

Infections of Orthopaedic Implants and Devices

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Abstract Prosthetic implantation is rising in popularity in the United States, and use of prostheses will only continue to increase with the aging of the Baby Boomer generation. A concurrent rise in the number of infections of these implants is also being seen. Prosthetic implant infection (PII) can be caused by direct inoculation of bacteria to the implant or by seeding from the blood (hematogenous). Because the implant is quickly coated by host connective tissue upon implantation, bacteria such as the staphylococcal species *S. aureus* and *S. epidermidis* are able to readily gain a foothold in the host. *S. aureus* is able to quickly develop antibiotic resistance and methicillin-resistant strains (MRSA) are considered endemic in hospitals. This bacterium features a myriad of virulence factors that allow it to colonize and damage the host, as well as avoid the host immune response. These virulence factors are largely regulated through population-based quorum sensing via the *agr* system. *S. aureus* is also able to form biofilms, microbial communities encased in a polysaccharide matrix, which allows the bacteria within to persist in the face of antimicrobial therapies and the host response. Diagnosis of PII is difficult with many false results and confusion with aseptic loosening of the implant. Because of ineffective means of diagnosis, combined with *S. aureus*' high incidence of antibiotic resistance and its ability to evade both antibiotics and the host response through biofilm formation, treatment for PII is often inadequate and infection can become chronic. Therefore, PII has a high rate of morbidity and mortality for patients, as well as an extreme economic burden on the US healthcare system.

1 Introduction

Prosthetic implant infection (PII) is a medical problem that is rising in importance as usage of artificial joints, intramedullary rods, plates, and screws increases. The total number of hip and knee replacements alone is over 500,000

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in the USA, and this number is expected to rise. Though only occurring in a minority of patients, PII is distressing due to a high rate of mortality and the high cost of treatment associated with these infections (Trampuz and Zimmerli 2005). Treatment of PII costs as much as \$50,000 per patient (Hebert et al. 1996), creating a large economic burden on the US healthcare system. In this chapter, we will first outline the ways in which PII can be initiated and the means of diagnosing and treating these infections. Next, we will detail aspects of the host that can contribute to the acquisition of PII, including some common risk factors. The most common etiologic agents of PII will be discussed, including the virulence factors that allow these bacteria to colonize, cause damage, and persist in the host. Finally, the host–pathogen interaction will be highlighted through outlining the normal host response to PII.

2 Routes of Infection

Prosthetic implant infection can originate from perioperative or postoperative exposure of the patient to the etiologic agent. Most infections that occur within 3 months of implantation are due to perioperative inoculation, where bacteria are introduced directly into the patient during, or soon after, surgery (Kaltsas 2004). This can occur due to the presence of commensal skin flora around the surgical site or from contamination from the healthcare provider. The usage of laminar flow in the operating room as well as antibiotic prophylaxis has decreased the incidence of microbial contamination of the surgical site during surgery (Hebert et al. 1996). The conditions of a surgical wound, including clotted blood and compromised soft tissues, also make it ideal for colonization. As well, infections can be caused by an associated bacteremia, where bacteria are seeded into the area of the implant, leading to an acute hematogenous infection. In these cases the prosthesis will function normally for some time before the onset of pain and swelling. Minor trauma likely predisposes the patient to infection by producing a small hematoma, vascular obstruction, and a subsequent bone necrosis that is susceptible to inoculation (Morrissy and Haynes 1989). Acute infection initially produces a local cellulitis that results in a destruction of leukocytes, increased bone pressure, decreased pH, and decreased oxygen tension. The collective effects of these physiologic factors further compromise the medullary circulation and enhance the spread of infection. Infection may proceed laterally through the Haversian and Volkmann canal system, perforate the bony cortex, and lift the periosteum from the surface of the bone. When this occurs in the presence of medullary extension, the periosteal and endosteal circulations are compromised; capillaries are lost, and large segments of cortical and cancellous bone die. If diagnosed more than 1 month after introduction into the body, the infection, whether caused peri- or postoperatively, is considered to be chronic and is often confused with aseptic loosening of the implant (Kaltsas 2004).

3 Diagnosis and Treatment of PII

Diagnosis of PII can be difficult. Because there is a lack of studies researching PII diagnosis and treatment, these often depend on the physician's personal knowledge, as well as tradition, and, ever increasingly, issues of liability (Hebert et al. 1996). Often, infection is only obvious if other indications are present, such as pain and fever. Aspiration of the suspected infected joint can aid in diagnosis, as can tests for PMN levels, C-reactive protein, and erythrocyte sedimentation rate (Bernard et al. 2004). However, sensitivities and specificities of these methods vary widely. PMN counts have a sensitivity that ranges from 20 to 83% and specificity from 80 to 100% (Bernard et al. 2004; Duff et al. 1996; Flivik et al. 1993; Lachiewicz et al. 1996; Spangehl et al. 1999). Measurement of C-reactive protein features sensitivity and specificity rates of 96–100% and 81–100%, respectively (Bernard et al. 2004; Sanzen and Carlsson 1989; Shih et al. 1987; Spangehl et al. 1999). The sensitivity and specificity of erythrocyte sedimentation rate varies from 29 to 100% and 0 to 100%, respectively (Bernard et al. 2004; Cuckler et al. 1991; Duff et al. 1996; Flivik et al. 1993; Lachiewicz et al. 1996; Levitsky et al. 1991; Roberts et al. 1992; Sanzen and Carlsson 1989; Shih et al. 1987; Spangehl et al. 1999; Teller et al. 2000; Thoren and Wigren 1991). Histopathological examination of the tissue surrounding the implant in order to gauge inflammation from infiltration of neutrophils can also aid in diagnosis and has a sensitivity of >80% and a specificity of >90% (Hebert et al. 1996).

Because aseptic loosening of the implant due to mechanical failure can closely resemble infection, it is of great importance to diagnose PII prior to corrective surgery (Bernard et al. 2004). There are several imaging methods that allow clinicians to envisage the region of possible infection. Conventional radiography is often used. However, radiographic changes to bone are often difficult to interpret, and can take up to 2 weeks following the onset of infection to reach a level that can be visualized because a 30–50% loss in bone density is often required (Butt 1973). Aseptic loosening can cause bone resorption, leading to false results as this will mimic infection radiographically (Bernard et al. 2004). Also, it can be difficult to determine the extent of infection. Ultrasound is another option, which is efficacious in showing fluid surrounding the artificial joint in suspected septic arthritis; however, this cannot differentiate between infection and joint effusion as a result of aseptic loosening (Bureau et al. 1999). Computed Tomography (CT) scans can be of better use due to their high level of detail. However, due to the problem of scatter in the presence of metal, this method is not always useful in the case of infected implants. It is also a very expensive procedure, which further limits its usefulness. Radionuclide scans are widely employed, and these help to identify areas of inflammation better than radiography alone. This method also has the benefit of being useful because there are no issues with scatter. One technique, technetium-99m methylene diphosphonate (Tc-99m MDP) scintigraphy, has proven to be effective and relies on pharmaceutical accumulation at areas of increased blood flow and bone repair. While sensitivity is high, specificity is lacking due to bone remodeling from the implantation of the

prosthetic device (Zimmerli et al. 2004), as well as new bone formation, fracture healing, heterotopic ossification, arthritis, and local minor trauma (Datz et al. 1984). Another radionuclide technique exploits indium-111 (In-111) labeled white blood cells, in which patient leukocytes are isolated, tagged with In-111, and injected back into the patient. This method works well in cases of suspected prosthetic implant infection when combined with bone marrow imaging with Tc-99m sulfur colloid marrow scintigraphy, since leukocyte uptake around prostheses may be caused by surgery. When an accumulation of leukocytes is seen, coupled with noncongruent bone marrow patterns and absent marrow uptake, an infection is likely. Tc-99m hexamethylpropylene amineoxime leukocytes (Tc-99m HMPAO WBC) are also employed to overcome the problems with In-111 WBC, such as the 24-h delay required for imaging, high levels of radiation in the spleen, and limited injection dose. Sensitivity of HMPAO WBC in diagnosis of PJI is 63%, while specificity is 96% (Sonmezoglu et al. 2001). The combination of these two scans leads to a sensitivity of 100% and a specificity of 94% (Palestro et al. 1992). MRI is the final imaging technique that can be utilized for diagnosis. This method allows differentiation between bone and soft tissue infection. However, MRI also has the issue of scatter when metal implants are present.

The gold standard of diagnosis for PJI is culture and identification of the infecting organism (Carek et al. 2001). Until a positive identification of the etiologic agent is made, the proper therapeutic treatment is delayed. Cultures cannot be taken from any superficial wounds or sinus tracts because they may feature contamination with skin flora (Hebert et al. 1996). Culture of the synovial fluid, in the case of infected joint replacements, can determine the etiologic agent 45–100% of the time (Hebert et al. 1996). Tissue biopsies taken via surgery are the most reliable, with accuracy ranging from 65 to 94% (Hebert et al. 1996). Once a positive diagnosis of infection has been made, treatment for PJI often consists of antimicrobial therapy along with two-stage revision of the implant, where the infected implant is removed and replaced after antimicrobial therapy has ended and the infection is eradicated (usually >6 weeks) (Hebert et al. 1996; Zimmerli et al. 2004). If the infection is acute (less than 3 weeks duration), and the implant is stable, debridement and retention of the implant may be feasible (Hebert et al. 1996; Zimmerli et al. 2004). Debridement consists of the excision of all infected tissue, including bone, soft tissue, and sinus tracts (Zimmerli et al. 2004). If the soft tissue around the implant is relatively undamaged, one-stage revision, where the implant is replaced during the same surgery as the removal, is possible (Hebert et al. 1996). In patients who are severely immunocompromised, abuse IV drugs, or for whom no functional improvement after surgery is expected, the infected implant is often removed and not replaced. Alternatively, long-term antibiotic therapy can be commenced if the patient is inoperable, is on long-term bedrest, or is debilitated (Hebert et al. 1996). In all cases, a broad-spectrum antibiotic is given until culture results are obtained to cover the most common pathogens, and then specific therapy is begun once the infecting agent is identified.

Antibiotic treatment must be administered for at least 4–6 weeks, with 3 months being suggested for hip prosthesis infection and 6 months for infected knee

replacements (Zimmerli et al. 2004). When a two-stage revision is being performed, antibiotic therapy is administered for 6 weeks, with 2–3 weeks without antibiotics following the treatment before replacement of the device (Trampuz and Zimmerli 2005). For staphylococcal infections, the regimen of choice includes rifampin, often combined with quinolones. Because staphylococci are beginning to show increased resistance to quinolones, fusidic acid is an alternate option for combination with rifampin (Zimmerli and Ochsner 2003). For *Staphylococcus aureus* infections specifically, particularly methicillin-resistant *S. aureus* (MRSA), the antibiotic of choice is vancomycin, with teicoplanin, trimethoprim-sulfamethoxazole, or minocycline plus rifampin being alternatives (Carek et al. 2001). Beta-lactam antibiotics are most often administered for streptococcal infections, either alone or in conjunction with rifampicin (Everts et al. 2004).

4 Host Factors Involved in Prosthetic Implant Infection

These will be discussed in more detail in Chap. 12. In general, it is clear that the host immune system responds to both the implant and to the presence of colonized bacteria on the implant, yet this response is not effective at clearing the infection. This is most likely due to the fact that biofilms are inherently resistant to killing by factors of the host immune system. Avoidance of these host factors is discussed later.

4.1 Microbial Species Responsible for Prosthetic Joint Infection

Postoperative PJI is generally monomicrobial in nature; that is, a single bacterial species is isolated from the infected region. Polymicrobial hematogenous PJI is rare (Waldvogel et al. 1970a–c; . Staphylococci cause approximately 75% of infections, and almost all PJI are caused by Gram-positive, aerobic cocci (Segawa et al. 1999). Coagulase-negative staphylococci are present in 30–43% of cases, while *S. aureus* is attributed to 12–23% (Zimmerli et al. 2004). *Staphylococcus spp.* are capable of causing PJI in immunologically normal children and adults, as well as in immature and immunocompromised individuals. Incidences of *S. aureus* infection are becoming more worrisome with the emergence of multiple-antibiotic-resistant strains such as MRSA. Until recently, the only drug to which all *S. aureus* was susceptible was vancomycin, but vancomycin-resistant strains are beginning to be isolated as well (Pechous et al. 2004). *S. aureus* can cause infections when acquired in the community rather than in the hospital. Though these infections tend to be skin-related, community-acquired strains also acquire methicillin resistance and are becoming of greater concern, not only because they are beginning to become more virulent (Lindsay and Holden 2004), but also because these strains are able to infect hosts with no predisposing risk factors outside of the hospital setting. Community-

acquired strains are beginning to play a more prominent role in deep tissue infections. In one study, the community-acquired MRSA strain USA300 was shown to cause more than half of the PII seen in subjects (Kourbatova et al. 2005). *Staphylococcus epidermidis* causes the majority of PII, and the acquisition of bio-film-mediating and antibiotic-resistant genes contributes to its ability to do so. Though there are many strains of *S. epidermidis*, one study (Kozitskaya et al. 2005) showed that the majority of infections in a particular hospital were caused by one clone that was able to form biofilms and was resistant to beta-lactam antibiotics. This suggests that clinical isolates have an advantage when they contain these genes, which may be acquired by horizontal transfer (Kozitskaya et al. 2005). Overall, other pathogenic microorganisms associated with PII include *Enterococcus spp.* (3–7%), *Streptococcus spp.* (9–10%), Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Enterobacter spp.* (3–6%), *Mycobacterium spp.*, as well as anaerobic and mycoidal species (specifically *Candida spp.*) (Zimmerli et al. 2004). Each of these pathogenic species, individually, represents a small minority of infections. The immature or compromised immune status of the host is the primary cause of initial infection and development into a persistent and chronic PII infection by these other species.

4.1.1 The MRSA Epidemic

Once considered an aberrant, rare strain, MRSA is now the most commonly isolated nosocomial bacterial pathogen in most of the world (Grundmann et al. 2006). Approximately 40–60% of all nosocomially acquired *S. aureus* are methicillin-resistant, and these strains are now considered endemic in hospitals (Lindsay and Holden 2004). These infections can be devastating. A recent report of the Journal of the American Medical Association (JAMA) stated that approximately 94,000 individuals were diagnosed with invasive MRSA in 2005, leading to 19,000 deaths (Klevens et al. 2007). These numbers make deaths due to MRSA higher than those caused by HIV infection. The infection rate of 31.8 per 100,000 for invasive MRSA is also higher than the rates for invasive pneumococcal disease, Group A streptococcus, meningococcal disease, and *Haemophilus influenzae* infection combined (Camargo and Gilmore 2008). This is an increasing burden on the healthcare system as MSSA (methicillin-susceptible strains) infections are not concurrently decreasing in number (Gould 2005). Colonization with MRSA does seem to be correlated with nosocomial infection, and patients who get infected while admitted often have a recurrence once discharged (Huang and Platt 2003). As mentioned, MRSA is also rampant in the community; CA-MRSA is now also the most common cause of skin and soft tissue (SSTI) infections in the USA (Moran et al. 2006). CA-MRSA can cause significant mortality via invasive illness as well. CA-MRSA is all the more worrisome due to the fact that it is able to easily infect healthy young adults with no risk factors. This is exemplified in the rising incidence of CA-MRSA among young athletes (Centers for Disease Control 2003). As well, multidrug resistance also

seems to play a role in promoting biofilm formation (discussed later). In a study of 101 clinical isolates, 38% of MRSA strains could form biofilms, while only 14% of MSSA isolates could do the same (Kwon et al. 2008). This illustrates the idea that MRSA strains may be more apt to cause chronic infections such as PII than their MSSA counterparts. With the increasing age of the US population as the Baby Boomer generation nears retirement, the need for prosthetic implants will rise. This, combined with MRSA's increasing incidence in the community – both due to colonization acquired in the hospital and due to community-acquired disease – means that MRSA infections likely will continue to be a leading healthcare problem.

4.1.2 Virulence Factors of Staphylococci

S. aureus has a large number of virulence factors that allow it to cause a wide range of diseases. The *S. aureus* virulence factors are responsible for colonization and damage to the host, as well as avoidance of the immune response. These factors include regulatory systems that control virulence factor expression, biofilm formation, adherence proteins, toxins, and immuno-avoidance factors.

4.2 Regulation of Virulence Factors (*Quorum Sensing*)

Cell-to-cell communication between bacteria is a sophisticated process resulting from environmental cues, leading to the release of diffusible signal molecules that bind to a receptor, with the end result of modulated gene expression. This process, which is cell-density dependent (Miller and Bassler 2001), is termed quorum sensing, an expression first coined by Fuqua et al. (Fuqua et al. 1994). Quorum sensing depends upon the production and release of small molecules called autoinducers. For a molecule to be considered a cell-to-cell signal molecule (CCSM), it must meet several criteria, including the following: production during specific growth stages or environmental conditions; extracellular accumulation and the ability to bind to a receptor; and the generation of a concerted response when a critical concentration is reached, which extends beyond that required to simply metabolize or detoxify the molecule itself (Winzer et al. 2002).

There are currently four different identified autoinducer types utilized by bacteria for quorum sensing. AI-1, utilized by some Gram-negative species such as *P. aeruginosa*, is an *N*-acyl-homoserine lactone (AHL) (Pearson et al. 1994). AI-2, first discovered in *Vibrio harveyi*, is a furanosyl-borate-diester (Sun et al. 2004). As this molecule has been found to be produced by many bacteria (Sun et al. 2004), it is hypothesized that it may play a role in interspecies communication, as was shown in multispecies dental biofilms (McNab et al. 2003). This could have a significant implication in the formation of the multispecies biofilm infections common to contiguous focus osteomyelitis in the diabetic foot. Another autoinducer, AI-3, has been shown to modulate quorum sensing in Enterohemorrhagic *E. coli* (EHEC).

AI-3 has been shown to activate the transcription of the virulence genes of the LEE (Locus of Enterocyte Effacement) and can cross-talk with the human hormones epinephrine and norepinephrine to activate these genes (Sperandio et al. 2003). It is not currently understood whether norepinephrine triggers LEE expression in EHEC by directly substituting for AI-3, or whether it promotes endogenous AI-3 production or perhaps the synthesis of autoinducer(s) by the endogenous microflora (Vlisidou et al. 2004). As norepinephrine has been shown to increase adherence of EHEC to intestinal epithelium (Chen et al. 2003), perhaps AI-3 itself can also act in this same fashion to help increase colonization. Also, a study has found that incubation of *S. epidermidis* with norepinephrine significantly increased biofilm formation on polystyrene (Lyte et al. 2003). Norepinephrine also decreases bacterial clearance (Koch et al. 1996). The final autoinducer system is found in Gram-positive bacteria and involves a signal peptide. In this section, we will focus on the latter system in *Staphylococcus spp.*

During early exponential growth, *S. aureus* has a low cell density and produces proteins involved in adherence and colonization (discussed later). However, in late exponential phase, the cell density increases, and *S. aureus* begins secreting proteins involved in acquiring nutrients, damaging the host, and disseminating to new areas (Shirliff et al. 2002). Gram-positive bacteria utilize peptides typically structured as a 16-membered side chain to tail macrolytic peptide, with a short linear peptide attached to its amino-terminus (Miller and Bassler 2001). In *S. aureus*, quorum sensing is under partial or complete control of the staphylococcal accessory regulator (*sar*) and the accessory gene regulator (*agr*) systems. The *agr* locus, which is approximately 3 kb in size (Novick 2003), consists of two divergent transcription units, driven by promoters P2 and P3. The P2 operon contains four genes, *agrB*, *agrD*, *agrC*, and *agrA*, while the P3 operon codes for RNAIII (Novick et al. 1995). An octapeptide with a unique thioester ring structure (referred to as the *agr* autoinducing peptide (AIP)) is generated from its precursor, AgrD, and secreted out of the cell through the action of AgrB (Ji et al. 1997). AgrD is a propeptide that must be altered to become the active AIP with a unique thiolactone ring between a conserved central cysteine and the peptide's carboxy terminus (Novick 2003). This ring structure is vital for its function, as a replacement of the thiolactone by a lactone abrogates the activation properties of AIP (Novick 2003). Also, artificial compounds imitating this thiolactone structure were able to inhibit quorum sensing (Scott et al. 2003). It is believed that this processing is mediated by AgrB (Ji et al. 1997; Saenz et al. 2000; Zhang et al. 2002). AgrB is associated with the cytoplasmic membrane, with the N-terminus and C-terminus both being found within the cytoplasm, and six transmembrane segments in between (Zhang et al. 2002). Recently, AgrB was shown to be an endopeptidase (Qiu et al. 2005). It was also determined that two amino acids were required for this function – His⁷⁷ and Cys⁸⁴ (Qiu et al. 2005). Zhang et al. recently discovered that the AgrD propeptide is anchored into the cytoplasmic membrane via its amphipathic, α -helical N-terminal region, which stabilizes it for its interaction with AgrB (Zhang et al. 2004). AIP is sensed by the two-component signal regulatory system that is composed of AgrC and AgrA (Miller and Bassler 2001). As the concentration of AIP increases in the

extracellular microenvironment, the interaction between AIP and the histidine kinase receptor, AgrC, also increases. This interaction possibly acylates AgrC and enables it to phosphorylate and thereby activate an intracellular *agr*-encoded protein (AgrA) (See figure of *agr* system) (Mayville et al. 1999; Morfeldt et al. 1996). AgrA subsequently positively regulates transcription from P2 and activates transcription from P3 (Novick 2003) (Fig. 1).

The intergenic region between P2 and P3 likely houses the transcription factor binding sites necessary for activation of the operons. In fact, there is a 17-bp inverted repeat in this region that may be a bidirectional regulatory binding protein site (Bayer et al. 1996). However, AgrA does not contain a DNA binding domain. On the other hand, SarA, encoded by the regulatory locus *sar*, does contain a putative DNA-binding domain, and has been shown to bind a consensus motif within the *agr* promoter, leading to initiation of both RNAII (*agrBDCA*) and RNAIII transcription (Heinrichs et al. 1996). Recently, however, AgrA has been shown to bind to the P2-P3 promoter region via electrophoretic mobility shift assay (Koenig et al. 2004). SarA has also been shown to directly interact with the promoter regions of a number of other genes, including Protein A, fibronectin binding protein, and alpha-hemolysin (Chakrabarti and Misra 2000; Wolz et al. 2000). However, the interactions with this complex system are still being elucidated. Since *sar* and RNAIII homologs have been identified in a number of coagulase-negative *Staphylococcus spp.*, including *S. lugdunensis* (Chan and Foster 1998), *S. epidermidis* (Lina et al. 1998; Van Wamel et al. 1998), *S. simulans*, and *S. warneri* (Van Wamel et al. 1998), this regulation system is also used by the other members of the staphylococcal genus. Therefore, it is

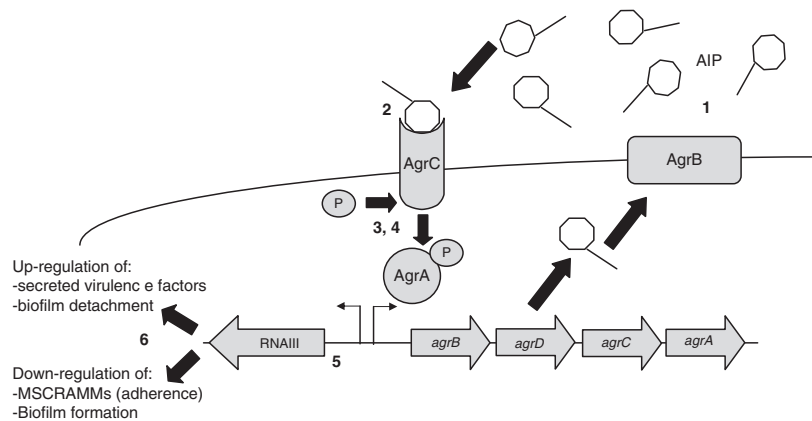


Fig. 1. The *agr* staphylococcal quorum sensing system. As the bacterial population grows, AIP molecules (produced from *agrD* and secreted via AgrB) accumulate in the extracellular milieu (1). These peptides then bind to AgrC (2), leading to the proteins autophosphorylation (3). This phosphate is then transferred to AgrA (4), which can bind to the intergenic region containing P2 and P3, leading to increased transcription of RNAIII (5). In this way the *agr* system is able to regulate the expression of genes for downstream effectors (6)

hypothesized that AgrA and SarA work cooperatively to bind within the *agr* promoter to initiate transcription. This is supported by a study in which RNAII and RNAIII levels decreased 2.6-fold in a *sar* mutant, suggesting that SarA is necessary for wild-type levels of transcription (Dunman et al. 2001).

During early logarithmic growth, a protein encoded by *rot* (repressor of toxins) inhibits the expression of *agr*-activated virulence factors (McNamara et al. 2000). Once activation of the *agr* and *sar* regulatory loci occurs during late exponential phase, there is increased transcription of an *agr* regulatory RNA molecule known as RNAIII (Balaban and Novick 1995). RNAIII, the product of the P3 promoter, is the real effector of the Agr response, in that it positively controls the expression of secreted proteins and negatively regulates cell surface-associated proteins (Zhang and Ji 2004). RNAIII immediately blocks the production of cell wall proteins that are upregulated in early exponential phase, such as coagulase, Protein A, and the fibronectin binding proteins (Chan et al. 2004), and, with a hypothesized timing signal, upregulates transcription of extracellular pathogenicity factors (such as exotoxins). The chief regulatory function of RNAIII is at the level of transcription by an unresolved mechanism, but may involve one or more regulatory proteins (Morfeldt et al. 1996). This RNA molecule is also capable of controlling production of at least two virulence factors, alpha-hemolysin (*hla*) and Protein A (*spa*), at the level of translation. Recently, a *hla* knockout was shown to be deficient in biofilm production at the level of microcolony formation, suggesting that this protein may be necessary for cell-to-cell communication (Caiazza and O'Toole 2003). At the beginning of exponential phase growth, the expression of alpha-hemolysin is normally inhibited through intramolecular base pairing that blocks the ribosomal binding site (Morfeldt et al. 1995; Morfeldt et al. 1996). Later in exponential phase, RNAIII is expressed and folds into a stable but inactive regulatory molecule. After a significant lag, the secondary structure of RNAIII changes through an unknown agent, and the 5' region of RNAIII is then able to hybridize with a complementary 5' untranslated region of *hla* mRNA, thereby making the transcripts accessible for translation initiation (Morfeldt et al. 1995). Conversely, the 3' region of RNAIII contains sequences complementary to the leader sequence of *spa*, and hybridization is believed to inhibit translation of Protein A. In addition, SarA (the primary product of *sar*) has been shown to have an inhibitory effect on the expression of a number of genes, including *cna*, *sea*, *sar*, and the *agr* operon (Wolz et al. 2000). Therefore, *sarA* expression may be autoregulated, but the interactions with this complex system are still being elucidated. Another product of the *sar* locus, SarU, has been shown to be a positive regulator of RNAIII (Manna and Cheung 2003).

As the concentration of AIP rises in the extracellular environment, the level of RNAIII also increases. As mentioned, RNAIII is the intracellular effector of the *agr* system (Novick et al. 1993), enabling the growth phase-dependent reduction in adherence factor production and increase in extracellular pathogenicity factor production. AIP is not only capable of activating the *agr* regulon in self strains, but can also inhibit the *agr* activation of other *S. aureus* strains. Based on *agr* sequences and precise recognition between the AIP and AgrC, there are four identified groups of *S. aureus* (Jarraud et al. 2000). The groups are defined by the ability to mutually

inhibit one another's *agr* response, resulting in a unique regulation in which *agr* activity, but not growth, is inhibited (Ji et al. 1997). In one study, chimeric AgrB molecules were created in which sections of AgrB2 were swapped into AgrB1 and vice versa, and processing of AgrD1 and AgrD2 was examined. The first transmembrane α -helix and the extracellular loop 1 of AgrB1 were the most vital segments in determining the precise processing of AgrD1. On the other hand, the same segment in AgrB2 was not imperative in its group-specific contact with AgrD2. In its place, the two hydrophilic segments (methionine 67 to glycine 75 and alanine 126 to lysine 141) in AgrB2 were necessary for its specific processing of AgrD2 (Zhang and Ji 2004). In another study, AgrC chimeras were constructed and tested for activation or inhibition specificity against different AIPs. This work showed that AgrC/AIP binding is hydrophobic in nature, but activation or inhibition of *agr* via this binding is allele-specific (Wright et al. 2004). It has been shown that the AIP from group I (AIP1) is able to potently antagonize AgrC from groups II and III (Fig. 2) (Bhasin et al. 1998; Boles and Horswill 2008). This brought about the idea that AIPs from one group of *S. aureus* could be used therapeutically against infections by other groups. In a mouse protection test, skin abscesses were attenuated when Group II AIP was coadministered with Group I *S. aureus* in a subcutaneous infection (Mayville et al. 1999). In a more recent study, it was shown that *agr* antagonists work to prevent *agr* activation during the initial growth phase of the bacteria. In a mouse abscess model, this was able to abrogate virulence, suggesting that the *agr* quorum sensing that occurs in the early part of infection is critical for bacterial survival and abscess formation (Wright et al. 2005). While these AgrC antagonists may help to eradicate systemic *S. aureus* infections, they are unlikely to be successful against biofilm infections such as PII (discussed later). Reactive oxygen species released from phagocytes have been shown to interfere with the Group I autoinducer peptide, suggesting that these cells are able

Staphylococcus aureus - Thioester-containing octapeptide

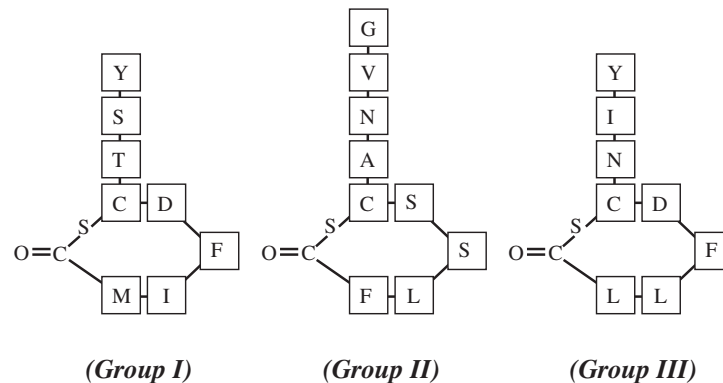


Fig. 2. Autoinducer peptide (AIP) structures. The three major groups of AIP molecules involved in quorum sensing are shown

to target quorum sensing in order to protect the host (Rothfork et al. 2004). This idea could also lead to novel therapeutic strategies in treating *S. aureus* infection.

The interaction of the quorum sensing system with certain host factors can also have a role in pathogenicity. Rothfork et al. showed that fibrinogen, a component of the inflammatory response that can cause staphylococcal clumping, was able to increase the virulence of wild-type *S. aureus* but not of an *agr* mutant. It was shown that fibrinogen's effect was on the delivery of AIP to the two-component AgrC-AgrA regulatory system that causes an increase in RNAIII transcription. The authors concluded that fibrinogen-induced clumping of *S. aureus* creates a microenvironment within the host that aids quorum sensing-dependent virulence, resulting in augmented bacterial burden and host morbidity and mortality (Rothfork et al. 2003).

Another two-component regulator, *saeRS*, has been shown to interact with Agr. This system represents the second two-component regulator involved in global virulence of *S. aureus* (Novick 2003). In a *sae-agr* double mutant, neither complementation with *agr* nor *sae* could restore production of secreted proteins (Novick 2003), suggesting that the two work together. Transcription of *sae* requires RNAIII, but *sae* is not required for *agr* activation, meaning that *sae* must be downstream of *agr* in the regulatory cascade (Novick and Jiang 2003). As varying environmental conditions, such as osmolarity and pH, seem to affect *sae* transcription, this locus may bridge the gap between cell density and environmental cues (Novick 2003).

4.3 Biofilm Formation

As mentioned previously, several bacteria responsible for PII are able to grow and persist chronically as a biofilm. Prosthetic implantation provides an ideal environment for biofilm infections to flourish, as the incision offers a route into the host, and the implant becomes an ideal attachment surface (Stoodley et al. 2005). A biofilm is defined as a microbially derived sessile community, typified by cells that are attached to a substratum, interface, or each other, are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression, and protein production (Donlan and Costerton 2002). Biofilm depth can vary from a single cell layer to a thick community of cells surrounded by a substantial polymeric milieu. Structural analyses have shown that these thick biofilms possess a complex architecture in which microcolonies can exist in distinct pillar or mushroom-shaped structures (Costerton et al. 1995), through which an intricate channel network runs. These channels provide access to environmental nutrients even in the deepest areas of the biofilm.

Within this complex architecture, various microniches of biofilm bacteria can exist. For example, DNA and protein production seems to be restricted to areas at the air/nutrient interface, and most of the biofilm's mass is metabolically inactive but viable (Rani et al. 2007). As well, biofilm-upregulated proteins have been shown via immunofluorescence confocal microscopy to be produced in some microcolonies but not others, or even within some individual cells within micro-

colonies (Brady et al. 2007). This indicates that protein production is heterogeneous, likely due to the varying levels of nutrient and oxygen availability within various areas of the biofilm.

By adopting this sessile mode of life, biofilm-embedded microbes benefit from a number of advantages over their planktonic counterparts. One advantage is the capability of the extracellular matrix to seize and concentrate a number of environmental nutrients, such as carbon, nitrogen, and phosphate (Beveridge et al. 1997). Another benefit to growing as a biofilm is the facilitation of resistance to a number of removal tactics, such as elimination by antimicrobial and antifouling agents, shear stress, host phagocytic clearance, and host oxygen radical and protease defenses. This innate resistance to antimicrobial factors is mediated through very low metabolic levels and radically downregulated rates of cell division (e.g., small colony variants) of the deeply entrenched microbes (Brown et al. 1988). One study even concluded that *P. aeruginosa* stationary phase cells and biofilm cells have comparable resistance to killing by antimicrobials (Spoering and Lewis 2001). While low metabolic rates may explain a great deal of the antimicrobial resistance properties of biofilms, other factors likely play a more major role. One such feature may be the capability of biofilms to act as a diffusion barrier to slow down the infiltration of some antimicrobial agents (Xu et al. 2000). For example, reactive chlorine species (such as hypochlorite, chloramines, or chlorine dioxide) in a number of antimicrobial/antifouling agents may be deactivated in the surface layers of the biofilm before they are able to disseminate into the lower layers (De Beer et al. 1994). In another study, alginate (a component of *P. aeruginosa* exopolysaccharide) was shown to be able to induce an α -helical conformation in antimicrobial peptides and likely entraps these peptides, preventing their diffusion into the biofilm (Chan et al. 2004). However, investigations of *S. epidermidis* biofilms with fluorescent molecules such as rhodamine demonstrated that these molecules were able to rapidly diffuse into the biofilm. Because the tracers used mimic the size of many antibiotics, at least in the case of *S. epidermidis*, prevention of antibiotic infiltration as a means of antimicrobial resistance is unlikely (Rani et al. 2005). As well, the nature of the biofilm allows for heterogeneous areas of oxygenation within the biofilm, with pockets of bacteria existing within an anaerobic environment (Stoodley et al. 2005). This can lead to the inactivation of antibiotics that are efficacious in physiologic conditions. Importantly, a recent study has shown that low-level exposure of *P. aeruginosa* to aminoglycoside antibiotics actually leads to the induction of biofilm formation (Hoffman et al. 2005). Resistance to the host response may include the prevention of host inflammatory molecules from entering the biofilm, although white blood cells are not limited in their ability to penetrate into staphylococcal biofilms (Leid et al. 2002). As well, the host response can cause host cell lysis and subsequent damage to the host tissue. This can lead to the release of host cell components, which serve as nutrients for the bacteria (Stoodley et al. 2005).

The final benefit to the biofilm manner of growth is the potential for dispersion via detachment. As mentioned, microcolonies exist in discrete mushroom-shaped or tower structures. These microcolonies may detach under the direction of mechanical fluid shear or through a genetically programmed response that mediates

the detachment process (Boyd and Chakrabarty 1994). Under the direction of fluid flow, this microcolony travels to other regions of the host to attach and promote biofilm formation in previously uninfected areas. In *S. aureus*, this movement is via *tethered rolling* of biofilm flocs via viscoelastic attachments to the substratum (Rupp et al. 2005). In addition, detachment and seeding of virgin surfaces may be accomplished by the migration of single, motile cells from the cores of attached microcolonies (Sauer et al. 2002). Therefore, this advantage allows an enduring bacterial source population that is resilient against antimicrobial agents and the host immune response, while simultaneously enabling continuous shedding to encourage bacterial spread.

Patients with PII display many of the signs currently associated with biofilm-associated infections in other diseases, including negative culture results even in the face of signs of infection; chronicity of infection with periodic acute, systemic disease; and minimal responsiveness to antibiotics (Stoodley et al. 2005). However, because these symptoms can closely resemble those of aseptic loosening, it is of paramount importance to continue researching novel means of diagnosing these implant-associated biofilm infections (discussed earlier).

Staphylococcus spp. produce a multilayered biofilm embedded within a glycocalyx, or slime layer (Gristina et al. 1985). The glycocalyx develops on devitalized tissue and bone (such as the involucrum), or on medically implanted devices, to produce an infection (Akiyama et al. 1993). The presence of implants is a predisposing factor in the development of infection since they are coated in host proteins, including fibrinogen and fibronectin, soon after implantation, and this host protein coating provides an excellent source of attachment for any bacteria remaining after debridement surgery (Herrmann et al. 1988). Once attached, the bacteria can form the glycocalyx, which protects the bacteria from normal host defenses and systemic antibiotics (Oie et al. 1996), particularly those active against cell wall synthesis (Cerca et al. 2005). Though one study shows that biofilm formation is not necessary to cause persistent infections (Kristian et al. 2004), biofilms are difficult to eradicate and thus deserve special attention.

Early studies described the solid component of the glycocalyx as primarily composed of teichoic acids (80%) as well as staphylococcal and host proteins (Hussain et al. 1993). Host-derived proteins, such as fibrin, may result from the conversion of fibrinogen by the staphylococcal coagulase–prothrombin complex (Akiyama et al. 1997). In later studies, a specific polysaccharide antigen named polysaccharide intercellular antigen (PIA) was isolated. PIA is composed of β -1,6-linked *N*-acetylglucosamine residues (80–85%) and an anionic fraction with a lower content of non-*N*-acetylated d-glucosaminyl residues that contains phosphate and ester-linked succinate (15–20%) (Mack et al. 1996). PIA is a polymer of approximately 130 residues, but other sizes of this β -1,6-linked *N*-acetylglucosamine have been identified, termed PNAG-I (the immunogenic 460-kDa compound), II (100 kDa), and III (21 kDa) (Maira-Litran et al. 2002). Depolymerization of PIA was recently shown to disrupt biofilms of *S. epidermidis*, supporting the role of this substance in biofilm formation (Itoh et al. 2005). PIA is produced in vitro from UDP-*N*-acetylglucosamine via products of the intercellular adhesion (*ica*) locus

(Cramton et al. 1999). The genes and products of the *ica* locus [*icaR* (regulatory) and *icaADBC* (biosynthetic)] have been demonstrated to be necessary for biofilm formation and virulence, and are upregulated in response to anaerobic growth, such as the conditions seen in the biofilm environment (Cramton et al. 2001). Though not found in all PII-causing clinical isolates of *S. epidermidis*, when the *ica* locus is present, it is always in its entirety, with every gene present (Arciola et al. 2005). This locus has been shown to be necessary for biofilm formation in *S. epidermidis* and for increased virulence in a rat model of infection (Li et al. 2005). Other studies, however, do not see a significant correlation between the presence of the *ica* locus and biofilm formation, but do find a relationship between the transcription of *icaA* and *icaD* and biofilm production (Cafiso et al. 2004). In another study, analysis of 112 *S. aureus* clinical isolates showed that, while all had the *ica* genes, none produced biofilms (Kim et al. 2008). Therefore, the relationship of the presence of *ica* and biofilm production is still being debated. In *S. aureus*, the *ica* locus seems to be dispensable, as the activation of the *ica* locus in four clinical isolates did not always lead to an increase in biofilm formation (Fitzpatrick et al. 2005a). This alludes to a possible *ica*-independent mechanism of biofilm formation. Deacetylation of PIA via IcaB has been shown to be important for biofilm formation (as an *icaB* mutant was unable to attach), resistance to phagocytosis by neutrophils, and colonization in a mouse model of infection (Vuong et al. 2004b). The regulation of *ica* in *S. epidermidis* is via reversible inactivation by insertion sequence (IS256) phase variation in 25–33% of variants (Conlon et al. 2002), and this has recently been observed in some *S. aureus* strains as well (Kiem et al. 2004). Recently, phase variation due to IS256 insertion was shown to be regulated via the stress-response sigma factor SigB (discussed later) (Valle et al. 2007). The protein GdpS has also been shown recently to be involved in biofilm formation through regulation of *ica*. While its means of *ica* regulation remains unclear, it has been demonstrated that GdpS, which is involved in c-di-GMP synthesis in other species of bacteria, is working in a c-di-GMP-independent manner in staphylococci (Holland et al. 2008). Regulation of PIA synthesis also seems linked to the TCA cycle, as when the TCA cycle was disrupted, PIA production was decreased (Vuong et al. 2005). Changes in the cycle may be utilized by the bacterial cells to detect alterations in the environment. Temperature may also play a role, as it has been shown that both elevated and decreased temperatures can induce *S. epidermidis* biofilm formation (Fitzpatrick et al. 2005b). Other levels of control in *S. epidermidis* are accomplished through IcaR-mediated transcriptional repression (relieved by ethanol stress) and the *sigB* operon product σ^B (regulated by operon genes *rsbU* and *rsbV*). Jefferson et al. (Jefferson et al. 2003) recently discovered that IcaR binds to a 42-bp region just upstream of *icaA*, and hypothesize that its role is to sterically hinder the binding of σ^B , thus preventing activation of the *ica* locus. Because σ^B regulates the expression of many genes involved in surviving in times of environmental stress, it has been hypothesized that PIA serves to protect *S. epidermidis* cells from these stresses (Jager et al. 2005). However, further studies are needed to evaluate this theory. Another component of *S. epidermidis* biofilms is poly-dl-glutamic acid (PGA), which is encoded by the *cap* locus. This locus is ubiquitous among *S. epidermidis* strains, unlike the *ica* locus, and was found to increase

resistance to high salt and the innate host immune response (Kocianova et al. 2005). Expression of PGA was also shown to be necessary for infection of a PII mouse model in this study.

In *S. aureus*, several virulence factors are σ^B -regulated genes, including clumping factor, fibronectin binding protein A, and coagulase (Nair et al. 2003; Nicholas et al. 1999), all of which are positively controlled, as well as alpha- and beta-hemolysin, enterotoxin B, SplA (a serine protease), cysteine protease (SplB), the metalloprotease Aur, staphopain, and leucotoxin D, all of which are negatively regulated (Kullik and Giachino 1997). Thus, the genes needed for attachment and biofilm formation are upregulated by σ^B . However, biofilm formation in vivo does not necessarily require σ^B , as a recent study showed that in a catheter infection model, both wild type and SigB-deficient mutants were able to form biofilms equally well (Lorenz et al. 2008). Also, IcaR is a strong negative regulator of the *ica* locus, as deletion of *icaR* augmented PIA production by nearly tenfold, and increased transcription of the *ica* locus approximately 100-fold (Jefferson et al. 2004). Another gene, *rbf*, has recently been identified by transposon mutagenesis. The Rbf protein was shown to be important in multicellular aggregation during biofilm formation, and also in the induction of biofilm formation by NaCl and glucose, but had no effect on *ica* transcription (Lim et al. 2004).

In addition to PIA, a number of other studies have elucidated vital genes and their products in the development of staphylococcal biofilms. There is recent evidence that attachment of bacterial cells to a polymer surface, a prerequisite for biofilm formation, may be promoted by an autolysin of *S. epidermidis* (Heilmann et al. 1997); the homologue in *S. aureus* (*atl*) may also function in this manner. In fact, a regulatory system termed WalK/WalR has been shown to upregulate AtlA and biofilm formation in *S. aureus* (Dubrac et al. 2007). Recently, an *ica*-independent biofilm phenotype was determined to be due to FnBPA and B, two proteins involved in initial ligand binding. This biofilm growth was limited to MRSA strains and could be activated through increased glucose concentrations in vitro (Huang and Platt 2003). A two-component regulatory gene locus that mediates adhesion and influences biofilm formation in *S. aureus* has also been studied recently. This locus is a system encoded by *arlRS*, a member of the OmpR-PhoB family of response regulators, that is regulated by the *agr* and *sarA* loci (Fournier and Hooper 2000; Fournier et al. 2001). When upregulated, the product of *arlS* prevents biofilm formation and may mediate attachment to polymer surfaces by affecting peptidoglycan hydrolase activity. Recent work determined that *arlRS* negatively regulates genes involved in the early attachment of *S. aureus* to surfaces, and that its repression is in effect under both static and flow growth conditions. In an *arlRS* mutant strain, biofilm thickness is significantly greater than wild type, but this increased density is not due to increased PIA production, *agr* activity, or autolysin (Toledo-Arana et al. 2005). Extracellular teichoic acids have been shown to be crucial in the development of *S. epidermidis* biofilms (Sadovskaya et al. 2005), and their structure is also crucial. Specifically, the addition of D-alanine esters to teichoic acids via *dltA* may be an important factor in imparting the proper charge balance on the Gram-positive cell surface, enabling initial attachment and subsequent biofilm formation. The accumulation associated protein (Aap) of *S. epidermidis* is implicated in biofilm

formation in strains that lack the *ica* locus. In particular, Aap seems to be processed to a truncated form by host proteases, and this smaller form is able to induce biofilm formation, thus aiding the bacterium in avoiding the host response (Rohde et al. 2005). Monoclonal antibodies generated against Aap are able to block biofilm formation by *S. epidermidis*, supporting the protein's importance (Sun et al. 2005). A similar protein in *S. aureus*, SasG, has been implicated in nasal colonization in vivo (Corrigan et al. 2007). Another *S. aureus* gene, biofilm associated protein (Bap), which was required for biofilm formation on inert surfaces, was discovered via transposon mutagenesis. This protein is found on the cell surface and its gene expression is positively regulated by the SarA protein (Trotonda et al. 2005). Found in mastitis-causing *S. aureus* strains, biofilm production has been shown to be inhibited through the binding of calcium ions to this protein, illustrating the idea that mastitis may be exacerbated by a lack of biofilm formation (Arrizubieta et al. 2004). This gene is encoded on the pathogenicity island SaPIbov2, and evidence of horizontal gene transfer of this island between staphylococcal species is beginning to be uncovered (Tormo et al. 2005a). However, testing of 262 clinical and animal isolates showed that none carried the *bap* gene; thus, the in vivo significance of this protein may be doubtful (Vautor et al. 2008). Lipoteichoic acid (LTA) also seems important, as an LTA mutant completely lost its ability to form biofilms, likely due to changes in the surface hydrophobicity of the cells. Because prosthetic implants are often hydrophobic in nature, this could have important implications for potential biofilm therapies (Fedtke et al. 2007).

Besides polysaccharide and proteins, extracellular DNA (eDNA) has also been shown to be important for biofilm formation. Rice et al. recently showed that eDNA is important for *S. aureus* adherence and biofilm formation and that its release is due, at least in part, to the *cidA* murein hydrolase regulator (Rice et al. 2007). It is thought that the CidA protein is a holin that allows for bacterial cell lysis. The autolysin AtlE in *S. epidermidis* has also been implicated in this process for the release of chromosomal DNA and early attachment (Seol et al. 1997). The *cidA* gene's expression has been shown to be upregulated by CcpA, a protein involved in catabolite repression that augments biofilm production in the presence of glucose. CcpA has also been shown to upregulate *ica* (Seidl et al. 2008). Though also present in *S. epidermidis* biofilms, eDNA seems to play a more vital role in *S. aureus* biofilms, as DNase I was able to prevent biofilm formation by this species, as well as promote detachment of preformed biofilms and render *S. aureus* biofilms sensitive to detergents, while *S. epidermidis* biofilms were less affected (Izano et al. 2008).

In another study, the differential gene expression in planktonic (shaken) vs. biofilm (static) *S. aureus* cultures was evaluated, and five genes whose expression was increased in biofilms were identified (Becker et al. 2001). These included the gene encoding threonyl-tRNA synthetase (upregulated by amino acid starvation), three oxygen starvation response genes, and the ATPase ClpC. This protein has been shown to be involved in regulating the TCA cycle and entry into death phase, as well as the response to environmental stresses (Chatterjee et al. 2005; Frees et al. 2004). Another study employing microarrays to study differential gene expression between these conditions found 48 genes that were enhanced at least twofold in the biofilm

compared to planktonic conditions (Beenken et al. 2004). Resch and colleagues recently discovered that, besides the genes mentioned earlier, other genes upregulated under biofilm conditions include *sdrC*, which is involved in binding to bone sialoprotein; staphylococcal secretory antigen A; and staphyloxanthin, a pigment involved in protection from UV radiation (Resch et al. 2005). Yao et al. performed analogous studies on *S. epidermidis*, and found that, like in *S. aureus*, many metabolic and secreted virulence genes are downregulated, while expression of genes involved in salt protection, stress response, and resistance is increased under biofilm conditions (Yao et al. 2005). As mentioned earlier, research has shown that biofilm formation is upregulated by anaerobic, osmotic, and ethanol stress due to the stress-induced alternative sigma factor, σ^B (Rachid et al. 2000). The overriding theme is that proteins involved in cell wall synthesis and protection from environmental stresses are upregulated in biofilm conditions, while virulence factors and secreted toxins are increased during planktonic growth.

4.4 Adherence to Host Tissues/Implants

The ability of *S. aureus* and *S. epidermidis* to adhere to host cells and implanted biomaterials is crucial in initiation of infection. This adherence is what allows *Staphylococcus spp.* to subsequently colonize in the form of a biofilm. *Staphylococcus spp.* have several proteins, termed MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) that can bind to a variety of host proteins that comprise the extracellular matrix, including host proteins such as fibrinogen, fibronectin, collagen, and elastin (Williams et al. 2002). Soon after implantation, foreign material will be coated with these host proteins. Because *S. aureus* and *S. epidermidis* have adhesins that specifically bind to these host factors, the implant becomes an ideal environment for bacterial attachment and subsequent infection. The MSCRAMM proteins include the fibrinogen binding proteins Fib, ClfA, and FbpA; the fibronectin binding proteins FnbA and FnbB; the collagen receptor Cna; and EbpS, which is important in binding elastin. All of these adherence proteins are alike in that they possess a LPXTG motif in their C-terminal domains, which is cleaved by sortase so that the N-terminal ligand-binding domain is attached to the cell wall surface (Harris et al. 2002). Besides initial adherence, these proteins can also be important for other functions. For example, fibronectin binding protein has been shown to be necessary for invasion of host cells by *S. aureus* (Dziewanowska et al. 1999). This protein mediates invasion via the $\alpha 5 \beta 1$ integrin (Sinha et al. 1999). Eap, an extracellular protein that can bind to a variety of host proteins, has also been shown to be important for eukaryotic cell invasion (Haggart et al. 2003).

Because these proteins are vital for infection, several therapeutic strategies are currently under development to target the MSCRAMMs, particularly ClfA. Humanized monoclonal antibodies against ClfA have been shown to have efficacy in binding fibrinogen and in preventing MRSA infection in animal models of sepsis and endocarditis (Patti 2004; Vernachio et al. 2003). A mouse monoclonal antibody

against ClfA has also demonstrated an ability to block *S. aureus* adhesion, to displace previously adherent *S. aureus*, and to prevent infection in a murine sepsis model (Hall et al. 2003). In another study, monoclonal antibodies raised against a peptide fragment of Cna were shown to be able to block binding of collagen by the receptor, and to also block collagen binding to intact *S. aureus* cells and attachment of *S. aureus* to a collagen substrate (Visai et al. 2000).

4.5 Avoidance of the Host Immune Response

S. aureus has several tools to prevent clearance by the immune system. Capsular polysaccharides prevent the bacterium from being phagocytosed by immune cells such as macrophages and PMNs. Though complement proteins and antibodies can be deposited on the bacterial surface, the capsule prevents the interaction of these proteins with their receptors on the host's phagocytic cells (O'Riordan and Lee 2004). Most *S. aureus* strains are encapsulated. There are currently 11 capsular serotypes, with serotypes 5 and 8 being most prevalent. These two polysaccharides are processed by proteins of the *cap5* and *cap8* loci, respectively. Strains that express the type 5 capsule are more virulent than acapsulated strains in animal models of infection (Tzianabos et al. 2001), and phagocytosis of capsulated strains is lessened due to the need for anticapsule antibodies and not simply complement binding (Bhasin et al. 1998; Cunnion et al. 2001; Tzianabos et al. 2001). The virulence of strains expressing these two capsules has been shown to differ, with *S. aureus* that expresses type 5 capsules being more virulent than strains expressing type 8 (Tzianabos et al. 2001). Isogenic mutants lacking type 5, type 8, or both capsules supported the idea that the difference in virulence is due to the difference in capsule type, and that cells expressing type 5 capsule are more resistant to in vitro killing by whole blood and human PMNs than are cells expressing type 8 capsules (Watts et al. 2005). Adherence and capsule production seem to be inversely regulated, with adherence being maximal at times when capsule production is lessened. This could be expected since a thick capsule would effectively mask adherence proteins on the cellular surface (O'Riordan and Lee 2004). Capsules have also been correlated with abscess formation in rat models of *S. aureus* infection (Tzianabos et al. 2001).

Protein A (Spa) is another mechanism employed by *S. aureus* to avoid the host immune response. This 47-kDa, cell wall-associated protein binds the Fc portion of host immunoglobulins, thus rendering them ineffective by pointing the Fab fragments (antigen binding domain) out, away from the bacterium, consequently preventing opsonization (Gao and Stewart 2004). Spa is expressed maximally during exponential growth, unlike most staphylococcal exotoxins, and seems to be negatively regulated by the *agr* system (Recsei et al. 1986). During active infections, Spa can be released from the bacterial surface (Silverman et al. 2005). Spa has been shown to be important in contributing to staphylococcal sepsis through binding to the TNF α receptor and mimicking TNF α (Gomez et al. 2004).

This leads to a strong proinflammatory response in the host and subsequent damage to host tissues (Fournier and Philpott 2005). Spa has also been shown to target marginal zone (MZ) B cells, which are then apoptotically deleted (Goodyear and Silverman 2004). This function has given Spa an identity as a sort of B cell *superantigen* (superantigens discussed later) (Silverman et al. 2005). Thus, Spa may not only function in preventing opsonization via the adaptive immune response, but also stop the B cells before they can even produce antistaphylococcal antibodies, as well as the more predetermined response of *innate-like* B-1 B cells (Silverman et al. 2005).

Another means of avoiding the host immune response is via invasion of host cells. In general, *Staphylococcus spp.* are considered noninvasive, extracellular organisms, but in recent years it has been demonstrated that these bacteria can enter eukaryotic cells (Alexander and Hudson 2001), including fibroblasts (Murai et al. 1992), epithelial cells (Bayles et al. 1998), and osteoblasts (Bost et al. 1999; Jevon et al. 1999). Adherence to cells is a prerequisite for invasion, and this adherence is mediated through the MSCRAMM proteins (discussed earlier). In particular, fibronectin binding protein (FnBP) seems to be the most important for invasion (Alexander and Hudson 2001). It is proposed that fibronectin serves as a sort of bridge, binding to FnBP on *S. aureus* and to $\beta 1$ integrins on the host cell surface (Ruoslahti 1996), leading to receptor-mediated endocytosis (Alexander and Hudson 2001). Once inside the cell, *S. aureus* is able to avoid the humoral immune response as well as clearance by antibiotic treatment. The *agr* and *sar* quorum sensing systems seem to be implicated in causing apoptosis of invaded host cells (Wesson et al. 1998), and may provide a means of escape for the bacteria. Osteoblasts that have been invaded by *S. aureus* produce heightened levels of the proinflammatory cytokines IL-6 and IL-12 (Bost et al. 1999), which could aid to further destroy infected bone tissue during PII or osteomyelitis. Often, the invading *S. aureus* are *small colony variants*, which feature a defect in the electron transport chain (McNamara and Proctor 2000), leading to a slower metabolic rate. Small colony variants have also been implicated in chronic infection and show superior resistance to vancomycin and oxacillin (Chuard et al. 1997).

One of the key strategies of the innate response is production of nitric oxide (NO), which is able to modify numerous cellular targets such as lipids and DNA (Radi et al. 1991) (Wink et al. 1991). Though there is evidence that NO is found at high concentrations in areas where staphylococcal colonization occurs (Andersson et al. 2002) *S. aureus* is able to resist this compound. It has been shown that this resistance is due to *S. aureus*' ability to adapt to growth in a NO-rich environment via increased lactate dehydrogenase activity (Richardson et al. 2008). This gene is encoded by the *hmp-ldh1* cassette, and mutation of *ldh1* leads to decreased virulence and a competitive disadvantage in a murine model. Therefore, *S. aureus* is uniquely suited to thrive in the face of the innate NO response by converting its metabolism to homolactic fermentation during nitric stress (Richardson et al. 2008).

Other immunoavoidance factors or strategies are the gamma-hemolysin and Panton-Valentine leukocidin. These exotoxins work to elicit an inflammatory

response by affecting neutrophils and macrophages (Dinges et al. 2000). Gamma-hemolysin is also able to lyse many types of mammalian erythrocytes (Dinges et al. 2000). These toxins work to lyse immune cells by inserting into the cell membrane and creating a pore. Each toxin has two components, S and F, which alone are inactive but together have lytic activity. Gamma-hemolysin has two genes encoding S components (*hlgA* and *hlgC*) while PV leukocidin only has one S component gene (*lukS-PV*). Each toxin only has one F component gene (Dinges et al. 2000). Because any of the S components can combine with any of the F components from either toxin, there are six possible forms of gamma-hemolysin/PV leukocidin. Gamma-hemolysin has been shown to be an important virulence factor when expressed in conjunction with alpha-hemolysin (discussed later) in a model of septic arthritis (Nilsson et al. 1999). PV leukocidin is able to cause granule secretion in PMNs and release of inflammatory mediators (Siqueira et al. 1997). Finally, biofilm formation (discussed earlier) is crucial for immune evasion as well.

4.6 Damage to the Host

S. aureus has a myriad of toxins and secreted enzymes with which it can damage the host. Several of these are enterotoxins, which cause food poisoning. Another toxin, toxic shock syndrome toxin (TSST), causes toxic shock syndrome. These toxins are superantigens, which bind simultaneously and nonspecifically to MHC class II and T-cell receptors, constitutively activating the T cells and causing massive T-cell proliferation (Baker and Acharya 2004) and a TH₁ response (Krakauer 1995). This also leads to a large increase in cytokine production and inflammation, eliciting damage that weakens the host. There are 17 staphylococcal superantigens, including enterotoxins A, B, C, D, E, and G–Q, as well as TSST (Baker and Acharya 2004).

Superantigens consist of N- and C-terminal domains with a long, solvent-accessible α -helix in the center (Baker and Acharya 2004). A flexible disulfide loop is present in several enterotoxins, and it is believed that this loop is responsible for the emetic properties of staphylococcal enterotoxins that are characteristic of food poisoning (Acharya et al. 1994). The main targets of superantigens are the CD4 T cells (Bavari and Ulrich 1995). Superantigens are not processed by antigen-presenting cells (APCs). These proteins instead can bind directly to MHC class II molecules on the APC surface. This binding is mediated, in the case of many enterotoxins, through the binding of a zinc ion to a zinc binding site on the toxin (Baker and Acharya 2004). Most superantigens bind to the V β region on the T-cell receptor (TCR), and up to 10% of resting T cells are stimulated to produce a TH₁ response (Baker and Acharya 2004). This response leads to the production of proinflammatory cytokines, including IL-2, IFN γ , and TNF α (Herrmann et al. 1992; Litton et al. 1994; Miethke et al. 1992), which leads to massive T-cell proliferation and the onset of shock (Johnson et al. 1992).

Another class, the exotoxins, includes four hemolysins (α , β , γ , and δ) as well as Pantone-Valentine leukocidin. Alpha-hemolysin inserts into the eukaryotic cell membrane, forming a pore and lysing the cell. It is specifically known to lyse erythrocytes (Dinges et al. 2000), and also has cytolytic, dermonecrotic, and lethal properties. It is this exotoxin that bestows upon *S. aureus* its beta-hemolytic property (Bhakdi and Tranum-Jensen 1991). Alpha-hemolysin is encoded by the *hla* gene, and the structure of the protein is mainly composed of beta sheets (Dinges et al. 2000). Expression of *hla* is under the control of the *agr* locus, leading to expression of alpha-hemolysin during late exponential phase. Though produced by many strains, it has been demonstrated that TSS isolates often contain *hla* but do not produce alpha-hemolysin due to mutations that prevent translation (Dinges et al. 2000). Alpha-hemolysin monomers insert into eukaryotic cell membranes, where they form a heptamer and a small pore (Belmonte et al. 1987). This pore then allows for rapid efflux of K^+ and influx of Na^+ , Ca^{2+} , and other small solutes. Osmotic swelling leads to lysis of the cell (Dinges et al. 2000).

Beta-hemolysin's function in disease is still being elucidated, but this toxin is found mostly in animal strains and shows specificity for sphingomyelin, which has high levels in macrophages (Dinges et al. 2000). It has been demonstrated that beta-hemolysin has phosphorylase C activity (Doery et al. 1963). Delta-hemolysin is encoded by *hld*, which is translated from RNAIII, the effector molecule of the *agr* quorum sensing system (Yarwood et al. 2004). It is capable of lysing macrophages and neutrophils, but, like beta-hemolysin, its role in disease is unclear (Dinges et al. 2000). The gamma-hemolysin and Pantone-Valentine leukocidin, as mentioned earlier, work to elicit an inflammatory response by affecting neutrophils and macrophages (Dinges et al. 2000).

S. epidermidis has another means by which it is able to elicit host damage. A cell wall-associated PAMP (pathogen-associated molecular pattern) called phenol soluble modulins (PSM) (Mehlin et al. 1999) has been shown to be proinflammatory and chemoattractive, stimulating innate immune cells to cause higher cytokine production, degranulation, enhanced respiratory burst, and inhibition of apoptosis in neutrophils (Liles et al. 2001). There are three PSM molecules (α , β , and γ) (Mehlin et al. 1999) and while these peptides show similarity to other staphylococcal proteins, their biological roles are still being determined. Otto and colleagues have shown that PSM production is not essential for infection, as approximately 22% of clinical isolates do not produce the peptides (Vuong et al. 2004a, b). As well, PSMs seem to inhibit biofilm formation, and the genes encoding these peptides are the most significantly downregulated under biofilm growth conditions when compared with planktonic; concentrations of these peptides within biofilm cultures were also significantly less than in planktonic cultures (Yao et al. 2005). The researchers also determined that PSM production is controlled by the *agr* system; indeed, PSM γ is encoded by RNAIII (Vuong et al. 2004a, b). *Agr* mutant strains showed markedly downregulated production of PSM peptides (Batzilla et al. 2006). This regulation leads to a delayed onset of PSM production until the bacterial population has reached a threshold and may help to keep the bacteria *hidden* until they are at a level to resist the proinflammatory response.

The reduced production of these peptides in a biofilm state illustrates the less inflammatory, chronic nature of these types of infections compared with acute, planktonic-associated disease.

4.7 *Quorum Sensing and Staphylococcal Biofilms*

One of the most exciting areas of current research involves determining what influence quorum sensing has on the growth, development, and pathogenesis of staphylococcal biofilms. There is growing evidence that the *agr* phenotype and expression patterns may impact several features of biofilm behavior, including attachment of cells to substrates, biofilm detachment and dispersal, and even the chronic nature of many biofilm-associated infections (Yarwood and Schlievert 2003). However, the relatively few studies that have been done on the relationship between quorum sensing and biofilms seem to give conflicting results. Pratten et al. showed that there was little difference in biofilm formation between wild type and an *agr* mutant of *S. aureus*, and that *agr* and *sar* were expressed most highly in the deepest areas of the biofilm, which the authors contend would be expected with genes that are expressed in a cell-density-dependent manner (Pratten et al. 2001). Shenkman et al. showed that expression of RNAIII increases the binding of *S. aureus* to fibronectin, but decreases its binding to fibrinogen (Shenkman et al. 2002). Another study looked at the effect of mutating *sarA*, and found that this mutant had a decreased ability to form biofilms, which could be due to decreased binding to substrates such as fibronectin (Beenken et al. 2003). An additional study in which *sarA* was mutated found a subsequent decrease in transcription of the *ica* locus, which is responsible for production of polysaccharide intracellular adhesion (PIA), a necessary component of biofilm formation (Valle et al. 2003). This was recently shown to be the case in *S. epidermidis* as well (Tormo et al. 2005a, b). However, a different report in which *ica* was mutated showed that this mutant was still able to form a biofilm (Beenken et al. 2004), which means that lack of PIA, or *sarA*'s effect on the *ica* locus, must not explain why a *sarA* mutant is attenuated in biofilm formation. It is suspected that the expression of the *ica* genes occurs early in biofilm formation and is needed for initial colonization, rather than persistence (Vandecasteele et al. 2003). A recent study of biofilm growth in iron-limited conditions contends that Agr and SarA are both required for the expression of two adhesive factors that are required for low-iron biofilm growth – Eap and Emp – thus making quorum sensing a requirement for biofilms under iron stress (Johnson et al. 2008).

Agr-mediated quorum sensing activates the transcription of secreted proteins in late exponential phase, while subsequently downregulating expression of adhesion proteins. Vuong et al. (Vuong et al. 2000) studied the difference in biofilm formation between *agr*-positive and *agr*-negative strains. Out of 105 *S. aureus* strains, 78% of those that were *agr*-negative could form biofilms, while only 6% of the *agr*-positive strains did so. These results were found to be independent of PIA levels. In another

study, Vuong and colleagues (Vuong et al. 2004c) showed that an *agr* mutant in *S. epidermidis* was able to form a significantly thicker biofilm in a static in vitro biofilm model than could the wild type. AtlE production was increased in this mutant, which promotes attachment (Vuong et al. 2003). These results were confirmed by another study in which an *agrD* deletion mutant of *S. aureus* was employed under static conditions (Yarwood et al. 2004). Vuong et al. also showed that, in the wild type, *agr* expression was limited to the outer areas of the biofilm (Vuong et al. 2003), unlike the aforementioned study by Pratten et al., where *agr* was expressed most highly inside the biofilm (Pratten et al. 2001). As planktonic bacteria in this study also showed *agr* expression, the researchers concluded that *agr* may be involved in promoting biofilm detachment, rather than its formation (Vuong et al. 2004c). Along these same lines, a very recent study indicated that a peptide that mimics the *agr* peptide AIP-I is able to augment biofilm formation (Fowler et al. 2008). This phenotype is consistent with blockade of the AIP-I receptor AgrC-I (Vuong et al. 2000). The global regulator protein CodY has also been implicated in repressing *agr* during biofilm growth (Majerczyk et al. 2008). Therefore, targeting *agr* for treatment of biofilms may actually exacerbate the biofilm. As well, clinical isolates of *S. epidermidis* obtained from prosthetic implant infections were found to have significantly less *agr* function than isolates obtained from skin, further illustrating the idea that *agr*-targeted therapies would not be effective against biofilm infections (Vuong et al. 2004c). It has been hypothesized that delta-hemolysin, which is encoded within RNAlII and has surfactant properties, may contribute to the detachment of cells from both *S. aureus* and *S. epidermidis* biofilms (Vuong et al. 2003; Vuong et al. 2000). This was supported recently when exogenous purified AIP-I was added to biofilms and caused detachment of the cells via activation of the *agr* locus. This effect could also be induced through reduction in available glucose in the growth media, illustrating how environmental cues can upregulate Agr and lead to detachment (Boles and Horswill 2008). If this is in fact true, it has significant clinical implications. Cells that are expressing *agr* and actively detaching from a biofilm may initiate additional infection sites while also playing a role in the toxemia linked to acute staphylococcal infections. It is probable that these cells would express secreted virulence factors, such as exotoxins and superantigens. Meanwhile, as there is a high frequency of strains that are naturally *agr*-null (Shirtliff et al. 2002) or have defective *agr* system (Cafiso et al. 2007), cells that do not express *agr* and remain part of the biofilm may be a factor in chronic, low-level infections (Yarwood and Schlievert 2003).

Recently a study emerged that may tie together the varying opinions of the role of *agr* in biofilms. Yarwood et al. showed that there are virulence factor variants within a biofilm. The population that dominated the biofilm was non-hemolytic and thus, Agr deficient, which could be due to the repression of *sarU*. However, the Agr-positive population does remain, albeit at lower levels, and likely reflects the population that is able to detach and cause an acute illness (Yarwood et al. 2007).

Recently, biofilm formation by *S. aureus* was shown to be augmented in the presence of heparin, a normal component of the eukaryotic cell membrane and a commonly administered anticoagulant drug (Shanks et al. 2005). It was hypothesized

that this mechanism may be via cell–cell communication, possibly by inhibiting *agr*, since, as discussed earlier, *agr* mutants also exhibit robust biofilm growth. As well, because staphylococcal species have been shown to contain a heparin-binding protein (Fallgren et al. 2001), it was also hypothesized that *S. aureus* bacterial cells may utilize heparin to cross-bridge in order to better adhere to each other (Shanks et al. 2005). The notion that heparin can increase biofilm formation is particularly troubling in light of the fact that heparin is regularly given in the case of catheterization, where bacterial biofilms are often found (Gorman et al. 1993).

Another facet of quorum sensing and its relation to biofilms could lie in antibiotic resistance. It has been shown that while wild-type *S. aureus* shows appreciable resistance to both oxacillin and rifampin, an *agr* mutant is sensitive to rifampin (Yarwood et al. 2004). Because rifampin is often used with other antibiotics to treat staphylococcal biofilm infections, this finding could have clinical relevance; if *agr* could be targeted therapeutically, rifampin may be more effective. Targeting quorum sensing could be a way to treat antibiotic-resistant biofilm infections. The quorum-sensing inhibitor RNAIII-inhibiting peptide (RIP), a heptapeptide (YSPWTFNH₂), is able to abrogate toxin production and biofilm formation (Balaban et al. 1998) by both *S. aureus* and *S. epidermidis* by preventing adhesion to epithelial cells and plastic polymers (Balaban et al. 2003). When rats were implanted with RIP-soaked grafts or injected with RIP and subsequently infected with *S. aureus* or *S. epidermidis*, the rats showed no infection compared with controls (Dell'Acqua et al. 2004). A more recent study further illustrated this idea by showing that soaking a graft with RIP and a variety of other antimicrobial compounds and then implanting it into a rat completely prevented the onset of biofilm infection on the graft by several *Staphylococcus* species (Balaban et al. 2005). Another study using a rat model of urinary catheterization showed that coating of the stents abrogated biofilm formation, particularly when combined with teicoplanin therapy (Simonetti et al. 2008). This implies that RIP may be successful if used therapeutically, perhaps in combination with antibiotics, but more studies will be needed to elucidate this.

4.8 Case Example of Normal Immune Responses in *S. Aureus* PII

During acute PII, the innate immune system reacts to the peptidoglycan wall (via *N*-formyl methionine proteins and teichoic acids) of *S. aureus* to create proinflammatory cytokines (such as IL-1, IL-6, and TNF α) and C-reactive protein. These factors allow the host to build up a protective inflammatory response that controls this pathogen and frequently clears the infection. However, when the infection is not resolved by the host's innate immune response, *S. aureus* is able to persist via a number of virulence factors and strategies, including its ability to invade and survive in mammalian cells, the production of a biofilm, or encasing itself within a thick, antiphagocytic capsule. As well, the cell-mediated (TH₁) and humoral (TH₂) adaptive immune responses are often inadequate. In a study by Yoon et al., using a murine model of

acute hematogenous osteomyelitis, the major cytokines of cell-mediated immunity (IL-2 and IFN γ) seemed to rise only transiently while inflammatory cytokines remained at elevated levels in infected bone (Yoon et al. 1999). This cytokine profile led to an early expansion and activation of T-cell subsets followed by apoptosis. Thus, *S. aureus* seemed to hinder the normal immune response by downregulating both T-cell immunity and the production of cytokines important in the adaptive response. While a staphylococcal infection typically pushes the immune system toward a TH₁ response, due to the low oxygen partial pressures of infected bone where immune cell function is inhibited, the efficacy of this response is debatable. Also, a study in mice showed that a high level of IFN γ (a TH₁ cytokine) plays a detrimental role in the eradication of staphylococcal infection, and the TH₂ response (evidenced through high levels of IL-4 and IL-10) is involved in host resistance to infection through regulation of gamma interferon (Sasaki et al. 2000). However, the requirement of the TH₂ response to clear *S. aureus* infection was questioned in a study using IL-4-deficient mice (Hultgren et al. 1999). It seems that a TH₂ response is only required for clearance of *S. aureus* infection in certain mice depending upon their genetic background. In addition, it has been determined that when IFN γ was administered to mice infected with *S. epidermidis* well after the initial inflammatory response, the animal was able to reduce the level of biomaterial-associated infection (Boelens et al. 2000). Also, while intracellular persistence occurred in untreated mice, those animals that received IFN γ did not demonstrate any Gram-positive intracellular invasion. Therefore, while an increase in TH₁ cytokines during the initial inflammatory response may often result in host tissue damage, pathogen eradication may occur when these cytokines are provided to the infected host after this early phase. This may be an important method to trigger a correct and appropriately timed TH₁ immune response.

In summary, upon infection with *S. aureus*, a strong native immune response, cytokine release, and high T-cell activation are elicited. This pathogen is able to use a number of immunoavoidance strategies during this time (discussed earlier), while the host's immune system concurrently causes damage to *self* tissues and blood vessels in the area of infection. This damage may lead to local circulatory and immune compromise. High levels of T-cell activation ultimately result in apoptosis and a helpless immune system, allowing *S. aureus* to persist. By artificially pushing the host immune system toward an effective TH₁ response (via administration of IFN γ) after the preliminary inflammatory response, this persistent pathogen may be cleared more easily by the host.

5 Special Case: Ilizarov Fixator

Though it used to be believed that adult bone was unable to grow, it is now known that such bones are able to be lengthened. Transosseous osteosynthesis, a method developed by G. Ilizarov, is now commonly used to treat many conditions involving bone nonunions (Ilizarov 1989a, b). A section of bone is removed, and a fracture is created above the empty space. An external metal frame, the Ilizarov fixator, is

applied to the limb, and screws are inserted from the frame to the bone. This holds the two bone segments apart, providing stabilization. The fixator is adjusted periodically to *stretch* the bone such that new bone will form in the fracture. This causes the bone segment to lengthen, and eventually the two segments will meet, thus filling the empty space.

The Ilizarov fixator is indicated in several clinical cases, often when sections of bone need to be removed. This includes compound fractures (Dagher and Roukoz 1991), osteomyelitis (Parsons and Strauss 2004; Pearson and Perry 1989), PII with subsequent prosthesis removal and debridement (Manzotti et al. 2001; Manzotti et al. 2002), and bone defects (Song et al. 1998). The device can also be useful in limb lengthening in children (Birch and Samchukov 2004), such as in the case of osteogenesis imperfecta (Saldanha et al. 2004). In cases of infection, the infected bone is debrided, leaving behind a dead space that can be large, depending on the extent of infection. The Ilizarov technique has been shown to be efficacious in lengthening bone and filling this space, with 100% union and >76% resolution of infection in one study (Parsons and Strauss 2004). However, because the device must be adjusted daily, it requires a good deal of labor and patient compliance to be effective.

Complications with the Ilizarov fixator can occur, and one such complication is the risk of pin site infection (Clasper and Phillips 2005; Naudie et al. 1998). Because the fixation device is external and must be attached to bone through the skin and soft tissues, the integrity of these tissues is compromised. This allows relatively easy access of bacteria into the soft tissues and bone. As well, the presence of the pin itself can prevent healing (Davies et al. 2005). Superficial pin site infections are quite common (Manzotti et al. 2001; Naudie et al. 1998; Tomak et al. 2005). Those infections that move deeper can be persistent and form sequestra and draining sinus tracts (Ring et al. 1996), necessitating removal of the pin (Tomak et al. 2005). In particular, those pins that are placed near joints are at a higher risk for causing sepsis, due to the amount of movement present at those sites (Hutson and Zych 1998). Infection is defined as the presence of tenderness, erythema, heat, and discharge (Hedin and Larsson 2004; Mahan et al. 1991). Severity of the infection is classified according to the Checketts-Otterburns scale, with a 1 being considered a minor infection and a 6 being a very severe infection of the pin tract that requires debridement (Dahl and Toksvig-Larsen 2004). Often these severe infections occur after removal of the device. Minor infections (1, 2, or 3 on the Checketts-Otterburns scale) can be managed with oral antibiotics and pin site care, and perhaps resiting of the pins; external fixation can continue. More severe infections involve multiple pin sites, severe soft tissue infection, or the spreading of the infection to the bone (Dahl and Toksvig-Larsen 2004). The most commonly found etiologic agents in pin site infections are *S. epidermidis* and *S. aureus* (Mahan et al. 1991). Because *S. epidermidis* is a skin bacterium, it is not surprising that it would be found in the pin tract, and may or may not cause infection. Proper care of the pin site can help to prevent these infections and also keep superficial infections from spreading to the deeper tissues or becoming systemic (Davies et al. 2005). However, the best course of treatment of pin sites is debatable, with some

advocating a nihilistic approach with simple daily showers for cleansing, and others suggesting a more aggressive treatment with antiseptics and bandaging (Hedin and Larsson 2004). When antiseptics were used, chlorhexidine proved to be more effective at preventing infection than saline solution. Chlorhexidine has antistaphylococcal properties, and using this solution for cleansing can help to lessen the need for prophylactic antibiotic treatment (Dahl and Toksvig-Larsen 2004; Hutson and Zych 1998).

6 Conclusion

Prosthetic implantation is an ever-increasing medical practice. With a high level of mortality and the cost of treatment being approximately \$50,000 per patient (Hebert et al. 1996), PII is a distressing public health concern. The most common etiologic agents of PII are *Staphylococcus spp.*, including *S. aureus* and *S. epidermidis*, which often harbor resistance genes allowing them to persist in the face of commonly used antibiotics. Staphylococci possess a number of virulence factors that allow them to colonize and damage the host, all the while avoiding the host's immune response. Quorum sensing allows for tight regulation of virulence factor expression. Perhaps the most important staphylococcal virulence factor is its ability to form a biofilm. This mode of growth allows the bacteria to persist in the presence of antimicrobials and the host response, leading to a chronic state of infection that can only be eradicated through removal of the infected device. With the increased usage of intramedullary rods, plates, and screws, as well as prosthetic joints, the number of prosthetic implant infections (PII) will only rise as well unless significant research is dedicated to developing better ways of treating and preventing these infections. Because *Staphylococcus spp.* rely on biofilm formation to cause persistent infections and on quorum sensing to trigger the expression of virulence factors, therapeutics aimed at these phenomena could prove to be promising candidates for the treatment of PII.

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