigating the incidence of clinical isolates positive for PS/A and/or PIA/HA, have not been reported yet.

In most studies only the bacteria adherent to the biomaterial are (quantitatively) cultured. In our studies we quantitatively cultured standardized biopsies taken from the implantation site [27-31]. We found that \textit{S. epidermidis} was able to persist longer in tissue than on the implanted biomaterials. Around silicon elastomer (SE) and around polyvinylpyrolidone-grafted silicon elastomer (SEpvp) the tissues remained culture-positive for weeks to months whereas the implanted segment was culture-negative after 2 to 5 d [27,28]. This suggests that not the biomaterial surface is a niche for the bacterial persistence, but the tissue surrounding the implanted biomaterial. In addition, the greater than 90% reduction of adherence to the surface of the modified SEpvp and polyvinylpyrrolidone-grafted polyamide (PApvp) in vitro did was not result in a reduced infection rate in vivo [27,32]. Mice with SEpvp and PApvp were more susceptible to BAI than mice with SE. Moreover, implantation of SE and SEpvp with adherent \textit{S. epidermidis} showed that more SEpvp segments and tissues were culture positive for a significant longer period than SE, despite the fact that the number of adherent \textit{S. epidermidis} on SEpvp was 90% lower than on SE [29,32]. Moreover, several other studies indicated that reduced in vitro adherence does not predict reduced infection susceptibility in vivo [33-35]. Data from some studies, however, did show a reduced susceptibility to BAI when antimicrobial-catheters were used [36-38]. The use of rifampicin / minocyclin impregnated or antiseptic-impregnated intravenous catheters [36,37] significantly reduces the risk on a catheter-associated bloodstream infection. It should be noted, that although no catheter-associated bloodstream infection occurred, after a average period of implantation of 6 d, still respectively 8 % and 28 % of the above antimicrobial-catheters, were colonized. Tissue cultures were not performed. Although bacteria present on these novel antimicrobial-catheters did not cause a clinical infection, their persistence is potentially dangerous. If, the equilibrium existing between bacterium and the immune-defense is tipped in favor of the bacteria at a later time, the subclinical persistence of bacteria can cause infection.
Hence, we hypothesize that the ability of *S. epidermidis* to induce BAI is more likely due to an impaired host defense in the vicinity of an implanted biomaterial than due to the ability of *S. epidermidis* to adhere to biomaterial surfaces. The predominance on the skin of *S. epidermidis* strains is probably the main reason that these relatively non-pathogenic bacteria induce BAI. This is supported by the fact that the distribution of bacterial species causing a BAI is correlated with the anatomical site where the biomaterial is implanted or inserted and with the nursing ward where the patient is residing [1,39]. The expression of some virulence factor, such as slime, PS/A and PSA/HA may only partly contribute to the enhancement of susceptibility to infection.

**Enhanced tissue reactivity favors bacterial persistence**

Injury due to the implantation or insertion of a biomaterial and the biomaterial itself provokes an inflammatory response to biomaterials [3,40,41]. The intensity and duration of this foreign body response is largely dependent on the size, shape, chemical and physical properties of the implanted material [1,42]. We demonstrated in an experimental animal model that injection of small amounts of bacterial cell wall components in tissue surrounding an inert and approved biomaterial, enhanced the inflammatory reaction, resulting in abscess formation and delayed encapsulation of the biomaterial [27,28]. Bacterial components and dead bacteria are equally potent in inducing host mediators and subsequent host response as viable bacteria [43-46]. When, in the same model, very low numbers of *S. epidermidis* were injected along the implant the induced abscess formation was associated with persistent infection [27,28]. This indicates that the enhanced inflammatory environment increased the susceptibility to infection. Thus, novel antimicrobial-biomaterials, such as the antibiotic and/or antiseptic-impregnated catheters, which will partly eradicate bacteria present on or around the implant, will leave bacterial cellular components [36,37]. An enhanced inflammatory environment, probably even further enhanced due to the released antibiotic [44,46-48], could arise. The enhanced inflammatory environment around these novel materials may be more prone to infection, particularly when antibiotic is not released anymore.
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Polymorphonuclear Cells and biomaterial-associated infection.
The function of Polymorphonuclear Cells (PMNs) in the environment of an implanted biomaterial has been studied extensively. Normally, neutrophils contribute to phagocytic host defense by ingesting and killing invading microorganisms [49-51]. Intracellular killing occurs as a consequence of reactive oxygen intermediates formed during phagocytosis and due to bioactive constituents from granules, such as myeloperoxidase and defensins. In addition, activated PMNs will release part of their granule content, contributing to extracellular killing of microorganisms [49-51].

Using a guinea pig model of foreign body implantation, Zimmerli et al. observed that complement-mediated opsonic activity in tissue fluid surrounding implanted tissue cages containing biomaterials was substantially reduced. PMNs harvested from these tissue cages had decreased bactericidal activity in comparison to PMNs from peripheral blood or peritoneal exudate. In addition, PMNs from tissue cages had defective oxidative metabolism and granulocyte enzyme content [5,52]. Similar impairment of the bactericidal capacity has been described for PMNs exposed to non-phagocytosable surfaces [53-59]. PMNs rapidly become associated with the implanted biomaterial, and under normal conditions these high numbers should be capable of sufficient phagocytic host defense. However, various reports indicate, that PMNs become activated by contact with the biomaterials, and thereby lose the capacity to respond to subsequent stimuli like invading microorganisms [42,55,57,60-63]. In addition, and probably more importantly, these biomaterial-associated PMNs induce a dysfunctional impaired activation of incoming fresh PMNs [5,52,63], due to the abundant release of defensins [54,63]. High concentrations of defensins were shown to be cytotoxic for eukaryotic cells and for PMNs [60,64,65]. Despite the fact that defensins contribute to create a hostile environment for bacteria in tissue surrounding an implanted biomaterial, the reduced activity of PMNs may favor bacterial persistence.

Mononuclear cells and biomaterial-associated infection.
Impairment of phagocytic and/or intracellular killing due to biomaterials has also been reported for monocytes and macrophages [4,66-69]. MHC class II (1a) expression is an index for cellular immunity. Decreased 1a expression is associated with increased susceptibility to infection as found in trauma patients, patients with bleeding disturbances and sepsis patients [70,71]. In clinical series and experimental animal models, Henke et al described a locally
suppressed la expression by monocytes in S. epidermidis infected perigraft fluid compared to the la expression by peripheral monocytes, [4,68,69]. Others described that low expression of la was associated with a very low lysosomal activity around collagen implanted in rat [69].

The la suppression around infected perigrafts, was associated with an enhanced pro-inflammatory environment [4], and with inhibition in biomaterial incorporation [72]. This finding matches observations in humans; biomaterials evoking strong tissue reactions generally have higher infection rates than biomaterials with less tissue reaction [39,73-75]. For instance, phlebitis associated with an intravascular catheter disposes for enhanced susceptibility to BAI [76-78]. Also in our mice model with implanted SEpvp, strong tissue reactivity was associated with delayed encapsulation of SEpvp [28]. Macrophages play an important role in the initiation of the encapsulation of a biomaterial [34-36].

We recently observed that S. epidermidis is able to persist in macrophages in mouse subcutaneous tissue surrounding three different biomaterials, SE, SEpvp and PApvp [29]. Moreover, S. epidermidis was able to survive for months in tissue surrounding these materials and induced bacteremia and sepsis in mice carrying the PApvp segments challenged with S. epidermidis, 2-3 weeks after implantation. This suggests that macrophages may be a niche for S. epidermidis, and that S. epidermidis intracellular survival can finally result in BAI. However, the number of infected macrophages and the number of persisting bacteria per cell depended on the type of biomaterial used. Apparently, the extent of intracellular survival is dependent on the physico-chemical characteristics of the biomaterial.

**Cytokines and biomaterial-associated infection.**

The difference in inflammatory reactions around various biomaterials is related to the local production of cytokines. The putative role of cytokines in BAI have been investigated in a number of in vitro and in vivo studies, discussed below.

**Cytokine responses in vitro.** Several in vitro studies showed that exposure of human monocytes or macrophages to biomaterials induces the production of cytokines [79-85]. However, detectable levels of cytokines, specifically of the pro-inflammatory cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α and IL-6, were only found in the presence of lipopolysacharide (LPS), a major cell wall component of gram-negative bacteria.
Moreover, only in the presence of LPS, biomaterials differed significantly in their cytokine-inducing properties. This indicates that the presence of bacteria or bacterial components can modulate the inflammatory tissue reaction caused by the implanted biomaterial. Pro-inflammatory cytokines are initiating the inflammatory response and are therefore suggested to play a role in bio-compatibility of biomaterials. Exaggerated production of pro-inflammatory cytokines may result in enhanced leukocyte activation causing bio-incompatibility [82]. The anti-inflammatory cytokines IL-4 and IL-13 have been studied extensively as well. IL-4 is an anti-inflammatory cytokine and was demonstrated to induce macrophage fusion on biomaterial surfaces [86-91]. Also the anti-inflammatory cytokine IL-13, was demonstrated to induce monocyte / macrophage fusion in vitro [92]. Apparently, these anti-inflammatory cytokines are modulating mediators in the chronic inflammatory response, characterized by the foreign body giant cells and encapsulation of the biomaterial.

Cytokine responses in vivo. Little is known regarding the role of the various cytokines in the inflammatory response to biomaterials in vivo. Vaudaux et al demonstrated that TNF-α production in a tissue cage model has a role in the local host defense against bacterial challenge [59]. Increasing the local TNF-α concentration prevented experimental S. aureus infection. Henke et al described that the suppressed MHC class II antigen (1a) expression by mononuclear cells neighboring an implanted biomaterial was associated with an enhanced pro-inflammatory status of the tissue, characterized by enhanced prostaglandin, especially prostaglandin E2 (PGE2), and IL-1 production [4]. Blocking the activity of these cytokines with indomethacine or anti-IL-1 respectively, prevented macrophage 1a suppression and increased the clearance of bacteria. This indicates that protracted high levels of IL-1 supports bacterial persistence.

In our subcutaneous mouse model for BAI, we described the cytokine profiles of tissue surrounding the biomaterials SE, SEpvp and PApvp [28,29]. Although sterile implantation of these materials induced a “normal” foreign body reaction, the cytokine profiles of tissue around these biomaterials differed significantly [28,29]. None of the cytokines tested surrounding sterile PApvp were increased. In contrast, around SE and SEpvp a significant increase in the production of the pro-inflammatory cytokines IL-6, TNF-α, IFN-γ and IL-1β was observed. The IL-1β levels remained elevated over time in tissue surrounding SEpvp. IL-
10, an anti-inflammatory cytokine, was not detected around any of these 3 biomaterials. IL-4, another anti-inflammatory cytokine, was only produced around SE [31].

When, in the same mouse model low numbers of \textit{S. epidermidis} were injected along the 3 implanted materials, persistent infection was induced around SEpvp and around PApvp but not around SE. Histologically, the foreign body response around SE remained normal. Also the cytokine profile did not change significantly, compared to those around sterile SE [28]. In contrast, around SEpvp an enhanced inflammation associated with abscess formation and persistent infection was observed histologically. This was associated with exaggerated and protracted IL-1 production. In IL-1 receptor type I gene deficient (IL-1R-/-) mice, a situation in which the IL-1 activity is blocked, no abscess formation nor persistent infection occurred [31]. Thus, sustained IL-1 levels, and the resulting protracted inflammatory response, apparently supported the bacterial persistence. The finding that sustained IL-1 levels may impair bacterial clearance was described by others as well [93-96]. Kanangat et al described that high concentrations of LPS, IL-1\(\beta\), IL-6 and TNF-\(\alpha\) stimulated intracellular growth in monocytes as well as extracellular growth of \textit{Staphylococcus aureus}, \textit{Pseudomonas aeruginosa} and \textit{Acinetobacter sp.}, which are all important nosocomial pathogens [94]. Various observations in humans also support a role for increased pro-inflammation in persistent infection; (i) patients suffering from acute respiratory distress syndrome, caused by acute development of diffuse lung inflammation, have persistently elevated levels of pro-inflammatory cytokines associated with an increased rate of nosocomial infections [97,98]; (ii) patients with long-term exposure to intravascular catheters exacerbated complications associated with sepsis [99]; (iii) patients who had an extracorporal circulation [100] as well as patients undergoing hemodialysis [101-104] with enhanced levels of circulating pro-inflammatory cytokines were highly susceptible to infection. Apparently, a threshold of cellular activation exists, at which phagocytic cells effectively kill ingested bacteria.

In our mouse model of BAI we observed a remarkable inflammatory response around PApvp after injection of \textit{S. epidermidis} [29]. Histologically a “normal” foreign body reaction was seen, however, large numbers of bacteria persisted in the tissue surrounding the implanted PApvp segment. Extended histological, immunohistochemical and electron-microscopical examinations demonstrated that the persisting bacteria were located intracellularly and associated with a granuloma [29]. The number of persisting \textit{S. epidermidis} remained stable.
during the first week, but gradually increased thereafter. During the first week the persistence was associated with a significant sustained increase in IL-1. The significant increase in persisting bacterial numbers after the first week coincided with an enhanced production of the anti-inflammatory cytokines IL-4 (data not shown) and IL-10 [29]. From various studies it is known that anti-inflammatory cytokines, such as IL-4, stimulate bacterial growth by reducing intracellular killing in macrophages without affecting phagocytosis [105-111]. In vivo, this intracellular bacterial growth stimulation is mainly seen in infections caused by intracellular pathogens [105,109,112,113], and is a novel observation in the pathogenesis of biomaterial-associated infection.

Apparently a variety of inflammatory responses can arise from the implantation of a biomaterial. Orchestrated interplay between cellular environment and biomaterial is required for proper functioning of inflammatory cells and for encapsulation in the host tissue.

Modulation of host inflammatory response to prevent biomaterial associated infections. Recently, promising results were obtained from animal studies modulating the inflammatory response to biomaterials. Rozalska et al demonstrated that granulocyte-macrophage colony-stimulating factor-coated implants reduced bacterial survival around an infected biomaterial in neutropenic mice, although abscesses remained present [114]. Henke et al found that mice injected with indomethacin or anti-IL-1, inhibiting and blocking the PGE2 and IL-1 activity respectively, were less susceptible to infection [4]. Similarly, we found that mice deficient for the IL-1 receptor type I are less susceptible to abscess formation and persistent infection. In addition, mice receiving interferon (IFN)-γ injections, to restore deactivated macrophages in the vicinity of the implanted biomaterial, were less susceptible to infection [30,31]. Histologically, no intracellular persistence of bacteria was observed in IFN-γ treated mice, while abundant intracellular persistence was observed in non-treated mice. These data clearly demonstrate that through local immunomodulation, BAI can be prevented in mice.

Conclusion.
The inflammatory response arising from the implantation of a sterile biomaterial varies significantly. The presence of low amounts of bacterial components or low numbers of bacteria can enhance the inflammatory response, making some biomaterials more prone to infection. Alterations in the host defense in the vicinity of an implanted biomaterial
compromise the local host-defense, resulting in a marked increase in the pathogenic potential of relatively non-pathogenic bacteria, such as *S. epidermidis*. Not the surface of a biomaterial, but the surrounding tissue, especially the intracellular environment of a macrophage seems to be an important niche for these bacteria to persist. To circumvent the enhanced susceptibility to BAI, a successfully balanced immune response is required. The balance is apparently strictly regulated, because an appropriate response can easily convert into an inappropriate reaction and pathology, causing an opportunistic infection. Rather than inhibiting adherence through surface modification or by developing biomaterials coated with antimicrobials, immunomodulating the local host inflammatory response may be more effective in preventing BAI.

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