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The Basic Science of Musculoskeletal Infections

Mark E. Shirtliff, Jeff G. Leid, and J. William Costerton
Montana State University, Bozeman, Montana, U.S.A.

I. INTRODUCTION

The occurrence, type, severity, and clinical prognosis of bone and joint infections depend upon the interplay within a factor triad that includes the characteristics of the infecting pathogen, the properties of the host, and the source of infection. In order to describe accurately why certain types of microbes cause certain types of musculoskeletal infections (MSIs), the relative contribution of each one of these factors must be taken into account (Fig. 1). Therefore, we first discuss the various species of microbes responsible for MSIs. The virulence factors of the pathogens responsible for the majority of musculoskeletal infection cases are also addressed within this introductory chapter, with specific emphasis on Staphylococcus aureus, the most commonly isolated bacterial species in these infections. We also discuss host factors, including normal properties such as blood supply and its relation to infection, as well as host defects such as local trauma, age, vascular insufficiency, immunocompromise, phagocyte defects, and implanted medical devices. The final part of the infection triad to be discussed is the source of infection, whether it be via hematogenous introduction, extension of a contiguous focus of infection, or direct inoculation from penetrating trauma, compound fracture, bites, or surgical contamination.
II. MICROBIAL SPECIES RESPONSIBLE FOR MUSCULOSKELETAL INFECTIONS

Virtually every bacterial species has been reported to cause MSIs. In a 1999 prospective review of patients \((N = 164)\) suffering from long bone osteomyelitis between 1994 and 1996, \textit{Staphylococcus} spp. represented 53\% of all isolated bacteria (1). Specifically, \textit{S. aureus} constituted nearly 80\% of all \textit{Staphylococcus} spp. isolated from patients with osteomyelitis. \textit{Staphylococcus} spp. are capable of causing osteomyelitis in immunocompetent hosts as well as in immature and immunocompromised individuals. Some other pathogenic microorganisms asso-
associated with osteomyelitis are *Enterococcus* spp., *Streptococcus* spp., *Pseudomonas aeruginosa*, *Enterobacter* spp., *Mycobacterium* spp., as well as anaerobic and fungal species (specifically *Candida* spp.). Each of these pathogenic species individually represents a very 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 osteomyelitis infection by these other species.

Vertebral osteomyelitis is usually hematogenous in origin but may also be secondary to trauma. Most hematogenous infections are monomicrobial. In the normal host, *S. aureus* remains the most commonly isolated organism. However, aerobic gram-negative rods are found in 30% of cases. Usual sources of infection include the genitourinary tract, skin and soft tissue, respiratory tract, infected intravenous catheter sites, postoperative wound infections, endocarditis, dental infection, and unknown sources. However, the primary infection focus is usually unknown. Intravenous drug abuse causes a high incidence of infection by *P. aeruginosa* and *Serratia marcescens* (2).

Most instances of long bone hematogenous osteomyelitis occur in children after a bacteremic event. A single pathogenic organism is almost always recovered from the bone (3–5). The most common bone isolates are *Staphylococcus* spp., the most common gram-negative organism is *P. aeruginosa*, and the most common anaerobes are *Peptostreptococcus* spp. (Table 1). However, in the immunocompromised patient, other organisms must also be considered including fungi and mycobacteria. In contrast to hematogenous osteomyelitis, in contiguous focus osteomyelitis multiple organisms are usually isolated from the bone. *S. aureus* and coagulase-negative *Staphylococcus* spp. account for 75% of the bacterial isolates (3–5). However, gram-negative bacilli and anaerobic organisms are frequently isolated. A higher rate of nasal and skin colonization with *S. aureus* defects in host immunity, and impaired wound healing all play roles in foot infection, especially in an immunocompromised host such as a diabetic patient. Superficial fungal skin infections, which are common in diabetic patients, may also allow bacterial entry through macerated or broken skin.

Multiple organisms are found in patients with osteomyelitis involving the small bones of the foot, including *S. aureus*, coagulase-negative *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., gram-negative bacilli, and anaerobes. Aerobic gram-negative bacilli are usually a part of mixed infection (6).

The virulence and tropism of the microorganisms combined with the resistance or susceptibility of the synovia to microbial invasion are major determinants of joint infection. *S. aureus*, *Streptococcus* spp., and *Neisseria gonorrhoeae* are examples of bacteria that have a tropism for the synovia, probably related to adherence characteristics and toxin production. Aerobic gram-negative bacilli such as *Escherichia coli* rarely infect the synovia except in the presence of an underlying and compromising condition. Once the organism
Table 1  Osteomyelitis: Commonly Isolated Organisms

| Long bone hematogenous osteomyelitis (monomicrobial infection) |  |
|---|---|---|
| Infant | Childhood | Adults |
| < 1 Year | Staphylococcus aureus | Staphylococcus aureus |
| Group B Streptococcus | Streptococcus pyogenes | Staphylococcus epidermidis |
| Staphylococcus aureus | Haemophilus influenzae | Gram-negative bacilli |
| Escherichia coli |  | Pseudomonas aeruginosa |

| Contiguous focus osteomyelitis without vascular disease (polymicrobial infection) |  |
|---|---|---|
| Staphylococcus aureus |  |
| Staphylococcus epidermidis |  |
| Streptococcus pyogenes |  |
| Enterococcus species |  |
| Gram-negative bacilli |  |
| Anaerobes |  |

| Diabetic foot osteomyelitis (polymicrobial infection) |  |
|---|---|---|
| Staphylococcus aureus |  |
| Streptococcus species |  |
| Enterococcus species |  |
| Proteus mirabilis |  |
| Staphylococcus epidermidis |  |
| Peptostreptococcus species |  |
| Diphtheroids |  |
| Pseudomonas aeruginosa |  |
| Anaerobes |  |
| (e.g., Bacteroides spp.) |  |

| Vertebral osteomyelitis (monomicrobial infection) |  |
|---|---|---|
| Staphylococcus aureus |  |
| Staphylococcus epidermidis |  |
| Pseudomonas aeruginosa |  |

is inside the joint, the virulence of the organism varies. In rabbits, intra-articular injection of $10^2$ S. aureus into the knee joint resulted in major joint destruction, but identical injections of N. gonorrhoeae or S. epidermidis caused no joint inflammation (7).
The most common etiological agent of all septic arthritis cases in Europe and all nongonococcal cases in the United States is *S. aureus* (8–12). The representation of *S. aureus* is more pronounced in patients with either rheumatoid arthritis or diabetes. After *S. aureus*, *Streptococcus* spp. are the next most commonly isolated bacteria from adult patients suffering from septic arthritis (11–16). Whereas one study showed a high representation of *Streptococcus pneumoniae* (13), *Streptococcus pyogenes* is usually the most common streptococcal isolate, often associated with autoimmune diseases, chronic skin infections, and trauma (11–14). Groups B, G, C, and F, in order of decreasing preponderance, are also isolated, especially in patients suffering from immunodeficiency, diabetes mellitus, malignancy, and severe genitourinary or gastrointestinal infections (11–14). Gram-negative bacilli account for approximately 10%–20% of cases (10–14,16). Patients with a history of intravenous drug abuse, extremes of age, or immunocompromise display a higher prevalence of gram-negative infection. The most common gram-negative organisms are *Pseudomonas aeruginosa* and *E. coli*. Anaerobes are also isolated in a small percentage of cases, usually those of diabetic patients and patients with prosthetic joints. Approximately 10% of patients with nongonococcal septic arthritis have polymicrobial infections.

Historically, *Haemophilus influenzae*, *S. aureus*, and group A streptococci were the most common causes of infectious arthritis in children below 2 years of age. However, the overall incidence of *H. influenzae* as a cause of septic arthritis is decreasing because of the *H. influenzae* type b (Hib) vaccine now given to children (17). A 1999 study of 165 cases of acute hematogenous osteomyelitis or septic arthritis treated in the years before and after the advent of the Hib vaccine demonstrated that musculoskeletal infection due to this bacterial species was reduced to nearly nonexistent levels (18). Therefore, the coverage of *H. influenzae* as part of the empirical antibiotic coverage may no longer be needed in the management of acute septic arthritis in Hib vaccinated children. While *H. influenzae* has lost its predominance as the most commonly identified gram-negative pathogen in pediatric populations, the normal oropharyngeal resident of young children, *Kingella kingae*, may have taken its place, specifically in patients less than 24 months of age (19–22). In fact, a 1995 study found that the nearly half of the clinical isolates from acute septic arthritis patients less than 2 years old were *K. kingae* (21). However, these results have yet to be repeated in other centers. Clinical data suggest that the organism may gain access to the bloodstream in the course of an upper respiratory infection or stomatitis (23). In children above the age of 2, *S. aureus*, streptococci, *H. influenzae*, and *N. gonorrhoeae* have usually been isolated (24–26), although *H. influenzae* may have also lost its predominance in this patient age group (19).
Microbiological associations exist with concomitant disease states. Septic arthritis that follows cases of infectious diarrhea may be caused by *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., or *Yersinia* spp. (27,28). However, these cases may reflect a form of reactive arthritis. A rare form of migrating polyarthritis may be caused by *Streptobacillus moniliformis*. In human immunodeficiency virus-(HIV)-infected patients, *S. aureus* continues to be the most common isolate (approximately 30%) (29). However, there is an increased number of opportunistic pathogens isolated from this patient subset, including *S. pneumoniae*, mycobacterial species, and fungal species (29,30).

Relatively rare in Western Europe, the diplococcus gram-negative bacterial species *N. gonorrhoeae* is the most common cause of septic arthritis in the United States (11,12,31). The number of cases of gonorrhea decreased by 72% between 1975 and 1997 and this decrease was correlated with a reduction in disseminated gonococcal infection and arthritis (32). However, the reported rate increased by 9.2% between 1997 and 1999 and in 2000 stood at 133.2 cases per 100,000 per year (32). Specifically, the rate of gonococcal infection among homosexual men has demonstrated an alarming increase. These increased incidence rates may also cause higher numbers of observed gonococcal arthritis cases.

A. Properties of the Microbes in the Development of Musculoskeletal Infections

Since *S. aureus* has been extensively studied with regard to its role in MSIs and causes the majority of the infectious cases, we use this bacterial species as the “typical” pathogen in our discussion of bone and joint infections. Other representative species, including *N. gonorrhoeae* and *P. aeruginosa*, are also described in their relation to MSIs.

1. *Staphylococcus aureus*

Since *Staphylococcus aureus* spp. demonstrate a wide diversity of infections (tropical pyomyositis, lower respiratory tract infections [pneumonia], superficial skin infections [boils, sties, carbuncles], localized abcesses, endocarditis, osteomyelitis, toxic shock syndrome, serious skin infections [pyodermitis], food poisoning, bacteremia, empyema, pyopneumothorax, and exfoliative diseases), it is not surprising that *S. aureus* has evolved a wide variety of virulence products to cause disease. The pathogenesis of staphylococcal infections is multifactorial, and it is difficult to determine the precise role of any given factor in infection. Most of the virulence factors seem to be specifically adapted to survival and infection within the host. Staphylococcal products that have a role in infection may be classified as virulence factors responsible for adherence, direct host damage, or immunoavoidance. There are also a number of enzymes and extracellular proteins
that may have a role in virulence. These factors have a specific role in the colonization and infection process in bone and joint infections, and their expression is coordinated throughout the various stages of infection (Figs. 2 and 3). Therefore, the differential regulation of these virulence factors due to staphylococci population levels and environmental factors is extremely important in the development of infection.

a.) Regulation. *Staphylococcus aureus* produces a large number of extracellular and cell-associated products that may contribute to virulence and development of persistent infections. Most of these virulence factors seem to be specifically adapted to survival and infection within the host.

During early exponential growth when cell density is low, proteins that promote adherence and colonization (such as fibronectin binding protein, protein A, staphylococcal nuclease, and coagulase) are expressed. When cell growth reaches high densities, the production of the adherence and colonization factors is suppressed, while secreted toxins and enzymes are expressed (such as enterotoxins B, C and D, epidermolytic [exfoliative] toxin A, α, β- and δ-hemolysin, serine protease nuclease, type 5 capsular polysaccharide, clumping factor, leukocidin, phospholipase C, fatty acid modifying enzyme, lipase, hyaluronate lyase [hyaluronidase], and toxic shock syndrome toxin 1). Many of these post-exponential phase proteins are involved in damaging the host, obtaining nutrients from the host for pathogen growth, and disseminating after the organism has adequately colonized and increased in number to promote an active infection.

The expression of most of these staphylococcal products is under partial or complete control of the staphylococcal accessory regulator (sar) and the accessory gene regulator (agr) system. During early logarithmic growth, a protein encoded by repressor of toxins (rol) inhibits the expression of agr-activated virulence factors (33). Once activation of the agr and sar regulatory loci occurs during the late exponential phase, there is an increased transcription of an agr regulatory RNA molecule known as RNAIII (34). RNAIII immediately blocks transcription of surface protein genes and, with a hypothesized timing signal, upregulates transcription of extracellular pathogenicity factors (such as exotoxins). The primary regulatory function of RNAIII is at the level of transcription by an undetermined mechanism that may involve one or more regulatory proteins (35). This regulatory RNA molecule is also capable of controlling production of at least two virulence factors, α-hemolysin (*hla*) and protein A (*spa*), at the level of translation. At the beginning of exponential phase growth, the expression of α-hemolysin is normally inhibited through intramolecular base pairing that blocks the ribosomal binding site (35,36). Later in exponential phase growth, 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
S. aureus expressing adherence factors attaches to host extracellular matrix proteins

Localized multiplication of S. aureus on tissue surface and constitutive low-level secretion of the agr-derived auto-inducing thiolactone quorum sensing peptide (AIP)

AIP concentration triggers S. aureus agr system (adherence factor production and toxin/capsule production)

Host Extracellular Matrix Proteins
- collagen
- elastin
- osteopontin
- bone sialoprotein
- fibronectin
- vitronectin
- fibrinogen
- laminin
5' untranslated region of α-hemolysin messenger RNA (mRNA), thereby making the transcripts accessible for translation initiation (36). 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 (37). Therefore, sarA expression may be autoregulated, but the interactions with this complex system are still being elucidated.

It has been hypothesized that the activity of RNAIII is regulated through a population-sensing autocrine system (quorum sensing) that involves the products of the agr locus (38–41). This locus consists of two divergent transcription units, driven by promoters P2 and P3. The P2 operon contains four genes, agrB, agrD, agrC, and agrA, and the P3 operon codes for RNAIII (see previous discussion) (42). 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 the AgrB membrane protein (Fig. 4) (43). As the concentration of AIP increases in the extracellular microenvironment, the interaction between AIP and the histidine kinase receptor protein, AgrC, also increases. This interaction possibly acylates AgrC and enables it to phosphorylate and thereby activate an intracellular agr-encoded protein (AgrA) (35,44). AgrA~P then increases transcription at the P2 promoter. With SarA (the major transcript of the sar operon), AgrA~P also increases the transcription at the P3 promoter, resulting in elevated intracellular levels of RNAIII (45). Therefore, as AIP concentrates in the extracellular environment, the level of RNAIII increases, allowing the growth phase–dependent reduction in adherence factor production and increase in extracellular pathogenicity factor production. AIP not only is capable of activating the agr regulon in self strains, but can also inhibit the agr activation of other S. aureus strains. SarA (the primary product of sar) has been found to be autoregulatory, and it mediates virulence factor expression through agr transcription by binding to the promoter region as a

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Figure 2  Diagrammatic representation of S. aureus attachment to tissues due to high-level expression of adherence factors to host extracellular matrix proteins. After attachment and localized multiplication, the constitutive secretion of the quorum sensing autoinducing peptide (AIP) allows the concentration of this quorum sensing signal. As the microbial population increases, individual cells within the population begin to compete with one another for nutrients. At this time the AIP is at high enough levels to activate the production of a wide variety of staphylococcal virulence factors that enable the microbe to damage the host and obtain more nutrients or explore new niches. At the same time, capsule production increases while adherence factor production is reduced, thereby enabling the S. aureus to become able to resist phagocytosis.
Initial Attachment to Host Extracellular Matrix Proteins

Localized Replication and Population Density Increase – Triggering of the S. aureus Quorum Sensing System

Toxin and capsule production increase and S. aureus embeds within a biofilm
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Figure 3  Scanning electron microscope (SEM) and transmission electron microscope (TEM) images of the progression of S. aureus osteomyelitis from colonization, to localized multiplication, to embedding within a dense glycocalyx to form a biofilm that is resistant to removal by the host immune system and antimicrobial therapy. (Photographs courtesy of Mary Elizabeth Powers, Dissertation, 1995. University of Calgary.)

dimer (46). SarA has also been shown to interact directly with the promoter regions of a number of genes, including the coding regions for the P2 and P3 promoter of agr, protein A, fibronectin binding protein, and α-hemolysin (37,47). 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 (48), S. epidermidis (49,50), S. simulans, and S. warneri (50), this regulation system is also used by the other members of the staphylococcal genus.

Another regulatory locus, termed S. aureus exoprotein expression (sae), was discovered in 1997 (51). This system is composed of two genes, saeR and saeS, encoding a response regulator and a histidine protein kinase, respectively.

Gram Negative Bacteria

X1 = CH₃ or (CH₂)₃CH₃
X2 = O or H

Inositol Lactones

Gram Positive Staphylococcus spp.

Thiolactones

Gram Positive Streptomyces

γ-butyrolactones

Fungi

PsIA of Aspergillus nidulans

Figure 4  Structures of some common microbial signaling molecules.
with significant homology to other bacterial two-component regulatory systems (52). Its control is mediated at the transcription level, and mutation of the \textit{sae} locus lowered in vivo virulence by drastically diminishing levels of α- and β-hemolysins and coagulase and slightly reducing levels of protein A (53,54). Several environmental signals have also been implicated in the \textit{sar} or \textit{agr}-dependent or -independent regulation of virulence factors. These signals include nonmaintained pH (55), osmolarity (56,57), glucose (58), deoxyribonucleic acid (DNA) topology (56), NaCl, sucrose (59), temperature (60), amino acid availability (61), and presence of O$_2$ and CO$_2$. Also, a homolog to the ferric uptake regulator (Fur) of gram negatives was isolated in 2000 and found to regulate the staphylococcal iron regulated (\textit{sir}) operon, which has been proposed to constitute a siderophore-transport system in \textit{S. aureus} (62). Many of the reactions of \textit{S. aureus} to these environmental cues are classified as stress responses, and they are believed to be regulated by sigma factors that control gene expression. In this species, there are two varieties of sigma factors, the housekeeping sigma factor (\(\sigma^A\)) and the alternative sigma factor (\(\sigma^B\)), that are expressed and activate response genes upon entry into stationary phase and in response to environmental stress. \(\sigma^B\) is believed to be regulated by the product of the gene directly upstream of \(\sigma^B\) coding region, \textit{rsbW} (63). This protein is able to bind to the alternative sigma factor, thereby posttranslationally inhibiting its activity until a stress response is needed.

\textbf{b) Adherence Factors.} As stated earlier, the pathogen must colonize the target tissue through adherence in order to initiate infection. \textit{Staphylococcus} spp. have a variety of receptors for host proteins, termed \textit{microbial surface components}, that mediate adherence to the extracellular matrix in bones and joints or implanted medical devices by recognizing adhesive matrix molecules (64–66). Some of the host matrix proteins are fibronectin and laminin (adherence proteins), elastin (which imparts elastic properties), collagen (which provides structural support), and hyaluronic acid (a glycosaminoglycan that is abundant in the joints and the matrix and provides cushioning through hydration of its polysaccharides). There are also a number of bone- or joint-specific matrix proteins. These include osteopontin (a soluble phosphoprotein that acts as a cytokine and osteoclast attachment protein and is needed for bone injury repair and remodeling), bone sialoprotein (which interacts with osteoblasts and acts as a nucleator for calcium hydroxyapatite formation), and vitronectin (an adhesive glycoprotein that regulates adhesion and the coagulation, fibrinolytic, and complement cascades and also allows bone resorption when bound to osteoclasts). Eight adhesin genes have been determined: genes encoding fibrinogen binding proteins (\textit{fjb, cflA, and }\textit{fhpA}) (67–69), fibronectin binding protein (\textit{fnbA and }\textit{fnbB}) (70), a collagen receptor (\textit{ena}) (71), an elastin binding protein (\textit{ebpN}) (72), and a broad specificity adhesin (\textit{map}) that mediates low-level binding of several proteins including osteopontin,
collagen, bone sialoprotein, vitronectin, fibronectin, and fibrinogen (73). Also, this microorganism has been shown to possess a number of other host protein-binding receptors in which the genes have not yet been determined. These include a laminin- (52 kd) (74), a lactoferrin- (450 kd) (75), and a transferrin- (42 kd) (76) binding protein. The staphylococcal receptor that binds laminin may be used in extravasation (77). These receptors were found in S. aureus but were absent from the noninvasive pathogen S. epidermidis (77). The lactoferrin and transferrin receptors bind to these host iron binding proteins and may be used as adhesins and/or as iron acquisition mechanisms. In addition, S. aureus expresses a 42-kd protein, protein A, which is bound covalently to the outer peptidoglycan layer of their cell walls. This adherence protein is able to bind to the host platelet gC1qR (a multifunctional, ubiquitously distributed cellular protein, initially described as a binding site for the globular heads of the complement complex C1q) (78). Therefore, protein A may be able to promote adhesion to sites of vascular injury and thrombosis and has been implicated as an important colonization factor. Protein A production is repressed by the sar locus via both RNAIII-dependent and -independent mechanisms during post-exponential phase growth (45). This protein is also associated with S. aureus immunomodulation (discussed later).

Many of these and other staphylococcal cell wall proteins must be exported out of the bacterial cell in order to interact with the extracellular environment. This export can be either a targeting process (the protein is exported and has binding domains for cell wall secondary polymers such as teichoic acids) or a sorting process (a C-terminal conserved amino acid sequence, LPXTG, that directs the export and covalent attachment to the peptidoglycan) (79).

Increasing evidence supports the importance of staphylococcal surface components as virulence determinants by allowing initial colonization. In a number of studies, mutants in these receptors strongly reduced the ability of staphylococci to produce infection. In addition, there was significant binding of S. aureus to bone sialoprotein, fibronectin, and collagen type 1 in a mouse model, indicating that adherence remains a key phase in the early stages of infection (80). Expression of adhesins permits the attachment of the pathogen to cartilage. In a murine septic arthritis model, inoculation of mice with mutants of the collagen adhesin gene showed that septic arthritis occurred 43% less often than in the corresponding wild type (81). Collagen adhesin positive strains were also associated with the production of high levels of immunoglobulin G (IgG) and interleukin-6 (IL-6) (81). Also, vaccination with a recombinant fragment of the S. aureus collagen adhesin was able to reduce the sepsis-induced mortality rate to 13%, compared with 87% in the control group (82). However, the role of collagen adhesion of S. aureus as a major virulence factor was questioned in 1999 since approximately 30%-60% of clinical isolates do not display collagen binding in vitro or the cna-encoded collagen adhesin (83). Staphylococcal fibronectin-binding proteins (FbpA and FbpB) may have a major role in the colonization
and virulence of MSIs. In a 2000 study, all of the tested clinical isolates (N = 163) contained one or both of the coding regions for these binding proteins and 95% of these strains had a comparable fibronectin binding capacity to that seen in a staphylococcal reference strain known to bind fibronectin efficiently (84). In addition, an in vivo study of endocarditis in a rat model showed that mutants deficient for fibronectin-binding protein were 250-fold less adherent to traumatized heart valves (85). Also, S. aureus adherence to mitoplasts from iliac bones of guinea pigs was three times higher than that of the fibronectin-binding protein-defective mutant strain (86). It is likely fibronectin-binding proteins play an important role in bone and joint infections, especially those associated with initial trauma or implanted medical devices (87). These receptors play an additional role in an intracellular immunoavoidance strategy (discussed later).

c.) Factors Causing Damage to the Host. During acute infection, the innate immune system responds to the peptidoglycan wall (via N-formyl methionine proteins and teichoic acids) of S. aureus to produce proinflammatory cytokines (such as interleukin-1β [IL-1β], IL-6, and tumor necrosis factor-α [TNF-α]) and C-reactive protein. Bacterial DNA (specifically unmethylated CpG motifs) has also been shown to elicit an intense inflammatory response (88,89). When bacterial DNA from S. aureus, E. coli, or synthetic, unmethylated oligonucleotides containing CpG motifs was injected into the knee joint of mice, the development of arthritis occurred quickly and lasted up to 14 days, whereas methylated DNA had no significant effect. Also, the affected tissue was characterized by monocyte and macrophage influx with the release of their associated cytokines and chemokines and the absence of T cells.

S. aureus also secretes a number of enterotoxins (A, B, C1–3, D, E, G, H, I, and J) and toxic shock syndrome toxin (TSST-1). The enterotoxins and TSST-1 have been shown to activate the immune system profoundly when evaluated in animal models, increasing mortality rates and exacerbating host inflammatory cell invasion, cytokine release, and tissue degradation in the acute phase of the infection (80,90). They act as superantigens by binding to the conserved lateral regions of the host major histocompatibility complex class II molecule and T cell receptor. Although only approximately 1 in 10,000 T cells is activated during normal presentation of a nonself antigen, 2%-20% of all T cells may be activated by a superantigen (90). These activated T cells are then able to increase the release of a number of cytokines, such as the interleukins (90), interferon-γ (IFN-γ), and TNF-α (91). This upregulated production of cytokines causes a significant systemic toxicity, suppresses the adaptive immune responses, and inhibits plasma cell differentiation. Also, the stimulated T cells proliferate and then rapidly disappear, apparently because of apoptosis (92). Therefore, immune suppression may be due to generalized immunosuppression and T cell deletion. Since
superantigen toxins are usually produced during the postexponential phase of an established infection, they may also aid in local immune deficiency and host damage seen in bone and joint infections.

The importance of these superantigen toxins in septic arthritis has been demonstrated in animal models of septic arthritis. Most animals infected with strains of S. aureus isogenic for TSSF-1 or enterotoxins (A–D) had frequent and severe joint infection (80). However, 80% of animals infected with strains devoid of these toxins had no symptoms, and those remaining animals demonstrating symptoms had only mild or transient arthritis infections (80). Vaccination with a recombinant form of staphylococcal enterotoxin A devoid of superantigenicity was able to demonstrate significant protection from S. aureus sepsis in mice (93). These enterotoxins and TSST-1 also subvert the cellular and humoral immune system and may thereby promote a sustained and more fulminating acute infection or enable a localized osteomyelitis to develop into a chronic infection. However, since a study in 2000 demonstrated that expression of TSST is negligible at low oxygen partial pressures (94), the importance of this toxin in the relatively O₂-deprived environment of an infected bone, when compared to that in superficial abscesses or cases of septic arthritis, is still a source of debate.

Staphylococcal hemolysin expression is increased during post-exponential phase growth. Among other stimulatory signals, the sar/agr regulon plays a role in this postexponential expression. α-Hemolysin is secreted as a monomer that attaches to host membranes and polymerizes into a hexameric ring channel (95) and has been found to be a significant mediator of virulence (96). Although this hemolysin only binds to human erythrocytes in a nonspecific manner, it can still mediate significant host cell lysis when produced in high concentrations in the infection environment (97). Also, α-hemolysin promotes significant blood coagulation by mediating neutrophil adhesion (98), platelet aggregation (via a fibrinogen-dependent mechanism) (99), and its nonlytic attack on human platelets (100). In addition, this hemolysin can form channels in nucleated cells (e.g., endothelial cells) through which calcium ions freely pass (101,102). The calcium influx is responsible for the vasoregulatory process and inflammatory response disturbances seen in severe infection (103). Finally, α-hemolysin has been shown to interfere with lymphocyte DNA replication (98). These multiple effects of α-hemolysin on the host contribute to the vascular disturbances and immunodeficiency seen in staphylococcal infections, thereby contributing to acute infections and the development of chronic MSIs. The pathogenic properties of α-hemolysin were found in 1999 to occur only when another staphylococcal toxin, the leukocyte-specific γ-toxin (discussed later), was also present in the infecting strain (104).

Another type of hemolysin, sphingomyelinase (β-hemolysin), has only weak cytotoxic effects on human granulocytes, fibroblasts, lymphocytes, and erythrocytes (105). Instead, this hemolysin specifically attacks and kills those
cells with membranes rich in sphingomyelin, such as monocytes. The death of monocytes reduces the effectiveness of the immune response and sponsors the release of cytokines (IL-1β, IL-6, and soluble CD14). These cytokine-related events may be important in the infectious process.

δ-Hemolysin, the translation product of RNAIII of the agr regulon, specifically binds to monocytes and neutrophils (106). δ-Hemolysin promotes the production and release of tumor necrosis factor-α in monocytes (106). Also, this toxin upregulates the expression of neutrophil complement receptor 3. Although this toxin was unable to prime neutrophils directly for an enhanced response, it enhanced neutrophil priming by lipopolysaccharide or tumor necrosis factor. Therefore, the simultaneous presence of monocytes, neutrophils, and δ-hemolysin-producing staphylococci may overactivate host inflammatory response, resulting in host tissue damage in the microenvironment of bone infection. However, the exact role of this toxin in infection remains to be elucidated adequately.

Leukocidin (LukSF-PV) and γ-hemolysin (HlgAB and HlgCB) specifically lyse leukocytes. Each of these toxins is composed of an interchangeable two-component system. The active toxin is formed by taking one protein from the S component family (LukS-PV, HlgA, and HlgC) and one from the F component family (LukF-PV and HlgB) (107,108). The S component is most likely responsible for the specific cytopathic effect of each of the toxins; the F component is responsible for the common leukocyte binding activity. Although LukF and HlgA proteins show very strong similarity, they are encoded on different gene loci (109). Since these cytotoxins specifically interact and lyse leukocytes, they contribute to the inhibition of infection clearance by the host immune system, thereby enabling staphylococcal species to persist.

The role of exfoliative (epidermolytic) toxins A and B is well demonstrated in cases of exfoliative dermatitis (i.e., staphylococcal scalded skin syndrome) in which the epidermis separates from the stratum granulosum. However, these toxins have not been studied in relation to their effect on *S. aureus* virulence in MSIs. Also, they have been shown not to be bacterial superantigens (110). They may still contribute to virulence since they were classified in 2000 as possible sircine proteases (110). In addition to this large array of toxins, a 2000 study has identified a novel gene locus that encodes five exotoxinlike proteins. The in vivo relevance of these proposed toxins must still be demonstrated (111).

d) Immunoavoidance Factors. The ability of the bacteria to evade clearance by the host immune response and to promote a sustained acute infection (e.g., septic arthritis) or a persistent, chronic infection (e.g., osteomyelitis) resides in a number of staphylococcal defense mechanisms, including, but not limited to, IgG inactivation (via protein A attachment), antiphagocytic capsule production, biofilm formation, and invasion and survival in mammalian cells.
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Protein A is bound covalently to the outer peptidoglycan layer of their cell walls. This receptor binds to the Fe portion of IgG and presents the Fab fragment of the antibody to the external environment. Therefore, the Fe portion is unable either to bind complement or to signal polymorphonuclear leukocytes, thereby interfering with staphylococcal opsonization and phagocytosis. This interference has been demonstrated in vitro and in animal models with subcutaneous abscesses and peritonitis. Protein A also coats the staphylococcal cell wall with host Fab fragments, and the ability of the immune system to recognize the pathogen as nonself is hindered. The importance of protein A in *S. aureus* septic arthritis was demonstrated in a 1997 study in which strains that obtained this virulence factor caused greater inflammation and cartilage destruction (96).

Capsular polysaccharide may interfere with opsonization and phagocytosis. Among the 11 reported serotypes, capsule types 5 and 8 (microcapsule producers) constitute the vast majority (75%–94%) of clinical isolates (112–114). The capsule of these two serotypes was found to be much smaller than the capsule of other serotypes of *S. aureus* (such as capsule type 1) or pathogenic species such as *Streptococcus pneumoniae*. Unencapsulated and microencapsulated strains demonstrated a high rate of serum clearance when compared to fully encapsulated strains. Therefore, the role of capsular polysaccharide in opsonization and phagocytosis was questioned (112). However, the thin capsule may be necessary in early infection stages in order to allow the interaction of staphylococcal adhesion factors with host proteins (such as fibrin and fibrinogen). In one study, it was shown that a small capsule was necessary for fibroblast attachment by protein A of *S. aureus*, and a fully encapsulated strain reduced binding efficiency (115). In another study, the thin capsule was shown to be necessary for binding to bone collagen type I, since high capsular expression actually inhibited binding (116). Once these microorganisms adhere to solid surfaces (such as bone, implants, or joint tissue), both in vitro and in vivo, staphylococci produce larger quantities of cell-associated capsule than those grown in liquid cultures (117). Specifically, type 5 and type 8 capsule production was shown to be strongly upregulated during postexponential growth (i.e., after adhesion and colonization) by *agr* regulation and perhaps other regulatory systems (118). This upregulated capsule production makes them more resistant to antimicrobial treatment and host immune clearance. Therefore, once staphylococcal adherence proteins establish the infection, the pathogen enters postexponential growth phase and begins producing a thicker capsule that covers and hides the highly immunogenic adherence proteins. This thicker type 5 and type 8 capsule has been found to be serum resistant through inhibition of phagocytosis and opsonization (112,119). The effect of this staphylococcal polysaccharide microcapsule in murine arthritis was explored in a 1997 study in which strains expressing type 5 capsule were shown to produce a higher rate of mortality, higher frequency of arthritis, and more severe form of the disease when compared
to capsule mutants (119). In a clinical trial, a vaccine (StaphVax) that consists of isolated type 5 and type 8 capsular polysaccharides was able to reduce infection rates significantly, by 57% in a high-risk population for as long as 10 months (120).

*Staphylococcus* spp. can also produce a multilayered biofilm. A biofilm is a modular community of microbes embedded within a host- and/or microbe-derived hydrated matrix (usually exopolysaccharide) that exists at a phase or density interface. This interface may be between a solid support (e.g., soft tissue or bone) and a liquid medium (e.g., extracellular fluid, blood, mucin) (121). Biofilm thickness can vary from a single layer to a thick community of cells embedded within a thick polymeric matrix. Structural analyses in 1995 demonstrated that biofilms possess a sophisticated architecture in which microcolonies exist in discrete pillar or mushroom-shaped structures (122). Between these structures, an intricate channel network provides access to environmental nutrients. It has been hypothesized that the development and maintenance of this phenotype may be mediated through the action of quorum sensing systems in biofilm-producing microbes (123–126).

The multilayered *S. aureus* biofilm is embedded into a glycocalyx (127). The glycocalyx develops on devitalized bone (such as the involucrum) or medically implanted devices (128). The presence of implants is a predisposing factor in the development of infection since they are coated with host proteins soon after implantation, and this provides an excellent source of attachment for any bacteria remaining after débridement surgery (64,69,129,130). Once attached, the bacteria can form the glycocalyx, or slime layer, which protects the bacteria from normal host defenses and systemic antibiotics (131–134). This pathogen usually grows in coherent microcolonies in the adherent biofilm, which is often so extensive that the underlying infected bone or implant surface is obscured. This layer prevents the inward diffusion of a number of antimicrobials, allowing bacterial escape from the bactericidal and bacteriostatic effects of antimicrobial therapy (131–134). Furthermore, those bacteria that survive antibiotic clearance often develop resistance to the impregnated antibiotic and regrow. This resistance has been clinically demonstrated by the isolation of small colony variants of *S. aureus* resistant to gentamicin from the wounds of patients treated with gentamicin-impregnated polymethylmethacrylate (PMMA) beads (135). Also, the glycocalyx displays antiphagocytic properties, thereby allowing the bacteria to evade clearance by the host’s immune system (136–138). The glycocalyx is mainly composed of teichoic acids (80%) and staphylococcal and host proteins (139). Host proteins such as fibrin are derived from the conversion of fibrinogen by the staphylococcal coagulase–prothrombin complex (discussed later) (140). It was found that the biofilm produced by *S. epidermidis* also contains the capsular polysaccharide/adhesin (PS/A) that mediates cell adherence to biomaterials and a polysaccharide intercellular adhesin (PIA) that
may mediate bacterial accumulation into cellular aggregates (141,142). PS/A is a high-molecular-mass (>250-kd) molecule that is composed of acid-stable polymers of β1,6-linked glucosamine. PIA is a polymer of β1,6-linked N-acetyl glucosamine residues with a molecular mass of less than 30 kD that is synthesized through genes present on the intercellular adhesion locus (ica) (63,141). S. aureus and other Staphylococcus spp. also contain an ica locus, and its deletion results in the loss of biofilm-forming ability (63). The presence of glyocalyx was noted in 76% of S. aureus, 57% of Staphylococcus epidermidis, 75% of Escherichia coli, and 50% of Pseudomonas aeruginosa clinical osteomyelitis isolates (143).

Clinical strains of Staphylococcus spp. are able to persist by a number of glyocalyx properties. First, this layer has been shown to protect the embedded pathogens from the action of antimicrobial agents and the host immune system by forming a mechanical barrier (144). Second, local immune deficiency often occurs through frustrated phagocytosis since the normal phagocytic processes are devoted to the removal of the glyocalyx and the implant, if present. Therefore, the energy and resources of the immune system that would normally be used to fight infection are subverted. Third, the glyocalyx may activate monocyte production of prostaglandin E2 to inhibit T cell proliferation indirectly (145). Finally, this glyocalyx has been shown to inhibit polymorphonuclear leukocytes directly (136).

S. aureus has also been shown to survive intracellularly after internalization by cultured osteoblasts (146). Type 5 capsule production of in vivo–grown S. aureus (i.e., internalized in cultured osteoblasts) was shown in 1998 to be upregulated when compared to S. aureus grown in vitro (147). Therefore, the capsule not only may resist phagocytosis and opsonization, but may also contribute to intracellular survival. In addition to osteoblasts, staphylococci have demonstrated internalization into other cultured mammalian cells, such as bovine mammary gland epithelial cells, human umbilical vein endothelial cells, and pulmonary epithelial cells isolated from a cystic fibrosis patient (148–150). Specifically, initial adherence to glandular epithelial cells has been shown to be mediated by fibronectin receptors on this pathogen (148), possibly using fibronectin as a bridge between the host cell and bacterial receptors for this host factor. After adherence, bacteria may be internalized by host mechanisms involving membrane pseudopod formation (seen in established bovine mammary epithelial cell lines) or through receptor-mediated endocytosis via clathrin-coated pits (seen in mouse osteoblasts and epithelial cells) (148,151). In either case, the dependence upon the action of host cytoskeletal rearrangements through microfilaments is evident.

After internalization, staphylococci may induce apoptosis (via a host caspase–dependent mechanism) or survive intracellularly (148,149,152,153). Induced apoptosis may further the host cell damage seen in MSIs without causing further inflammation. Also, staphylococci may escape clearance by the
immune system and antimicrobial therapy by persisting within these host cells. This survival was demonstrated in vivo in 2000 when S. aureus cells were found in the cytoplasm of embryonic chick osteoblasts and osteocytes in mineralized bone matrix (154). In another study, S. aureus was found within polymorphonuclear neutrophils in an in vivo infection model (155). These infected host cells were able to establish infection in naïve animals. Therefore, this pathogen may utilize invasion as an immunoevasion technique during the host inflammatory response. After the downregulation of the adaptive immune response through T cell apoptosis (mediated by superantigens, other toxins, and invasion), fulminant and/or persistent infection may result.

e.) Other Staphylococcal Enzymes. Staphylokinase binds to plasminogen, and this complex activates plasmin-like proteolytic activity that causes dissolution of fibrin clots. Although the activity of this enzyme has not been directly related to virulence, the importance of escaping fibrin clots to invade surrounding tissue is apparent. Another enzyme, fatty acid modifying enzyme (FAME), allows the modification of host derived antibacterial lipids in abscesses (156). Therefore, in the intramedullary abscesses associated with osteomyelitis (Brodie's abscess), this enzyme may be responsible for prolonged bacterial survival and the development of osteomyelitis. Coagulase (cna), although not an enzyme, is an extracellular protein that forms a complex called staphylothrombin by binding host prothrombin (157). This complex is able to convert host fibrinogen to fibrin in order to promote localized clotting. This protein is mainly expressed during the exponential growth phase but is thought to be downregulated during late exponential phase by direct binding and inhibition by SarA (158). Coagulase also has fibrinogen-binding capacity in the absence of thrombin (159). Although no differences in virulence were observed between wild-type strains and coagulase mutants in several infection models, the collagen binding adhesin was found to be important in the pathogenesis of corneal infection in the rabbit (160–162). Also, one can speculate as to the benefits derived from locally impeding blood flow and promoting a fibrin-rich area for augmented colonization by Staphylococcus spp. This genus also possesses a number of other enzymes (lipase, nucleases, serine protease, and metalloprotease) that are used to acquire host nutrients such as lipids, nucleotides, and amino acids, respectively. Staphylococcal iron acquisition is mediated through siderophores, such as the 42-kd transferrin binding protein and the 450-kd lactoferrin binding protein (composed of multimers of 62- and 67-kd subunits). Although the pathogenic effects of urease have not been evaluated in MSIs, it is a critical virulence determinant for colonization, urolithiasis, and severe acute pyelonephritis. It is an enzyme that converts urea to ammonia and carbon dioxide, thereby surrounding the bacterium in a protective layer of ammonia. The ammonia is also toxic to host cells, and urease has a direct inflammatory activity on epithelial tissue. Therefore, urease aids in penetration of
the bacterium into tissues and blood. The regulation mechanism is still being determined.

In summary, *S. aureus* infects and elicits a strong native immune response, cytokine release, and high T cell activation. This pathogen is able to use a number of immunoavoidance strategies while the host immune system causes damage to "self" tissues and blood vessels in the area of infection. Damage may cause local circulatory and immune compromise. The high T cell activation eventually results in apoptosis and a weakened immune system, enabling this pathogen to produce a sustained and destructive infection effectively. Although the bacterial products discussed have been shown to increase bone and joint damage in acute septic arthritis, many more *S. aureus* virulence factors have not yet been tested. Therefore, future studies will undoubtedly identify other factors that play a role in MSIs.

2. *Neisseria gonorrhoeae*

As mentioned previously, *N. gonorrhoeae* is the most common cause of septic arthritis in the United States (11,12,31). This diplococcus possesses a number of cell surface structures that have been implicated in virulence. Initial attachment to host epithelium is mediated by long, hairlike protein projections called pilis. Whether this membrane structure is assembled (Pil+) or not (Pil−), also known as phase variation, is determined by posttranslational proteolytic cleavage, variations in homologous recombination, and slipped strand DNA replication resulting in frameshift mutations (163). In addition, the antigenic character of the pilis is altered by homologous recombination between coding regions for the various pilin subunits.

Protein I is the main protein on the outer membrane. It is a porin that is expressed in two different forms, a protein IA variant that is almost always associated with disseminated infection and protein IB that is associated with strains causing localized infections. Those strains that are able to cause a disseminated infection in hosts with a normal immune system display serum resistance (164). Protein IA allows stable serum resistance by binding to host factor H. This bacterially bound host factor efficiently inactivates C3b (a central factor in both the classical and alternative complement cascade) into iC3b (165), thereby reducing the efficacy of the host complement system. This porin may also be responsible for the prevention of phagolysosomal fusion in polymorphonuclear leukocytes and a reduced oxidative burst, thereby allowing survival within these cells. Another extracellular gonococcal protein is protein II, which is also called Opa since colonies expressing protein II on their surface have a more opaque appearance. This protein is believed to cooperate in the more intimate attachment after initial pilus interaction. In addition, protein II is able to attach to the lipooligosaccharide (LOS) of other *N. gonorrhoeae*, thereby enabling the cells
to bind to one another and form microcolonies. These microcolonies may also aid in the initiation of mucosal surface attachment. Protein II is capable of avoiding clearance by the host immune system by phase and antigenic variation (166). Phase variation occurs through slipped strand synthesis that produces a frameshift mutation and produces a prematurely terminated form of the protein. In addition, multiple variants of the protein II gene exist, and, therefore, the antigenic character of protein II can be changed by homologous recombination of these variants. Although this protein is important for mucosal infections, most isolates from DGI patients are missing protein II from their outer membrane and grow to form transparent colonies. Protein III is another porin that is prevalent on the bacterial surface. The antibodies directed against protein III are not bactericidal, and they sterically inhibit antibody binding to protein I and unsialylated LOS that would likely result in bactericidal action (167). Therefore, the generation of these blocking antibodies may prevent serum bactericidal action.

LOS is like the lipopolysaccharide of other gram-negative bacteria except that its carbohydrate portion does not have the complex structure of the repeating O side chain. LOS has endotoxin activity and is largely responsible for the synovial damage in gonococcal arthritis (168,169). Although stable serum resistance is due to protein L.A., unstable resistance is mediated by the ability of some gonococcal strains to attach covalently activated forms of host sialic acid to the galactose residues on LOS (170). This covalent attachment coats the bacterial cell in host proteins and avoids complement activation. In addition, opsonization by complement components and formation of the membrane attack complex of the complement system are inhibited. N. gonorrhoeae also produces an IgA protease that may aid in colonization. However, the relevance of this potential virulence factor in gonococcal pathogenesis will need further study.

3. *Pseudomonas aeruginosa*

*P. aeruginosa* is a gram-negative, ubiquitous, free-living bacterial species that is able to survive in a wide variety of environmental extremes. It has a predilection for moist environments and can infect plants, insects, lower animals, and humans (171). It has been described as the quintessential opportunist in human infections and is capable of causing fatal systemic disease in certain conditions. Some of these conditions arise when normal cutaneous or mucosal barriers have been breached or bypassed (e.g., penetrating trauma, surgery, or intravenous drug abuse); when immunological defense mechanisms have been compromised (e.g., by chemotherapy-induced neutropenia, hypogammaglobulinemia, extremes of age, diabetes mellitus, cystic fibrosis, cancer, or acquired immunodeficiency syndrome [AIDS]); when the protective function of the normal bacterial flora has been disrupted by broad-spectrum antibiotic therapy; and/or when the patient has
been exposed to reservoirs associated with a hospital environment (172). Therefore, this pathogenic bacterial species is able to gain access to the musculoskeletal system by a number of different routes.

a.) Virulence Factors. Once introduced into a susceptible location within a host, a *P. aeruginosa* biofilm may develop on devitalized tissue or medically implanted devices to produce an infection. Whereas pseudomonal adherence is mediated by type IV pili, initial colonization is augmented by the activity of flagella, which allows motility and places the bacterium close enough to solid structures for the pili to adhere (173–176). Also, this organism produces neuraminidase that enhances pili binding by removing sialic acid residues from host glycoprotein *G*M<sub>1</sub>, making it a better receptor for the pili (177,178). Once attached, this organism can form a fully mature biofilm structure composed of a complex channel system that provides even deeply embedded bacteria access to nutrients in this modular community. It is believed that the channel system is maintained by cell-to-cell signaling (179,180).

High-molecular-weight alginate polymers are used to retain *P. aeruginosa* cells efficiently within the biofilm matrix. However, large conglomerates of cells often detach, diffuse away from the parental biofilm, and reattach to the surface, thereby allowing biofilm spread in the mature biofilm form. This detachment may be mediated by stress due to hydrodynamic flow and/or by the pseudomonal enzyme alginate lyase encoded by *algL*. This enzyme is capable of alginate degradation and therefore can induce biofilm sloughing and dispersion (181).

The bacterial biofilm phenotype has been shown to protect the embedded bacteria from normal host defenses and systemic antibiotics. Specifically, the biofilm protects the organism from direct antibody- and complement-mediated bactericidal mechanisms and from opsonophagocytosis (172). Also, the major constituent of pseudomonal biofilms, the bacterially derived extracellular polysaccharide matrix, displays antiphagocytic properties, thereby allowing the bacteria to evade clearance by the host's immune system (182). The biofilm also acts as a mechanical barrier and prevents the inward diffusion of a number of antimicrobials, thereby increasing minimal bactericidal concentrations by greater than 100 times and allowing bacterial escape from the bactericidal and bacteriostatic effects of antimicrobial therapy (183–185). Furthermore, those bacteria that survive antibiotic clearance often develop or increase their resistance to the antibiotic and regrow upon treatment cessation. Meanwhile, the organism produces a number of extracellular enzymes, including alkaline protease (*apr*), elastase (*lasB*), serine protease (*lasA*), and hemolytic phospholipase C (*plcS*). In addition, the PA-II and PA-III lectins (*lecA* and *lecB*) are produced and appear to function as adhesins as well as cytotoxins for respiratory epithelial cells. *P. aeruginosa* also produces the virulence factors exotoxin A and exoenzyme S (*toxA* and *exoS*). Exotoxin A adenosine diphosphate–(ADP)-ribosylates host
elongation factor-2, causing host translation inhibition (186, 187). This toxin is similar to diphtheria toxin and contributes to tissue damage and diminishes the activity of phagocytes. Exotoxin S has ADP-ribosylating activity similar to that seen in many exotoxins and has been found to ribosylate and inactivate G proteins like the pertussis and cholera toxins (188). In order to achieve full enzymatic activity, exoenzyme S must be activated by a host cell protein, termed factor-activating ExoS (EAS) (189). Exoenzyme S is extremely important for the ability of P. aeruginosa to cause disease since disrupting the gene increased the LD₅₀ by a factor of 10⁴ in burned mouse models (188). The breakdown of host tissues by these extracellular bacterial products creates conditions that enhance bacterial proliferation, invasion, and tissue injury. These activities may culminate in bloodstream invasion and dissemination.

b. Regulation. P. aeruginosa regulates these virulence factors in a complex, but coordinated, way in a microbial cell density-dependent manner through quorum sensing and response to environmental cues. The quorum sensing ability in P. aeruginosa is very different from that in the S. aureus system and is dependent upon two distinct but interrelated systems, las and rhl. There is a definite hierarchy of these systems, with the las system taking precedence. These two systems work in concert to upregulate a number of pseudomonal factors that enable this pathogen to survive in highly diverse environments. In the las quorum sensing system, the lasI gene product directs the formation of the extracellular autoinducing signal N-(3-oxododecanoyl) homoserine lactone (3-oxo-C₁₂-HSL) (Fig. 4), whose secretion to the extracellular environment is aided by P. aeruginosa efflux pumps encoded by the mexA-mexB-ompR operon (190). As 3-oxo-C₁₂-HSL concentrates in the extracellular environment, it is taken up by P. aeruginosa cells and interacts with the LasR transcriptional activator. This LasR-3-oxo-C₁₂-HSL complex is then able to activate the expression of a number of genes, including lasB (elastase), lasA (a serine protease that nicks elastin and works synergistically with elastase), apr (alkaline protease), toxA (exotoxin A), both xcp operons (xcpPQ and xcpR-Z, encoding the type II secretion apparatus), rhlR, and lasI itself (191). The formation of the LasR-3-oxo-C₁₂-HSL complex may be aided by GroESL chaperonins as seen in the lux system of V. fischeri (192). These chaperonins are upregulated in response to heat shock and the resultant protein misfolding via the RpoH and AlgU alternative sigma factors. Also, the transcription of lasR is induced in response to glucose limitation, and this induction is mediated through the virulence factor regulator-cyclic adenosine monophosphate (vfr-cAMP) complex (discussed later) (193).

The second P. aeruginosa quorum sensing system consists of the regulatory protein RhlR and the diffusible autoinducer N-butyril homoserine lactone (C₄-HSL) (Figs. 4 and 5) synthesized by the product of rhlI. In contrast to 3-oxo-C₁₂-
Figure 5. Model of the las/rid quorum sensing system in P. aeruginosa. See text for gene descriptions. (Adapted from de Kieft et al., 1999.)
HSL, C₄-HSL is freely permeable. Apparently the length and/or degree of substitution of the N-acyl side chain determines whether an autoinducer is freely diffusible or is subject to active efflux by *P. aeruginosa*. As in the homologous *las* system, once the diffusible autoinducer C₄-HSL attains adequate levels, it binds and activates the RhlR transcriptional regulator. RhlR-C₄-HSL has been shown to regulate the rhamnolipid biosynthesis operon *rhlAB*, alkaline protease, pyocyanin, PA-IL and PA-IIL lectins, *lasB*-encoded elastase, and *rhl* itself (191). The hierarchy of the *las/rhl* system is aided by the inhibitory action of the unbound *las* autoinducer, 3-oxo-C₁₂-HSL, on the binding of C₄-HSL to the RhlR transcriptional activator. The upregulation of *lasR* transcription and the resulting elevated concentrations of the LasR-3-oxo-C₁₂-HSL complex allow the *rhl* system to be subsequently activated. The *rhl* quorum sensing system was also shown in 2000 to be inhibited by the alternative sigma factor, RpoS (194), and activated by the gac two-component regulatory system that responds to growth phase (Figs. 4 and 5) (187). In addition, the formation of the RhlII-C₄-HSL complex has been shown to be aided by GroEL chaperonins as seen in the *lux* system of *V. fischeri* and possibly in the formation of the LasR-3-oxo-C₁₂-HSL complex (192).

Another factor in this quorum sensing system is the negative regulator, RsaL (195). In *P. aeruginosa*, LasR and 3-oxo-C₁₂-HSL globally regulate many products associated with virulence, as well as the second *P. aeruginosa* quorum sensing system. It has been theorized that at low cell density, RsaL inhibits transcription of *lasI* by binding to the *lasI* operator region, thereby blocking activation by LasR-3-oxo-C₁₂-HSL (195). As the cell density increases, so does the intracellular concentration of 3-oxo-C₁₂-HSL, which allows sufficient LasR-3-oxo-C₁₂-HSL formation to inhibit RsaL competitively for binding to the *lasI* operator. Thus, it appears that during the early stages of growth, RsaL blocks the quorum sensing cascade by inhibiting the transcription of *lasI*. Finally, it was found that this organism produces another intercellular signal, the *Pseudomonas* sp. quinolone signal (PQS), that was identified as a 2-heptyl-3-hydroxy-4-quinolone (196,197). PQS is produced maximally at late stationary phase and works by activating transcription of the *rhlI* gene (and to a lesser degree *lasR* and *rhlR*). It is not known what activates the production of PQS, but this molecule is probably not involved in sensing cell density.

The 3-oxo-C₁₂-HSL and the C₄-HSL of the *las* and *rhl* systems, respectively, have been well studied; other homoserine lactones may have been identified in *P. aeruginosa* by using a combination of reporters, thin layer chromatography, and comparison to HSL standards (198). In *P. aeruginosa* isolates derived from cystic fibrosis–related chronic lung infections, investigators were able to detect up to five additional HSLs: *N*-hexanoyl-L-homoserine lactone (C₅-HSL), *N*-3-oxohexanoyl-L-homoserine lactone (3-oxo-C₆-HSL), *N*-3-oxooctanoyl-L-homoserine lactone (3-oxo-C₈-HSL), *N*-3-oxodecanoyl-L-
homoserine lactone (3-oxo-C₁₀-HSL), and N-(3-oxotetradecanoyl)-L-homoserine lactone (3-oxo-C₁₄-HSL). This study also noted that the production of HSLs showed pronounced reduction when the patient was subsequently cocolonized with *Burkholderia cepacia*. Although only one cocolonized patient was evaluated, the properties of multispecies infections and potential interspecies communication may be important in cystic fibrosis and in biofilms.

Although the quorum sensing system is an extremely important determinant of virulence factor expression in *P. aeruginosa*, the system must be able to respond adaptively to environmental stimuli. Stimuli that have been shown to affect this system (either directly or indirectly) include heat shock, iron and glucose availability, RpoS-mediated inhibition (possibly due to specific amino acid starvation), and entry into stationary growth phase mediated by unknown stimulators of the gac two-component system.

The regulation of biofilm formation and virulence factor expression is a complex interaction among a number of regulatory cascades in *P. aeruginosa*. For example, exotoxin A, the diphtherialike toxin responsible for protein synthesis disruption in eukaryotic cells, is regulated by a number of environmental and quorum sensing signals (Fig. 6). Some of the environmental signals that *P. aeruginosa* responds to are the presence of glucose, iron and nitrogen availability, oxygen levels, temperature variations, pH, osmolarity, amino acid starvation, and ultraviolet damage.

The *P. aeruginosa* response to glucose limitation is mediated through the virulence factor regulator (Vfr) that demonstrates significant homology to the cAMP receptor protein (CRP) of *E. coli* (193). When glucose is in short supply, the intracellular concentration of cAMP is upregulated in most microbes. In *P. aeruginosa*, two cAMP molecules bind the inactive Vfr dimer. This cAMP-Vfr complex is then able to bind a consensus dyad symmetrical sequence in the promoter region of a number of operons, including the quorum sensing regulator (*lasR*) and genes required for the utilization of various carbon sources, through a helix-turn-helix binding motif (193). Upon binding, the complex promotes the localization of the RNA polymerase holoenzyme (RNAP) to the promoter region through interaction between the β-subunit of the RNAP and the cAMP-Vfr complex. As previously mentioned, the cAMP-Vfr complex is able to activate the transcription of *lasR*, thereby activating the quorum sensing cascade when the autoinducer, 3-oxo-C₁₂-HSL, is present at significant levels. It is interesting to note that the transcription of the *vfr* gene itself has recently been shown to be activated by both the *las* and *rhl* autoinducer-transcriptional regulator complexes.

Another carbon metabolite regulator, termed the *catabolite repression control* (*Crc*) *protein*, has been recently found. This protein is able to sense carbon source availability and affects expression of the type IV pili structural subunit PilA to promote microcolony formation on biofilms. As demonstrated in mutation studies, *P. aeruginosa* Crc mutants are only capable of forming thin biofilm
monolayers instead of conglomerating into a number of microcolonies through type-IV-dependent twitching motility (199). Therefore, Crc may represent a link between carbon availability and the decision on whether or not to enter into a biofilm mode of growth.

Since *P. aeruginosa* prefers aerobic metabolism that utilizes a number of iron-containing enzymes, this microbial species has evolved a number of strategies to obtain iron from its environment (200). In order to conserve energy and resources, *P. aeruginosa* tightly regulates the expression of its iron acquisition systems to limit their activity in iron-rich environments. This iron-dependent regulation centers on the activity of a recently isolated homologue of the ferric uptake regulator (Fur) in *Escherichia coli* (200,201). When iron is in ample supply, Fur binds Fe$^{2+}$ and is able to attach to a palindromic consensus sequence (termed the "Fur box") in the promoter regions of iron-regulated genes, thereby repressing their expression. When *P. aeruginosa* is grown in iron-limited conditions, Fe$^{3+}$ dissociates from the complex, causing Fur release and repression removal. Genes controlled either directly or indirectly by the Fur system include those that code for other regulatory proteins (e.g., the sigma factor PvdS), iron-scavenging proteins (e.g., pyochelin and pyoverdin siderophores), proteases that degrade the iron-binding host proteins, the cytotoxin exotoxin A that enables iron release from susceptible host cells to occur, proteins involved in basic metabolic processes (e.g., Krebs cycle), and proteins responsible for oxidative stress survival (e.g., superoxide dismutase) (202–204). Alterations in iron concentrations have been shown to affect the quorum sensing system; such interactions may be mediated indirectly through the vfr regulation system, as seen in *V. fischeri* (205).

In reference to respiration, *P. aeruginosa* can utilize inorganic electron acceptors (other than oxygen) for growth. However, this species is incapable of fermentative metabolism and generally grows more fastidiously in oxygenated environments since it prefers aerobic metabolism. Therefore, it is not surprising that this organism alters its gene expression in response to oxygen levels. This control is mediated through the oxygen-sensing transcriptional regulator protein, anaerobic nitrate respiration (ANR) protein, which is homologous to the FNR in *E. coli* (206). This protein forms a [4Fe-4S]$^{2+}$ cluster under conditions of low O$_2$. This cluster formation has been shown to promote dimerization and binding to promoter regions of genes whose functions facilitate adaptation to growth under anaerobic conditions (e.g., denitrification enzymes and/or their regulators) (207). There are no data to support the direct role of ANR in the regulation of the

Figure 6  The complex interaction of the *P. aeruginosa* quorum sensing system and environmental stimuli in the regulation of toxA (exotoxin A) transcription. See text for gene descriptions.
quorum sensing system. However, O2 levels must be taken into account when evaluating cell-to-cell signaling experimental data since ANR has been shown to activate the transcription of a large number of enzymes associated with anaerobic metabolism while repressing the expression of those enzymes responsible for aerobic metabolism. By taking into account the effect of oxygen-dependent regulation, one may prevent the incorrect assumption of causal relationships between gene expression and quorum sensing system activity.

This organism has been shown to adapt to amino acid starvation through a complex series of regulatory events termed the stringent response that has been described as a global regulation mechanism. Briefly, this response (well elucidated in E. coli) is mediated through the accumulation of uncharged cognate transcriptional RNA (tRNA). When the ratio of aminocyl-charged to -uncharged tRNA falls below a critical threshold, occupation of the vacant mRNA codon at the ribosomal A site by uncharged cognate tRNA leads to stalling of peptide chain elongation. Also, the synthesis of the pseudomonal nucleotide (p)pGpp from guanosine triphosphate and adenosine triphosphate (GTP) (ATP) is induced in a ribosomal-dependent idling reaction (208,209). It has been demonstrated in E. coli that (p)pGpp inhibits RNA polymerase, causing the downregulation of a wide range of energetically demanding cellular processes (e.g., the synthesis of stable RNA), stimulation of certain amino acid synthesis pathways (e.g., isoleucine), and induction of stationary phase-specific genes through the effects of the stationary-phase/stress-specific sigma factor (a.k.a. σ70 or RpoS) (208,210). Whereas the σ70 (a.k.a. σ70') factor is responsible for the transcription of constitutive-expressed and housekeeping genes, σ32 has been implicated in the transcription regulation of over 50 genes in E. coli in response to not only amino acid starvation, but also osmotic stress, acid shock, heat shock, oxidative DNA damage, and transition to stationary phase. Transcriptional regulation of rpoS expression has been demonstrated to be under positive control by (p)pGpp and negative control by the cAMP receptor protein. Also, translational control has been ascribed to a number of other factors including an RNA-binding protein (Hfq), a nucleoid histonelike protein (H-NS), and a small regulatory RNA (dsrA RNA) that destabilizes the secondary structure in rpoS mRNA to allow translational initiation. However, proteolysis of RpoS by the ClpPX protease (due to the removal of protection of RpoS by the chaperone protein, DnaK) seems to be the main regulation mechanism (211). This sigma factor was shown in 2000 to inhibit rhlH transcription, thereby reducing the level of C4-HSL- and RhlR-RhlH-regulated gene transcription (194).

P. aeruginosa also mediates changes in gene expression through a complex array of other alternative RNA polymerase sigma factors in response to a number of environmental stressors. Heat shock is one example of an environmental stress, and the pseudomonal response is mediated through the combined effect of the extracytoplasmic stress factor, σ8 (a.k.a. AlgU), and σ32 (a.k.a. RpoH or σH) (212). AlgU and RpoH respond to the accumulation of misfolded proteins in the
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periplasmic and cytosolic bacterial compartments, respectively. Specifically, AlgU is able to upregulate its own expression as well as the expression of genes coding for RpoH and the enzymes of the alginate biosynthetic pathway (212). The antisigma factor products of mucA and mucB normally inhibit the activity of AlgU (213,214). However, in patients suffering from cystic fibrosis, these anti-sigma factor coding regions are often mutated, resulting in the conversion of nonmucoid strains into the mucoid variety by allowing for the constitutive overproduction of alginate (214,215). This excess of alginate production in P. aeruginosa results in the formation of biofilm microcolonies consisting of exopolysaccharide-embedded cells. These biofilm microcolonies demonstrate high-level resistance to host or antimicrobial clearance strategies. In reference to RpoH, many of its regulatory effects can be linked to its activation of GroESL proteins (192). These proteins act as chaperonins that sequentially promote correct folding of a number of proteins and aid in the formation of protein complexes, possibly including the LasR quorum sensing regulator with the LasI product, 3-oxo-C12-HSL, and the formation of the RhlR-C4-HSL complex (191,192). Two other environmental stressors that regulate gene expression through alternative sigma factors are the need for flagella (mediated by σF, a.k.a. σ28 or RpoF coded by fliA) and nitrogen depletion (σN a.k.a. σ54 or RpoN) (216,217). All of the sigma factors discussed have been well studied in E. coli, but their role in pseudomonal stress response and biofilm formation is still unclear.

GacA and GacS are highly conserved among Pseudomonas spp. and demonstrate upregulation of expression upon entry into stationary phase as a result of an unknown signal (187). gacS Encodes the cognate sensor kinase that activates the response regulator coded for by gacA by phosphorylation (218). This GacS/GacA system strictly controls the expression of extracellular products (antibiotics, exoenzymes, and hydrogen cyanide) when cells are in the transition from exponential to stationary phase. This system has also been found to increase the production of the C4-HSL autoinducer of the pseudomonal rhl quorum sensing system (187). It was hypothesized that activated GacA, by virtue of its typical C-terminal helix-turn-helix DNA binding motif, regulated the transcription of target genes. However, 1999 evidence points to posttranscriptional control by interacting with the mRNA ribosomal binding site of GacA-controlled genes (219). The importance of this regulatory system can be demonstrated by a study in which a gacA mutant of P. aeruginosa has attenuated virulence in animal models (218).

III. PROPERTIES OF THE HOST

A number of virulence factors of pathogenic microorganisms allow persistent infections, and many host factors assume a significant role. The localization of many MSLs within specific sites of the host is due to the vascular architecture of
Table 2  Systemic or Local Factors That Affect Immune Surveillance, Metabolism, and Local Vascularity

<table>
<thead>
<tr>
<th>Systemic (Bs)</th>
<th>Local (Bl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
<td>Major vessel compromise</td>
</tr>
<tr>
<td>Renal, hepatic failure</td>
<td>Small and medium vessel disease</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Extensive scarring</td>
</tr>
<tr>
<td>Chronic hypoxia</td>
<td>Arteritis</td>
</tr>
<tr>
<td>Immunosuppression or</td>
<td>Radiation fibrosis</td>
</tr>
<tr>
<td>immune deficiency</td>
<td>Chronic lymphedema</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Venous stasis</td>
</tr>
<tr>
<td>Immune disease</td>
<td>Neuropathy</td>
</tr>
<tr>
<td>Extremes of age</td>
<td>Tobacco abuse (\geq 2) packs per day)</td>
</tr>
<tr>
<td>Chronic granulomatous disease</td>
<td>Presence of implants</td>
</tr>
<tr>
<td></td>
<td>Localized trauma</td>
</tr>
</tbody>
</table>

*Bs, Bl, *

these sites. In addition, any systemic or local factor of the host that affects immune surveillance, metabolism, and local vascularity reduces the ability of the host to resolve the infection and may result in the development of MSIs (Table 2). A number of these factors are discussed in the sections that follow.

A. The Normal Host Vasculature

In cases of long bone osteomyelitis, the metaphyses of the long bones (tibia, femur) are most frequently involved (1). The anatomical characteristics of the metaphyseal region seem to explain this clinical localization (220). The afferent artery ends in the metaphyses as narrow capillaries that make sharp loops near the growth plate and enter a system of large venous sinuses where the blood flow becomes slow and turbulent. These capillary loops are essentially the "end-artery" branches of the nutrient artery. This structure leads to a slowing of blood flow in the area and presumably allows bacteria to settle and initiate an inflammatory response. The histological features of the region may also be a contributing factor. The metaphyseal capillaries lack phagocytic lining cells and the sinusoidal veins contain functionally inactive phagocytic cells (221); this structure further allows growth of microorganisms. Any end-capillary obstruction could lead to an area of avascular necrosis. Minor trauma probably predisposes the infant or child to infection by producing a small hematoma, vascular obstruction, and subsequent bone necrosis that are susceptible to inoculation from a transient bacteremia (222). Vertebral hematogenous osteomyelitis loca-
lizes within the cancellous bones, particularly the lumbar and thoracic areas of the spine because of their rich blood supply and reduced shear, thereby allowing for colonization and infection.

In cases of septic arthritis, the architecture of the normal joint space allows for the easy hematogenous entry of bacteria since the well-vascularized synovial membrane has no limiting basement membrane. Bacteria may also gain entry into the joint by direct introduction or extension from a contiguous site of infection. Once bacteria are seeded within the closed joint space, the low fluid shear conditions allow bacterial adherence and infection. The virulence and tropism of the microorganisms combined with the resistance or susceptibility of the synovia to microbial invasion are major determinants of joint infection.

B. Generalized Vascular Insufficiency

Most patients who have MSIs and generalized vascular insufficiency suffer from diabetes (6). The diminished arterial blood supply has traditionally been considered to be the major predisposing factor in the initiation of infection and its progression to a chronic state (6). Observations in 1999 suggest that neuropathy may be an equally important factor (223). Identifiable neuropathy as a complication of diabetes mellitus is present in approximately 80% of patients with foot disease (223). Neuropathy may cause foot infection through three mechanisms. First, patients with decreased sensation suffer mechanical or thermal injuries without awareness, leading to skin ulcerations. Second, motor neuropathy affecting the intrinsic muscles of the foot predisposes the patient to gait disturbances and foot deformities, such as hammer and claw toes and Charcot foot. These anatomical alterations may lead to a maldistribution of weight, which elevates focal pressure over the bony prominences. The increase in focal pressure where the foot contacts the ground or footwear may lead to subsequent skin ulceration. Third, autonomic neuropathy contributes by interfering with sweating and causing dry, cracked skin, thereby breaching the integrity of the skin envelope, allowing entry of microorganisms into the soft tissue. All three mechanisms may cause skin ulceration with subsequent skin infection, which may lead to contiguous focus osteomyelitis. A higher rate of nasal and skin colonization with *S. aureus*, defects in host immunity, and impaired wound healing all play a role in diabetic foot infection (224). Superficial fungal skin infections, which are common in diabetic patients, may also allow bacteria entry through macerated or broken skin. Inadequate tissue perfusion in the area of trauma allows the infection to persist to a chronic state.

Decreased sensitivity, ischemia, and a decrease in the cellular response to bacteria are all characteristics of diabetic foot infection. The extent of infection depends on the type of bacteria and number of different organisms present as well as the local tissue response. The location of infection is most often the
toes/phalanges or the midfoot/metatarsals. Although osteomyelitis can develop in the diabetic foot with sensate, well-vascularized soft tissue, it is uncommon. Footwear that does not evenly distribute weight or restricts circulation in an area that is under weight bearing pressure may contribute to cellulitis and ulceration. Cellulitis is an inflammatory response that produces gross signs of redness and edema. At the cellular level there are decreased levels of blood flow, oxygen, phagocytosis, complement factors, and antibodies and increased levels of acid, microscopic debris, and bacteria. The cause of diabetic foot ulcers and/or osteomyelitis is typically a contiguous spread of bacteria and necrosis from an ulcer site. The neuropathic patient may be unaware of the problem until there is a large ulcer formation. An abscess is produced when bacterial colonization creates a local pocket of cell death that is due to acid and pressure. A soft tissue abscess, left untreated, has the potential to progress to infect the bone. Infection in the diabetic foot is caused by the combination of decreased sensitivity, ischemia, and decreased tissue response to bacteria. The extent of infection depends on the amount and type of bacteria and the way the tissue responds to it.

C. Localized Vascular Insufficiency

As with generalized vascular insufficiency, local circulation that is compromised by one or more factors (Table 2) can lead to a reduction in the ability of bone and joint tissues to prevent or eradicate infections effectively as a result of the obviated metabolic supply to tissues, reduced immune surveillance, and the accompanying inhibition of the local inflammatory response (225). Colonization may also be aided in cases in which the bone or joint has undergone recent injury. In this environment, the production of host-derived extracellular matrix proteins that aid in healing (e.g., fibronectin) may promote bacterial attachment and progression to infection. Clotted blood, dead space, and compromised soft tissues make a medium perfect for bacterial proliferation. Within the damaged bone, necrosis of the outer tangential lamella partly is promoted by partial periosteal retrogression. Necrosis of the fracture ends caused by a disturbance of the medullary blood circulation may follow (226). Compromise of soft tissue adjacent to bone is a major reason for continued drainage (226,227). Within the bone, necrosis of the outer tangential lamella is partly promoted by partial periosteal retrogression.

D. The Involucrum and Osteomyelitis

In normal tissues, necrosis is an important feature of infection. Dead bone is absorbed by the action of granulation tissue developing at its surface. Absorption takes place earliest and most rapidly at the junction of living and necrotic bone. If the area of the dead bone is small, it is entirely destroyed by granulation tissue,
leaving a cavity behind. The necrotic cancellous bone in localized osteomyelitis, even though extensive, is usually absorbed. Some of the dead cortex (cortical bone) is gradually detached from living bone to form a sequestrum. The organic elements in the dead bone are largely broken down by the action of proteolytic enzymes elaborated by host defense and mesenchymal cells (polymorphonuclear leukocytes, macrophages, or the osteoclasts). Because of lost blood supply, dead bone appears whiter than living bone. Cancellous bone is absorbed rapidly and may be completely sequestrated or destroyed in 2 to 3 weeks, but necrotic cortex may require 2 weeks to 6 months for separation from living bone. After complete separation, termed sequestration, the dead bone is slowly eroded by granulation tissue and absorbed.

When the area of dead bone is too large, or the host response is systemically or locally compromised (Table 2), the process of bone resorption may be inadequate and may result in the development of the involucrum. Involution may be defined as live, encasing bone that surrounds infected dead bone within a compromised soft tissue envelope (228). The involucrum is irregular and is often perforated by openings through which pus may track into the surrounding soft tissues and eventually drain to the skin surfaces, forming a draining sinus tract (229). This host response is the hallmark sign of chronic osteomyelitis and is an attempt by the host to isolate the infection process. The development of the involucrum occurs once the infection is established and fibrous tissue and chronic inflammatory cells surround granulations and dead bone (228). New bone forms from the surviving fragments of periosteum, endosteum, and cortex in the region of the infection and is produced by a vascular reaction to the infection. New bone may be formed along the intact periosteal and endosteal surfaces. New bone may also form from the periosteum. The involucrum may gradually increase in density and thickness to form part or all of a new shaft. New bone increases in amount and density for weeks or months, according to the size of the bone and extent and duration of infection. Endosteal new bone may proliferate and obstruct the medullary canal. After the infection is contained, there is a decrease in the vascularization and the metabolic demands of an effective inflammatory response cannot be satisfied. The revascularization and resorption of the dead bone and scar tissue are similarly affected. The process of resorption eventually subsides and the Haversian canals are sealed by scar tissue. The decrease in vascularization produces a low oxygen tension in infected tissue that interferes with the normal oxygen-dependent intracellular killing mechanisms of the polymorphonuclear leukocyte and the angiogenesis and wound healing activity of fibroblasts (230).

The coexistence of infected, nonviable tissues and an ineffective host response leads to the chronicity of this disease. The nidus of the persistent contamination must be removed before the infection can begin to regress (229,231). Therefore, a thorough débridement is mandatory for resolution of
chronic osteomyelitis in situations in which the host defense removal of the sequestrum is inadequate. Once the sequestrum has been surgically removed, the remaining cavity may be filled with new bone, especially in children. However, in adults the cavity may persist, or the space may be filled with fibrous tissue that connects with the skin surface through a sinus tract.

E. Age and Musculoskeletal Infections

There are basic differences in the pathological features of MSIs in infants, children, and adults. In infants, small capillaries cross the epiphyseal growth plate and permit extension of infection into the epiphysis and joint space (232). The cortical bone of the neonate and infant is thin and loose, consisting predominantly of woven bone, which permits escape of the pressure caused by infection but promotes the rapid spread of the infection directly into the subperiosteal region. A large sequestrum is not produced because extensive infarction of the cortex does not occur, but large subperiosteal abscess may form. In children older than 1 year of age, infection presumably starts in the metaphyseal sinusoidal veins and is contained by the growth plate; the joint is spared unless the metaphysis is intracapsular. The infection spreads laterally, breaking through the cortex and lifting the loose periosteum to form a subperiosteal abscess. In adults, the growth plate has resorbed and the infection may again extend to the joint spaces. Also, in adults, the periosteum is firmly attached to the underlying bone, so subperiosteal abscess formation and intense periosteal proliferation are less frequently seen. The infection may erode through the periosteum, forming a draining sinus tract(s).

The elderly are more susceptible to a number of infections than younger adults, and, therefore, the aged may be considered immunocompromised. The decline in natural and induced immunity seen in the elderly results in a generalized reduction in the immune response to foreign antigens. The greater susceptibility to infections is due to the effects of age on the immune system and immune suppression caused by age-related illnesses. Specifically, the deficient immune response to foreign antigens results from the loss of thymic and T-lymphocyte function (mainly related to the production and response to interleukin-2 (IL-2)) and associated decrease in antibody production by B cells (233).

F. Implanted Medical Devices

The increased use of implanted medical devices such as intramedullary rods, screws, plates, and artificial joints has provided a physiological niche for pathogenic organisms to cause MSIs. Some bacterial species may initially colonize these implants during surgical implantation or subsequently by hematogenous spread. If the infection is of recent onset (<3 months), it is likely the
result of surgical contamination. In this setting, *Staphylococcus epidermidis* predominates as the major isolate. However, late-onset infection is usually caused by hematogenous seeding and *S. aureus* is the most common isolate, followed by *Streptococcus* spp., gram-negative bacilli, and anaerobes. An inherent problem associated with implants is their propensity to be coated in host proteins such as fibrinogen and fibronectin shortly after implantation (129). In the short term, fibrinogen/fibrin seems to be the dominant coating host protein, whereas fibronectin becomes dominant in the long term since fibrinogen/fibrin is degraded. Implants can then act as a colonization surface to which bacteria readily adhere through the binding activity of fibrinogen and fibrin binding receptors of *S. aureus*. Also, implants are often responsible for reduced blood flow and local immunocompromise by impairing natural killer, lymphocytic, and phagocytic cell activities. These implanted devices have also been linked to decreasing the amount of superoxide, a mediator of bacterial killing within professional phagocytic blood cells (234). Another mechanism by which implanted medical devices produce local immune compromise is through frustrated phagocytosis (234). In this case, professional phagocytes may undergo apoptosis when encountering a substrate of a size that is beyond their phagocytic capability. The resulting release of reactive products of oxygen and lysosomal enzymes may cause accidental host tissue damage and local vascular insufficiency, thereby increasing the predisposition to chronic osteomyelitis development. Also, a portion of the normal phagocytic processes are devoted to the removal of the implant foreign material (particularly with metals, methyl methacrylate, and polyglycolic acid), thereby using the energy and resources of the immune system that would normally be used to fight infection (235–237). Therefore, prosthetic implants not only provide a substrate for bacterial adherence, but also limit the ability of the host to deal adequately with the infection. Once colonized, bacteria (such as staphylococcal species) are able to synthesize a “slime” layer, termed the *glycocalyx* or *biofilm*. This layer prevents the inward diffusion of a number of antimicrobials and host phagocytic cells, thereby allowing the bacteria to escape the effects of antimicrobial therapy and the host immune system (238). Once an implant is colonized and chronic osteomyelitis ensues, the only treatment option is implant removal.

The risk of implant infection may be increased by a number of factors. First, certain joint replacements (e.g., total elbow arthroplasties) are more susceptible to infection because they remain close to the surface and have poor soft tissue coverage (239). Second, certain patient populations are at increased risk because of underlying conditions or systemic diseases, including those patients suffering from diabetes mellitus and rheumatoid arthritis (240). Also, of patients who are elderly, obese, or malnourished or who have undergone prior surgery at the implantation site are also at risk. Third, polymethylmethacrylate (PMMA) bone cement may be inhibitory to the activity of white cells and
complement function. Also, the heat released during PMMA polymerization may kill the juxtaposed cortical bone, thereby creating a nonvascularized area. This provides the bacteria a lush growth environment in which they are sealed off from the circulating host defenses. Finally, it has been shown that patient nasal carriage was the most important risk factor associated with surgical site infection (241). Therefore, it may be a worthwhile goal to eliminate *S. aureus* carriage prior to invasive procedures.

**G. A Special Case: Inherited Forms of Phagocyte Defects**

Defects of phagocyte function are due to alterations in which the normal oxidative burst of the phagocytes or phagocyte adherence ability (required for exit from the vasculature to infected tissues and opsonization of complement-coated bacteria) is reduced. These inherited defects occur in a small proportion of the population. However, the faulty phagocyte function can result in inhibition of infection clearance and progression to deep infection such as osteomyelitis. Three phagocyte defect syndromes that have been associated with the development of chronic osteomyelitis are chronic granulomatous disease (CGD), myeloperoxidase (MPO) deficiency, and hyperimmunoglobulin-E-recurrent infection (Job's) syndrome (HIE).

CGD patients have a defective cytochrome (b245) in the electron transport chain used in the production of reactive oxygen molecules (242,243). These reactive molecules are normally responsible for the oxidative burst in phagocytes that kill ingested microorganisms (243). Since catalase-positive pathogenic species (such as *Staphylococcus* spp., *E. coli*, *Pseudomonas* spp., *Aspergillus* spp., and *Candida* spp.) are able to degrade the low levels of hydrogen peroxide present in the phagocytes of these patients, they are usually associated with the deep infections encountered in CGD sufferers (244). MPO-deficient patients often are undetected since they rarely have recurrent infections unless they have a concomitant disease such as diabetes mellitus (244). However, they may be predisposed to recurrent *Candida* spp. infection (245). HIE patients have defective interferon-γ production by CD4+ T helper cells that results in abnormal chemotaxis and elevated IgE levels (246). These patients are susceptible to skin infections with *S. aureus* (244). The absence of specific granules is rare, but these patients also have recurrent infections thought to be secondary to a chemotactic defect and a minor abnormality of neutrophilic killing of microbes. Other disorders that affect phagocyte function include diabetes mellitus, liver failure, glycogen storage disease, antibody deficiency (IgG, IgM), complement deficiency (complement proteins C3, C3b), leukocyte adhesion deficiency types 1 and 2 (LAD 1 and 2), glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency), and Chediak-Higashi syndrome (CHS).
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H. Case Example of Normal Immune Responses in *Staphylococcus aureus* Osteomyelitis

During acute osteomyelitis, the innate immune system responds to the peptidoglycan wall (via N-formyl methionine proteins and teichoic acids) of *S. aureus* to produce proinflammatory cytokines (such as IL-1β, IL-6, and TNF-α) and C-reactive protein. These factors enable the host to mount a protective inflammatory response that contains this pathogen and often resolves the infection. However, when the infection is not cleared by the host’s innate immune system, *S. aureus* is well equipped to persist by a number of virulence factors and strategies, including, but not limited to, invading and surviving in mammalian cells, hiding within a biofilm, or producing a thick, antiphagocytic capsule. Also, the cell-mediated T helper [TH1] and humoral (TH2) adaptive immune responses are often inadequate. In a 1999 study using a murine model of acute hematogenous osteomyelitis, the increase in the central cytokines of cell-mediated immunity (IL-2 and IFN-γ) seemed to be only transient, whereas the inflammatory cytokines remained at elevated levels in osteomyelitic bone (247). This cytokine profile resulted in an initial expansion and activation of T-cell subsets followed by apoptosis. Therefore, *S. aureus* seemed to interfere with the antibacterial immune response by downregulating both T-cell immunity and adaptive immune cytokine production. Whereas a staphylococcal infection usually directs the immune system to a TH1 response (i.e., T-cell-mediated immunity), this type of immune response has questionable efficacy in the low oxygen partial pressures of infected bone where immune cell function is inhibited. Also, it was found in a 2000 study in mice that a high level of interferon-γ (a TH1 cytokine) plays a detrimental role in staphylococcal infection, and IL-4 and IL-10 (TH2 cytokines) are involved in host resistance to infection through regulation of interferon-γ (248). However, the necessity of the TH2 response to clear *S. aureus* infection was questioned in a 1999 study utilizing IL-4-deficient mice (249). It seems that a TH2 response is only required for *S. aureus* infection clearance in certain mice, depending upon their genetic background. In addition, it was shown in 2000 that when interferon-γ was given to mice infected with *S. epidermidis* well after the initial inflammatory response, the animal was able to reduce the level of biomaterial-associated infection (250). Also, whereas intraphagocytic persistence occurred in untreated mice, those animals that received interferon-γ did not demonstrate any gram-positive intracellular invasion. Therefore, although an early increase in TH1 cytokines during the initial inflammatory response may often result in host tissue damage, pathogen clearance may occur when these cytokines are provided to the infected host after this initial phase (i.e., during the temporal stage when the immune system has become compromised as a result of overstimulation by superantigens). This may be a valuable method to activate a correct and properly timed TH1 immune response.
In summary, *S. aureus* infects and elicits a strong native immune response, cytokine release, and high T cell activation. This pathogen is able to use a number of immune-avoidance strategies during this time (discussed previously), while the host immune system causes damage to “self” tissues and blood vessels in the area of infection. This damage may cause local circulatory and immune compromise. The high T cell activation eventually results in apoptosis and an impotent immune system, enabling this pathogen to persist. By artificially activating the host immune system to an effective TH$_1$ response (via administration of interferon-γ) after the initial inflammatory response, this persistent pathogen may be cleared more easily by the host.

I. Case Example of Normal Immune Responses in *Staphylococcus aureus* Septic Arthritis

Once colonized, bacteria are able to proliferate rapidly and activate an acute inflammatory response. Initially, host inflammatory cytokines, including IL-1β and IL-6, are released into the joint fluid by synovial cells (251). These cytokines activate the release of acute phase proteins (e.g., C-reactive protein) from the liver that bind to the bacterial cells and thereby promote opsonization and activation of the complement system. In addition, there is an accompanying influx of host inflammatory cells into the synovial membrane early in the infection. Phagocytosis of the bacteria by macrophages, synoviocytes, and polymorphonuclear cells occurs and is associated with the release of other inflammatory cytokines, which include TNF-α, IL-8, and granulocyte-macrophage colony-stimulating factor, in addition to increasing the levels of already present IL-1β and IL-6. It was demonstrated in a 1998 clinical study that IL-6 and TNF-α concentrations were persistently high even 7 days after treatment was initiated, whereas IL-1β concentration decreased significantly after 7 days (252). Many of these cytokines and the associated immune response have been shown in animal models to be required for bacterial clearance and the prevention of mortality due to bacteremia and septic shock (253). Nitric oxide, a common mediator of inflammatory cytokines, is also required (254).

The T cell mediated (TH$_1$) and humoral (TH$_2$) adaptive immune responses may also play a role in the clearance and/or pathogenesis of acute septic arthritis. T cells enter the joint within a few days after infection (255). The role of CD4$^+$ T cells in joint destruction has been demonstrated since their in vivo depletion resulted in a considerably milder course of staphylococcal arthritis (255). These lymphocytes are specifically activated by bacterial antigens in association with host antigen presenting cells or nonspecifically in the case of bacterial superantigens (e.g., TSST-1). The cytokine produced by these activated T cells, IFN-γ, has been shown to reduce the level of mortality and joint destruction in a mouse model of group B Streptococcus spp. when delivered 18 hours after bacterial
inoculation (256). However, when *S. aureus* was used as the infecting organism in this model, IFN-γ was shown to increase the frequency and severity of septic arthritis while protecting mice from septicemia (257). Also, it was found in a 2000 study in mice that a high level of IFN-γ (a TH1 cytokine) plays a detrimental role in staphylococcal infection, and IL-4 and IL-10, both TH2 cytokines, are involved in host resistance to infection through regulation of IFN-γ (248).

However, the necessity of the TH2 response to clear *S. aureus* infection was questioned in a 1999 study utilizing IL-4-deficient mice (249). It seems that a TH2 response is only required for *S. aureus* infection clearance in certain mice, depending upon their genetic background. Therefore, the exact role of T cells in host tissue damage and infection clearance is still being elucidated.

Under most circumstances, the host is able to mount a protective inflammatory response that contains the invading pathogen and resolves the infection. However, when the infection is not quickly cleared by the host, the potent activation of the immune response with the associated high levels of cytokines and reactive oxygen species leads to joint destruction. High cytokine concentrations increase the release of host matrix metalloproteinases (including stromelysin and gelatinase A/B) and other collagen degrading enzymes. When monoclonal antibodies or steroids attenuate these cytokines, cartilage degradation is minimized. The joint is further damaged by the release of lysosomal enzymes and bacterial toxins (258). Host proteoglycan degradation is initiated, followed by collagen degradation. In fact, the polymorphonuclear response with subsequent release of these proteolytic enzymes can lead to permanent destruction of intra-articular cartilage and subchondral bone loss in as little as 3 days. Metalloproteinases and the antigen-induced inflammatory response may persist and continue to damage the joint architecture even after the infection has been cleared (259,260). The infectious process induces a joint effusion that increases intra-articular pressure, mechanically impeding blood and nutrient supply to the joint. Thus, increased pressure destroys the synovia and cartilage. Because of the proximity of the epiphyseal growth plate to the joint, direct extension of a joint infection to any of the articulating bones may lead to decreased bone growth in infants and children (261,262). While bone mineralization is preserved, cartilage destruction causes joint space narrowing and erosive damage to the cartilage and bone if left untreated (263). In addition, the infection can spread to surrounding soft tissue, form sinus tracts, and disrupt ligaments and tendons in the untreated patient (264).

The interaction of the bacteria and host is of the utmost importance in the initiation and prolongation of infection and cartilage damage. There is a subtle balance between an effective immune response to eliminate the infecting organism from the host and the overactivation of this response that causes the majority of infection-related joint destruction. Therefore, care must be exercised and further studies must be performed in regard to using agents that suppress the inflammatory response in the treatment of septic arthritis.
Case Example of Normal Immune Responses in Neisseria gonorrhoeae Septic Arthritis

Gonococcal arthritis occurs in approximately 42%–85% of patients suffering from disseminated gonococcal infection (DGI) and begins with a localized mucosal infection (31,265). DGI-producing strains are unusually sensitive to the in vitro killing of penicillin G and possess unique nutritional requirements for arginine, hypoxanthine, and uracil. N. gonorrhoeae possesses a number of virulence factors. It is the combined effects of these factors, their phase and antigenic variation, and properties of the host immune response that enable this pathogen to persist and localized infection to become DGI.

The host may contain a gonococcal infection through the action of the innate immune response with particular dependence upon the complement system. This system is largely responsible for attracting polymorphonuclear leukocytes and the resulting cascade of inflammatory cytokines and chemokines. However, during periods surrounding early pregnancy, puerperium, and menstruation, the accompanying alterations in vaginal pH, cervical mucus, and genital flora and the endometrium exposure of submucosal vessels may predispose the female patient to N. gonorrhoeae invasion and DGI (31,164). Defects in the complement and/or reticuloendothelial systems may also inhibit the host's ability to prevent gonococcal MSI.

IV. SOURCE OF THE INFECTING ORGANISM AND THE DEVELOPMENT OF MUSCULOSKELETAL INFECTIONS

The source of infection is extremely important in the development of MSIs since it determines the type of infecting microbial species, the compartment to which the organism is delivered, and the size of the microbial inocula. As previously mentioned, S. aureus is able to cause MSIs derived from multiple sources, often independently of host factors or point of entry considerations, because of this species's vast array of virulence factors. However, many other seemingly "nonpathogenic" microbial species are only able to produce MSIs when delivered in high concentrations to specific musculoskeletal sites. These opportunistic pathogens depend upon high inoculation numbers and/or host defects to cause disease.

MSIs can result from hematogenous seeding or direct seeding. When infecting microbes are derived from transient bacteremia, this infection is usually monomicrobial with S. aureus predominating. However, host defects and the large inoculum size delivered directly into the bloodstream enable P aeruginosa and Serratia marcescens to cause many MSIs in the intravenous drug user (2,266). Also, exotic isolates including fungi, mycoplasmas, and anaerobes can be found
in compromised patients. The pathogenic microbes can also cause infection by gaining entry to the circulatory system from a distal focus of infection. These types of infections are heavily dependent upon the ability of the microbe to cause an initial infection, enter the blood, avoid immune clearance, and colonize and infect another area. One example of this type of infection is the development of gonococcal septic arthritis after *N. gonorrhoeae* urogenital infection and DGI. In 0.5% to 3% of gonorrhea infections, the pathogen is able to gain access to the bloodstream from the primary mucosal site of infections and produce disseminated gonococcal infection (31,267,268). A number of risk factors have been epidemiologically associated with the development of DGI (Table 3). Females are four times more likely to have DGI than males (265). This prevalence in women may be due to the asymptomatic nature of gonorrhea infections in women and the associated delay in diagnosis, thereby providing time for the bacteria to gain access to the bloodstream. In addition, a high percentage of affected females are either pregnant or menstruating at the time of the infection (31). Also, since the clearance of gonococcal infection depends upon an effective complement mediated immunity and a functional reticuloendothelial system, complement deficiencies and systemic lupus erythematosus are risk factors in this patient subset. In addition, some cases of secondary hematogenous MSIs may arise after the dissemination of *S. aureus* from an endocarditis source or after cases of infectious diarrhea by *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., or *Yersinia* spp. (27). A rare form of migrating polyarthritis may be caused by *Streptobacillus moniliformis*. In addition, the dissemination of *Borrelia burgdorferi* (the causal microbial agent in Lyme disease) from the initial tick bite and infection may cause MSIs.

MSIs can also be caused by direct microbial seeding of the bone or joint. For example, a direct extension of a contiguous focus of infection enables microbes to be seeded into the bone or joint from adjacent soft tissue infections (e.g., diabetic foot infections). In these cases, the multiple species of seeded

**Table 3** Risk Factors for Disseminated Gonococcal Infection

| Infection with transparent, piliated *N. gonorrhoeae* strains capable of phase variation |
| Diagnosis delay (especially in females, because of asymptomatic nature on the infection) |
| Complement system deficiency |
| Systemic lupus erythematosus |
| Menstruation, pregnancy, and puerperium |
| Male homosexuality |
| Urban residence |
| Promiscuity |
| Low socioeconomic and educational status |
microbes exist in a compromised host environment that allows their persistence, growth, and dissemination. Therefore, these types of infections are usually polymicrobial, and although staphylococcal species predominate, *Enterococcus* spp., *Proteus mirabilis*, *Peptostreptococcus* spp., diphtheroids, *P. aeruginosa*, and *Bacteroides* spp. are also found in sizable proportions of infected patients.

Direct seeding of organisms may also occur during invasive surgical procedures, such as total hip or total knee arthroplasties. If introduced during surgery, the most common contaminating bacterial species is *S. epidermidis* since it is a part of the resident skin flora and may find its way from the skin of the patient or health care provider to the surgical wound. In addition, the clotted blood, dead space, and compromised soft tissues of a surgical site make a medium perfect for bacterial proliferation once the wound has been colonized.

Trauma is the last source of direct microbial seeding. Trauma can take the form of a penetrating wound such as a stab wound or an animal or human bite. In these cases, the penetrating foreign object both generates a pocket of clotted blood and compromised soft tissue and carries microbes into these deep areas. Osteomyelitis or infectious arthritis may develop, depending upon the inoculum size, health of the host, and introduced microbe. Trauma can also take the form of seeding during open fracture. Under these infection conditions, rare species (e.g., *Pasteurella multocida*) may be isolated.

V. CONCLUSIONS

The reason why certain bacteria cause MSIs is dependent upon a clinical triad that includes the properties of the infecting microbial species, properties of the host, and source of the infection. Although *S. aureus* is the most commonly isolated bacterial species in cases of musculoskeletal infection, virtually every microbial species has been reported as the causal pathogen. The dominance of *S. aureus* in these types of infections may be explained by its wide variety of virulence products. Staphylococcal products that have a role in infection may be classified as virulence factors responsible for adherence, direct host damage, or immunoevasion. There are also a number of enzymes and extracellular proteins that may or may not have a role in virulence. The expression of these factors is regulated throughout the various stages of infection through quorum sensing and environmental cues. However, defects in the host or a large organism inoculum is required for most other microbial species, such as the quintessential opportunistic pathogen *P. aeruginosa*, to cause a musculoskeletal infection. The localization of many MSIs within specific sites of the host is due to the vascular architecture of these sites. In addition, any systemic or local factor of the host that affects immune surveillance, metabolism, and local vascularity reduces the ability of the host to resolve the infection and may result in the development of MSIs. Finally,
the source of infection is extremely important in the development of MSIs since it
determines the type of infecting microbial species, the compartment to which
the organism is delivered, and the size of the microbial inocula. When these three
facets of the clinical triad are taken into consideration, clues to the occurrence,
type, severity, and clinical prognosis of bone and joint infections may be
ascertained.

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