

Immune Responses to Indwelling Medical Devices

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Abstract Implanted medical devices have offered clinical hope to patients who either have critical illnesses or have more chronic problems such as joint destruction. No doubt, these devices have saved many lives and improved the quality of life of hundreds of thousands of people. Indeed, the use of indwelling devices has reached epic proportions in human medicine over the last three decades. One of the unintended consequences has been an accompanying rise in the infection rate in patients, which is directly related to the presence of these devices in humans. This is problematic because the devices are colonized by communities of microorganisms, termed biofilms, that are highly resistant to antimicrobial challenge and to destruction from the human host and its defenses. Over the past decade, there has been much progress on understanding how and why these communities are less susceptible to antimicrobial agents. However, many questions regarding the resistance of these communities to human host defenses are still unanswered. This chapter discusses the current knowledge of how the human immune system responds not only to the presence of indwelling medical devices, but also to the communities that colonize them.

1 Introduction

As the chapters in this book have repeatedly demonstrated, biofilm infections on implanted medical devices cause tremendous problems in medicine. Much of these chapters have documented on the antibiotic resistant nature of these communities and have touched upon the resistance of these communities to human host defenses. Indeed, a quick survey of all biofilm literature demonstrates that there is a preponderance of studies on antimicrobial resistance, yet there is a much smaller volume

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of knowledge about host resistance factors of biofilms. This knowledge gap on host defenses against biofilm infections is intriguing, since all implanted medical devices are quickly conditioned with host factors, including those factors that display antimicrobial activity. Additionally, it is clear that the human immune system responds to the presence of the implants: in some cases, as a result of the surgical procedure, and in other cases, as a result of the implant itself. Nonetheless, relatively few publications have focused on the host defenses and their responses to microorganisms specifically living the biofilm lifestyle. Since many previous chapters have included some information about either the immune response to implanted devices or the mechanisms associated with general host resistance, this chapter will review the main components of host factors involved in implant infections and document in detail innate immune responses to biofilms. Although the adaptive immune system plays an important role in defense, the role of the innate immune system will be the focus of the chapter, since it is the first line of defense against microbial colonization.

2 Implant Device Infections

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 infections. Some bacterial species may initially colonize these implants during surgical implantation or subsequently by hematogenous seeding. An inherent problem associated with implants is their propensity to be coated by host proteins such as fibrinogen and fibronectin shortly after implantation (Francois et al. 1998). In the short term, fibrinogen/fibrin seems to be the dominant-coating host protein, while fibronectin becomes dominant in the long term, since fibrinogen/fibrin is degraded. Implants can then act as a colonization surface to which many bacterial species readily adhere. Implants are also often responsible for reduced blood flow and locally compromised immunity 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 (Roisman et al. 1983). Another mechanism by which implanted medical devices produce locally compromised immunity is through frustrated phagocytosis (Roisman et al. 1983). In this case, professional phagocytes may undergo apoptosis when encountering a substrate of size that is beyond its phagocytic capacity. 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 of chronic infection development. A portion of the normal phagocytic processes is also devoted to the removal of the implant foreign material [particularly with metals, polymethylmethacrylate (PMMA), and polyglycolic acid], thereby utilizing the energy and resources of the immune system that would normally be used to fight infection (Santavirta et al. 1991; Santavirta et al. 1990; Wang et al. 1997). Therefore, prosthetic implants not only provide a substrate for bacterial adherence, but also limit the ability of the host

to adequately deal 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 infection resolution by antimicrobial agents and host phagocytic cells (Brause 1986). Once an implant is colonized and chronic infection ensues, the only treatment option is implant removal. The best alternative for preventing these difficult-to-treat infections seems to be preventing the biofilm from forming in the first place. A strategy in which high risk patients (e.g., patients undergoing dialysis treatment, long-term intravenous catheterization, or joint arthroplasties) are administered an “anti-biofilm” vaccine may enable the immune system to quickly recognize early biofilm epitopes and remove the invading microbial community before the development of the fully mature community that is resistant to the host’s immune system and antimicrobial agents.

Not only is treatment of implanted medical device infections difficult, but diagnosing these infections is also extremely problematic. However, a great step may have recently been made in the ability to diagnose these infections. An Italian group has developed an ELISA assay that was over 90% effective in detecting graft infections through the detection of serum antibodies against *Staphylococcus epidermidis* biofilm polysaccharide antigens (Selan et al. 2002). However, it is unclear whether this assay would be effective in diagnosing infections caused by other bacterial species.

The risk of implant infection may be increased by a number of factors. First, certain joint replacements are more susceptible to infection because they remain close to the surface and have poor soft tissue coverage (e.g., total elbow arthroplasties) (Sourmelis et al. 1986). 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 (Dougherty and Simmons 1989). Patients who are elderly, obese, malnourished, or have undergone prior surgery at the implantation site are also at risk. Third, PMMA bone cement may be inhibitory to the activity of white cells and complement function. The heat released during PMMA polymerization may also kill the juxtaposed cortical bone, thereby creating a nonvascularized area. This provides the bacteria a lush growth environment while being sealed off from the circulating host defenses.

A number of bacterial species are particularly well suited to cause infections in artificial joints. Although coagulase-negative staphylococcal species are often isolated from perioperative infections, *S. aureus* was found to be the major mediator of prosthetic implant infection in a number of studies (Arciola et al. 2001; Sanderson 1991). *S. aureus* is a gram positive, ubiquitous bacterial species, with the predominant reservoir in nature being humans. The carriage rate of this organism in humans is reported to be between 11 and 32% in healthy adults (Millian et al. 1960; Tuazon and Sheagren 1974). In the pre-antibiotic era, *S. aureus* bacteremia resulted in a 90% death rate (Smith and Vickers 1960). Because of the increasing involvement of *S. aureus* in foreign body-related infections, the rapid development and exhibition of multiple antibiotic resistance by these organisms, and their great propensity to change from an acute infection to one that is persistent, chronic, and recurrent, this pathogen is once again receiving significant attention.

3 Host Factors Involved in Prosthetic Implant Infection

3.1 Host Factors and the Implant

Prosthetic implantation provides a physiological niche for pathogenic organisms to cause infection. As mentioned, some bacterial species may originally colonize these implants during surgical implantation or afterward by hematogenous seeding. An intrinsic problem connected with implants is their tendency to be coated in host proteins, such as fibrinogen and fibronectin, shortly after implantation (Francois et al. 1998). Earlier, fibrinogen/fibrin seems to be the dominant-coating host protein, while fibronectin prevails in the long-term, as fibrinogen/fibrin is degraded. Because of the ability of bacteria such as *S. aureus* to bind to fibrinogen and fibrin via receptors for these molecules, implants can become colonized, as bacteria easily adhere to these proteins. As well, implants are frequently responsible for reduced blood flow and compromise of local immunity by impairing the activity of natural killer, lymphocytic and phagocytic cells, as well as by reducing the amount of superoxide, a mediator of bacterial killing, within professional phagocytic blood cells (Roisman et al. 1983). Another means with which implanted medical devices create local immune compromise is through frustrated phagocytosis (Roisman et al. 1983), which occurs when professional phagocytes undergo apoptosis upon encountering a substrate of size that is beyond their phagocytic capability. The resulting release of reactive oxygen products and lysosomal enzymes may trigger accidental damage to host tissue as well as local vascular insufficiency, thereby increasing the predisposition for chronic infection development. Also, a portion of the normal phagocytic processes are devoted towards the removal of the implant itself (particularly with metals, methyl methacrylate, and polyglycolic acid), thus utilizing the energy and resources of the immune system that would usually be used to fight infection (Santavirta et al. 1991; Santavirta et al. 1990; Wang et al. 1997). Therefore, prosthetic implants not only present a substrate for bacterial adherence but also restrict the capability of the host to effectively deal with the infection. Once established, bacteria (such as staphylococcal species) are able to produce a “slime” layer, termed the glycocalyx or biofilm (discussed later). This layer prevents the inward diffusion of a number of antimicrobials and host phagocytic cells, allowing the bacterial population to escape the effects of antimicrobial therapy and host clearance (Brause 1986). Once an implant is colonized and chronic osteomyelitis develops, the only treatment option is implant removal.

The risk of implant infection may be increased by a number of factors. First, certain joint replacements are more vulnerable to infection because they stay near the surface of the body and have poor soft tissue coverage (e.g., total elbow arthroplasties) (Sourmelis et al. 1986). Second, PMMA bone cement may inhibit complement function and the activity of white blood cells. Also, the heat released during PMMA polymerization may kill the adjacent cortical bone, thereby creating a nonvascularized area. This offers the bacteria a lush growth environment while being sealed off from the circulating host defenses. Third, some patient populations are at elevated risk

due to underlying conditions or systemic diseases, including those patients suffering from diabetes mellitus and rheumatoid arthritis (Dougherty and Simmons 1989). Other risk factors include the development of infection at the site of the prosthesis that is not associated with the prosthesis itself, the presence of malignancy, and a history of joint arthroplasty (Berbari et al. 1998). A surgical patient index score of 1 or 2 also increases the risk of PII (Culver et al. 1991). As well, patients who are elderly, obese, malnourished, or who have undergone prior surgery at the implantation site are also at risk.

3.1.1 Elderly Patients

The elderly are more susceptible to many infections than younger adults, and thus may be considered immuno-compromised. These individuals have a lessening of innate and adaptive responses, which results in a generalized reduction in the response to foreign antigens. Specifically, there is a deficit in thymic and T lymphocyte function, mostly associated with the decreased production of, and reaction to, interleukin-2 (IL-2) and the associated decline in antibody production by B cells (Ben-Yehuda and Weksler 1992). Also, it has been postulated that the decrease in T cell activation could be due to a decrease in the expression of costimulatory molecules, such as CD28, on the T cell, which thus causes T cells to be tolerized in the absence of this “second signal” (Nociari et al. 1999). This decrease in immune function can be due to the effects of age on the immune system as well as suppression caused by age-related illness.

3.1.2 Phagocyte Defects

Defects of phagocyte function occur when the normal oxidative burst of the phagocytes, or their ability to adhere (for extravasation into infected tissues or for opsonization due to complement), is reduced. This decrease in proper function can lead to the inhibition of infection clearance as well as a series of deep infections such as PII. The three phagocyte defect disorders that have been linked with the onset of chronic osteomyelitis are chronic granulomatous disease (CGD), meloperoxidase (MPO) deficiency, and hyperimmunoglobulin-E-recurrent infection (Job’s) syndrome (HIE). While there is no research illustrating a role for these defects in the inception of PII, because osteomyelitis and PII have such a similar disease course and because osteomyelitis often starts as an extension of PII, it is safe to assume that these illnesses can contribute to PII.

CGD patients have a defective cytochrome in the electron transport chain, which leads to a lack of production of reactive oxygen molecules (Gill et al. 1992; Tauber et al. 1983). These molecules are responsible for the oxidative burst that eradicates ingested microorganisms in phagocytes (Cunnion et al. 2001). As catalase-positive bacteria (such as *Staphylococcus spp.*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus spp.*, and *Candida spp.*) are able to degrade the low levels of hydrogen

peroxide present in the phagocytes of CGD sufferers, these are the organisms often associated with deep infections in these patients (White and Gallin 1986). Patients deficient in MPO often go undetected as they do not generally get recurrent infections, unless they suffer from another disease, such as diabetes, simultaneously (Duff et al. 1996). HIE patients suffer from faulty interferon- γ (IFN γ) production by CD4 T cells. This leads to abnormal chemotaxis and heightened IgE levels (Donabedian and Gallin 1983). These individuals are susceptible to skin infections with *S. aureus* (White and Gallin 1986), which could spread to cause deeper infections such as PII, osteomyelitis, sepsis, and brain and bronchial abscesses (Khanna et al. 2005). These patients also have recurrent infections believed to be derived from a chemotactic disorder and a minor anomaly of neutrophilic killing of microbes. There are several other disorders that may affect phagocyte function, including diabetes, liver failure, antibody deficiency, complement deficiency, leukocyte adhesion deficiency types 1 and 2 (LAD 1 and 2), Chediak–Higashi syndrome (CHS), glycogen storage disease, and glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency).

3.1.3 Immune System Disorders

Individuals who lack a fully functional immune system are at increased risk for osteomyelitis (Bahebeck et al. 2004; Restrepo et al. 2004; Rodriguez 1998; Talpada et al. 2002; Tehranzadeh et al. 2004). HIV is a worldwide epidemic, with 1.1 million Americans infected with the virus as of the end of 2002 (Tehranzadeh et al. 2004). Osteomyelitis is coupled with mortality rates over 20% in HIV-infected patients (Vassilopoulos et al. 1997), and is the second most frequent musculoskeletal infection in HIV-positive patients. Bone infections seem to be a common manifestation in young male homosexuals and intravenous drug users (Vassilopoulos et al. 1997; Belzunegui et al. 1997). Most of these infections are caused by *S. aureus* (up to 48%), though other species, such as *P. aeruginosa*, have also been found (Biviji et al. 2002; Major and Tehranzadeh 1997; Steinbach et al. 1993). In some cases, musculoskeletal infections such as osteomyelitis are the initial manifestations of HIV infection (Biviji et al. 2002), though these infections are not as common as infections affecting other organs (Major and Tehranzadeh 1997). The most commonly infected bones include the wrist, tibia, femoral heads, and thoracic cage, but other atypical sites, such as the patella and the mandible, have also been reported (Tehranzadeh et al. 1996). Though there is no reference to a correlation between HIV infection and PII, with its common linkage to osteomyelitis infections, it is safe to assume that HIV would predispose one to PII infection as well.

4 Role of the Innate Immune System

The innate immune system represents the first line of defense against invading microorganisms. It represents the most ancient defense mechanism and is found in plants, invertebrates, and vertebrates. Often ignored in the last two decades, its

importance has once again been brought to the forefront of immunology, some of which many argue was the direct result of the late Charles Janeway and his lifelong journey to understand the complexity and importance of these ancient antimicrobial defenses. While this may be debated, it is clear from the initial observations of Methnikoff, to the many contributions by Janeway and others, that the innate immune system is vital to many forms of life on this planet.

The innate immune system is composed of both soluble mediators, such as serum, enzymes, and other proteins, and cellular mediators such as macrophages and polymorphonuclear leukocytes (neutrophils). The main difference between these components, and the B and T lymphocytes of the adaptive immune system, is that while the innate immune system is highly capable of killing invading microorganisms and protecting the host from disease or death, it does not have a memory for these pathogenic events and therefore must continually fight these challenges from scratch. A good analogy is the posit that understanding the history of events, and then being able to plan accordingly for future challenges, results in more efficient and productive activities. This is really a description of the adaptive immune system. However, the innate immune system must respond to each challenge as if being invaded by a microorganism for the first time. In this context, the power of the innate immune system is truly amazing in that, in most cases, it protects humans and other animals from disease and death. The remaining chapter will focus on the soluble mediators and the cellular mediators of the innate immune system as they relate to published reports of biofilm-mediated infections. Although the authors have tried in earnest to be inclusive, we have chosen to focus on the most common mediators and on those mediators that have been studied in the context of biofilm infections.

5 Soluble Mediators

5.1 *Lactoferrin*

Lactoferrin is an iron-binding glycoprotein synthesized by neutrophils and in glands of the exocrine system. Its ability to bind free iron often protects the host simply by reducing the amount of iron available for invading microorganisms. By chelating iron, lactoferrin stimulates twitching motility in *Pseudomonas aeruginosa*. This twitching is a specialized form of motility causing bacteria to wander across a surface rather than aggregating to form a biofilm (Singh et al. 2002). By twitching motility, bacteria attach to surfaces using the type IV pilus (Mattick 2002). Retraction of the pilus frees bacteria from a surface, allowing them to roam, potentially searching the body for niches, such as an implanted medical device, where a community of organisms can be established (Skerker and Berg 2001). In the absence of lactoferrin, the daughter cells of attached bacteria remain near the origin of parent cell division, forming microcolonies and eventually biofilms. Conversely, the presence of lactoferrin induces motility and daughter cells move away from

parent cell origins, preventing the formation of microcolonies. That surface locomotion prevents *P. aeruginosa* biofilm formation suggests that microcolony development forms from the division of attached cells rather than the active aggregation of bacteria. In regards to host defense, lactoferrin prevents bacteria that survive initial killing from forming biofilms, buying time for adaptive responses to be recruited, and for antibiotics to be administered. Moreover, lactoferrin may take advantage of an evolutionary bacterial response. Since iron is an essential and difficult-to-acquire nutrient for microorganisms, lactoferrin-induced motility may actually benefit the bacteria. Through iron sequestration, twitching motility prevents bacteria from forming sessile biofilm structures in locations where iron is limited (Singh 2004). However, increased levels of superoxide dismutase activity have been observed in *P. aeruginosa* upon exposure to an iron chelator, reducing the efficacy of host respiratory burst response (Hassett et al. 1996, 1999).

Another unique ability of lactoferrin is its capacity to amplify apoptotic signals in infected cells, limiting necrotic tissue damage (Valenti et al. 1999). Necrotic tissue, a situation associated with implanted medical devices, serves as a wonderful ecological niche for microbial attachment and expansion at the site of necrosis. In addition, lactoferrin works synergistically with other compounds, including lysozyme, complement and prescription antibiotics. For example, combinations of lactoferrin and vancomycin demonstrated synergistic effects on biofilm reduction of *S. epidermidis* (Leitch and Willcox 1999a,b). *S. epidermidis* vancomycin resistance is attributed to the overproduction of cell wall materials that bind the drug at sites unrelated to its target location (Sanyal et al. 1993). When paired with lactoferrin, however, vancomycin penetrates the glycocalyx. This is attributed to the cationic lactoferrin binding the anionic cell wall materials of *S. epidermidis*, allowing vancomycin for greater access to its target (Leitch and Willcox 1999a,b). A similar mechanism of charge compensation is seen in conjunction with lactoferrin and Lysozyme (discussed later). Lactoferrin binds teichoic acid of *S. epidermidis*, allowing greater penetration of lysozyme and dissolution of the bacterial cell wall (Leitch and Willcox 1999a,b). In addition, the presence of lactoferrin has shown an increase in efficacy of antibiotics against *Salmonella* species (Naidu and Arnold 1994) and sensitization of *E. coli* to rifampin (Ellison et al. 1988). More recently, studies on the *Burkholderia cepacia complex* (Bcc), as a pathogen in cystic fibrosis (CF) lungs, demonstrated that lactoferrin, combined with rifampicin, decreased the viability of in vitro grown biofilms (Caraher et al. 2007).

The inactivation of lactoferrin may contribute to the chronic infection of *P. aeruginosa* biofilms common to CF patients. Cathepsin, a protease expressed by macrophages, fibroblasts, and in the epithelial cells of the lungs, becomes activated under acidic conditions (Chapman et al. 1997; Kirschke and Wiederanders 1994). The pH of the epithelial lining fluid of a CF patient is ~5.8, decreasing to 5.3 during infection (Tate et al. 2002). This acidic environment provides optimal conditions for cathepsin activity – shown to be several hundred-fold greater in CF patients than in non-CF patients (Rogan et al. 2004; Taggart et al. 2003). In addition, bacterial lipopolysaccharide (LPS) is also known to stimulate cathepsin release (Petanceska et al. 1996). Therefore, it seems that an initial bacterial infection in a CF patient

may increase concentrations of cathepsin, which cleaves and inactivates lactoferrin, predisposing the patient to *P. aeruginosa* biofilm formation and chronic infection (Rogan et al. 2004). However, lactoferrin binding of LPS may indirectly work in favor of the bacterium, decreasing the negative charge of the lipid A domain, enhancing the subsequent activity. This would in turn hinder the affects of other cationic antimicrobial molecules (CAMs, discussed later) (Na et al. 2004).

One of the most important works on lactoferrin production and anti-biofilm activity was published by Pradeep Singh's group in the journal Nature (Singh et al. 2002). For this study, Singh and colleagues demonstrated that lactoferrin, in concentrations below those commonly associated with antimicrobial activity, blocked biofilm formation by impacting twitching motility. In the presence of lactoferrin, the *P. aeruginosa* bacteria continuously wandered across the substrate surface and were never able to firmly attach and form mature biofilm communities. Follow up studies have suggested that lactoferrin production is decreased in CF patients (Rogan et al. 2004) and that the mucus environment in the CF lung prevents high concentrations of lactoferrin from reaching the biofilm bacteria (Matsui et al. 2006). However, all these studies were specifically associated with CF patients, not implanted with medical devices, and so it is unclear what role lactoferrin specifically plays in device-mediated infections. We have recently shown that cytokine crosstalk occurs between cellular mediators of the innate immune system in response to *P. aeruginosa* biofilms and that cytokine crosstalk eventually leads to lactoferrin secretion by human neutrophils (Leid et al. 2007). In these studies, *P. aeruginosa* biofilm bacteria that lacked the *flgK* gene (partly responsible for flagella production) were susceptible to lactoferrin-mediated killing. Overall, it is clear that lactoferrin is an important part of the innate immune defenses and it is likely that novel biofilm-specific mechanisms exist to defend against its broad antimicrobial activity.

Some treatment therapies received in health care facilities may actually encourage biofilm formation. Catecholamine inotropes, received by up to 50% of intensive care patients (Smythe et al. 1993), stimulate *S. epidermidis* growth as a biofilm on biomedical materials (Lyte et al. 2003). This mechanism of growth stimulation is due to the ability of catecholamines to supply iron to bacteria through interaction with transferrin and lactoferrin, allowing *S. epidermidis* to overcome iron restriction (Lyte et al. 2003; Freestone et al. 2000).

5.2 Lysozyme

Lysozyme is similar in its activity to the beta lactam antibiotics, in that it targets the microbial cell wall (peptidoglycan). However, unlike the beta lactams, which are most active against rapidly reproducing microorganisms because they block cell wall synthesis, lysozyme breaks down the cell wall and therefore may be active against microorganisms in all phases of growth. One of the first studies on implanted medical devices, bacterial communities, and lysozyme was conducted by Busscher et al. on voice prostheses (Busscher et al. 1997). Their study concluded

that voice prostheses preconditioned with saliva, and presumably lysozyme, retarded colonization by Streptococcal, Staphylococcal, and Candida yeast strains. A decade later, Hatti and colleagues reported that toothpaste containing salivary substitutes prevent biofilm colonization of healthy human teeth (Hatti et al. 2007). Interestingly, very few other reports have directly measured the activity of lysozyme against biofilm communities. This is especially important because the human body has many sources of lysozyme, including saliva (relevant to dental implants), serum (relevant to most other prosthetic implants), and neutrophils (relevant to most implanted devices). There is a clear need to better understand the role of lysozyme and biofilm bacterial interaction, as this enzyme may be employed as a defense against device colonization.

5.3 Cationic Antimicrobial Molecules

A recent review discussed antimicrobial peptides and biofilms and focused on the role of the exopolymers common in the bacterial matrix (Otto 2006). The main hypothesis was that the exopolymers either sequester or repulse these charged molecules. This is an intriguing approach and may lead to new therapeutics that either enhance activity of defensins and cathelicidins or block biofilm formation on implants containing these compounds. Defensins and cathelicidins are soluble mediators secreted by skin epithelial cells and sweat glands that act as first line representatives of innate host defense (Turner et al. 1998; Harder et al. 2001). Human cathelicidins and β -defensin have been shown to work synergistically against *S. aureus* (Ong et al. 2002). Defensins, in particular, are small peptides that form ion permeable channels in bacterial and mammalian cell membranes (Eckmann 2005). They are also part of the oxygen independent bactericidal mechanism used by human neutrophils (Schroder 1999). As mentioned earlier, these cationic peptides, however, are ineffective against *S. epidermidis* due to the production of polysaccharide intercellular adhesin (PIA). This gene-regulated response contributes directly to virulence and is often found in correlation with infections originating from indwelling medical devices. Furthermore, PIA seems specifically suited to protect against the antibacterial peptides of the skin. This is achieved by sequestering bacteria from proinflammatory products and inducing electrostatic repulsion of cationic antibacterial peptides. Vuong et al. (2004) pointed out that the specific characteristics of PIA may contribute to the reason why *S. epidermidis* is the predominant microbe on the human skin and major microorganism in nosocomial infections.

One of the hallmarks of biofilm infections is that they exhibit distinct developmental stages as they progress from initial attachment, which usually lasts from 6–48 h, to mature biofilm communities, sometimes observed as early as 4 days post-attachment. During this time, it is likely that these bacteria are exposed to components of the host defenses, but these defenses do not eradicate the expanding microorganisms. Long ago, we hypothesized that the human immune system likely

responds differently to these stages of biofilm maturation (Leid et al. 2002). More recently, Eberhard and colleagues quantified the different immune responses to early and late polymicrobial biofilms more clearly by using dental implants and the human oral cavity as a model (Eberhard et al. 2008). In their study, antimicrobial peptides such as human β -defensin, and two other newly characterized antimicrobial peptides RNase 7 and PSO, were dramatically upregulated in human epithelial cells at days 1 and 3 post-implant. As the implants were left longer and the biofilms were allowed to form for 9 days, the innate immune response of the epithelial cells switched to the recruitment of neutrophils through the secretion of IL-8. This work is the first of its kind to report a differential immune response to in vivo biofilms and, more importantly, to polymicrobial biofilms that develop in the oral cavity. Further studies along these lines will no doubt shed light on how the innate immune system either provides a protective response, or more importantly, how biofilm communities regulate the innate response such that biofilm growth and colonization occurs.

Although AMP activity is a broad response against microorganisms because of their ability to disrupt the cell membranes, as a therapeutic choice, one of the downsides of administration of AMPs may be their activity against the normal flora. To combat this, some groups have engineered Specifically (or selectively) Targeted AntiMicrobial Peptides (STAMPs). In two different studies, Eckert and colleagues demonstrated that manipulation of STAMPs resulted in selective killing of biofilms of *S. mutans* and *P. aeruginosa* (Eckert et al. 2006a, b). Interestingly, the STAMP version of the natural AMP G10 had increased activity against *P. aeruginosa* biofilms than the natural compound (Eckert et al. 2006b). Additionally, when coadministered with tobramycin, biofilm bacterial killing was enhanced, likely due to greater uptake of the antibiotic in the presence of increased membrane disruption (Eckert et al. 2006b). The combination of STAMPs and antibiotics may be a viable approach to treat biofilm infections in humans.

LL-37, the antimicrobial portion of cathelicidin, was shown to stimulate IL-8 and IL-1 β secretion in human epithelial cells (Tjabringa et al. 2003; Elssner et al. 2004). This event indicates that LL-37 is able to mount an acute inflammatory response (Eckmann 2005). Furthermore, cathelicidins have shown to be effective at killing nosocomial pathogens such as *S. aureus*, *Enterococcus faecalis*, and *P. aeruginosa* that are resistant to most antimicrobial therapies (Zanetti et al. 2002). The mechanism of killing involves membrane disruption; however, some porcine cathelicidins have been shown to interrupt bacterial protein synthesis (Oren et al. 1999; Boman et al. 1993). LL-37 also acts to orient the immune system by promoting leukocyte recruitment to infection sites (Scott et al. 2002). Microbial killing is further amplified by increased cathelicidin production by activated neutrophils (Turner et al. 1998). Cathelin, a prodomain of cathelicidin, is a cysteine protease inhibitor that promotes both bacterial killing and host tissue injury prevention by inhibition of microbial and neutrophil derived proteases. Cathelicidin is also known to induce apoptosis in proliferating lymphocytes, thereby regulating the inflammatory response (Zaiou et al. 2003; Risso et al. 1998). It is important to note that cationic peptides are often inhibited by high salt concentrations, as that observed in the CF lung. LL-37 is no exception. This may be a contributing factor to the chronic

inflammation seen in CF patients, as the environmental conditions force a valuable protease inhibitor to become inhibited.

As a percentage of all implant-related infections, urinary catheters and central venous catheters (CVCs) make up a majority of cases in the hospital setting. The normal therapeutic treatment for these device-related biofilm infections is removal of the fouled catheter and replacement with a new, sterile catheter. However, this treatment regime adds enormous costs to health care and does not address the issue of catheter colonization and biofilm infections. A more appropriate approach would be catheters that are resistant to colonization, therefore, preventing infections. Cirioni and colleagues demonstrated that pretreatment of CVCs with the cathelicidin AMP BMAP-28, before implantation into a rat model of infection, markedly reduced colonization by challenge with *S. aureus* (Cirioni et al. 2006). In combination with antibiotics, BMAP-28 reduced bacterial colonization to less than 10 CFU. In another study, Burton and colleagues demonstrated that a GlnU enzyme inhibitor, in combination with the cationic antimicrobial peptide protamine sulfate, dramatically reduced colonization of urinary pathogens to urinary catheters when the compounds were impregnated into the catheter design (Burton et al. 2006). These two studies, directed related to biofilm growth on implanted medical devices, clearly demonstrate the potential role of antimicrobial peptides in the fight against device related infections.

One of the most interesting aspects of biofilm studies in the last decade has been the observation that not just bacteria form biofilms. Indeed, many other microorganisms, including protozoa, viruses, and yeasts have been shown to live in biofilm communities. Being that these other microorganisms are fairly new to biofilm study, there has been little work on innate immunity towards these types of infections, even though these microorganisms are a cause of implant-related infections. Of all non-bacterial microorganisms, the fungi are the best studied microorganisms in the biofilm mode of growth. Burrows and colleagues recently demonstrated that non-amphipathic cationic AMPs were highly active against biofilms of *Candida* species (Burrows et al. 2006). Impressively, in their study, even preformed fungal biofilms on plastic surfaces, directly relevant to implanted medical devices, were killed when these AMPs were administered. Martinez and Casadevall studied the efficacy innate immune AMPs to *Cryptococcus neoformans* biofilms in a model of medical device infections (Martinez and Casadevall 2006). Their study demonstrated that many purified AMPs, including recombinant human β -defensins, were highly inactive against the biofilm form of this fungus, especially when compared to their planktonic counterparts (Martinez and Casadevall 2006). These conflicting results are a clear demonstration of the need for further studies on biofilms of non-bacterial origin and the resultant innate immune responses that may or may not protect the host from the development of chronic biofilm infections.

5.4 Complement

The complement system in humans is composed of greater than 20 serum proteins that act in a cascade fashion to induce inflammation through C3a and C5a, increase

phagocytosis of microorganisms primarily through coating with C3b, and by the formation of the membrane attack complex as the complement cascade proceeds. Complement activation is stimulated by such components as bacterial LPS within *P. aeruginosa* biofilm matrices. Despite activation, *P. aeruginosa* biofilms persist in CF patients, resulting in diminished neutrophil responses. Therefore, biofilms may induce a constant low-grade complement response, contributing to chronic lung inflammation (Jensen et al. 1993). Furthermore, phagocytic killing of mucoid *P. aeruginosa* requires complement opsonization of the bacteria. Non-mucoid exopolysaccharide (MEP)-specific opsonins deposit complement component C3 on cell surfaces below the MEP layer. As a result, it is hypothesized that MEP serves as a barrier, preventing cell-surface bound complement opsonins from binding complement receptors on phagocytes (Meluleni et al. 1995).

The Meluleni et al. (Meluleni et al. 1995) study tested this hypothesis by tracking C3 deposition of MEP-specific and non-MEP-specific opsonins. They determined that location of C3 deposition more strongly influenced opsonization than did amount to C3 present in sera. MEP-specific opsonin action by C3b occurs broadly throughout the biofilm, whereas non-MEP specific opsonization by C3b occurs in clusters around small patches of bacterial cells. The inability of CF patients to produce MEP-specific opsonins likely contributes to the persistence of *P. aeruginosa* infection. It may be possible, through active and/or passive immunotherapy, to improve production of MEP-specific opsonins in CF patients. Doing so may limit lung destruction from chronic infection (Meluleni et al. 1995). Complement activation might further be hindered by the fact that some surfaces, such as those of manmade biomaterials, may not permit formation of C5 convertase. This would inhibit complement activation and thus cleavage of C3 to C3a and C3b (Janatova 2000).

More recently, Hoffmann and colleagues demonstrated that Azithromycin activity against mucoid *P. aeruginosa* biofilms was multifactorial and included an increased susceptibility of biofilm microorganisms to complement and hydrogen peroxide mediated killing (Hoffmann et al. 2007). Although complement itself did not result in increased biofilm killing, the combination with the antibiotic Azithromycin did. The synergy between antimicrobial, biofilm-specific killing and components of the human innate immune system are ripe for further exploration. As expected, complement by itself does little to enhance biofilm-specific killing. Simmons and Dybvig recently confirmed what has been known for some time in other microorganisms (Janatova 2000), when they demonstrated that biofilms of *Mycoplasma pulmonis* were protected against the lytic effects of complement (Simmons and Dybvig 2007). Finally, in a biochemical study of sialic acid bacterial intake in *H. influenzae*, Johnston et al. demonstrated that the ability of biofilm bacteria to take up sialic acid directly related to the ability of complement to kill biofilm bacteria (Johnston et al. 2008).

We have, in collaboration with Dr. Samuel Silverstein's laboratory at Columbia University, looked at both complement and antibody penetration (and eventual coating) and killing of biofilms of *S. epidermidis*. Although these studies are not complete, they do suggest that biofilm bacteria are easily opsonized by antibody and complement and that biofilm killing does occur if the appropriate conditions are present. Fig. 1 demonstrates the opsonization of *S. epidermidis* biofilm bacteria

by antibodies and complement. Although these biofilms were very mature (10 days old), it is clear that both antibodies and complement can opsonize the individual bacteria within the biofilms. However, for reasons yet to be elucidated, this interaction does not always lead to effective bacterial killing. We are currently investigating the mechanisms behind complement mediated opsonization and biofilm bacterial killing.

Bacteria have developed clever methods of evasion to escape host immune responses. CHIPS (chemotaxis inhibitory protein of *S. aureus*), for example, is a small protein secreted by *S. aureus* that blocks chemotaxis receptors, including complement C5a receptor (de Haas et al. 2004). In addition, *S. aureus* secretes staphylokinase to inhibit defensins (Jin et al. 2004). Bacteria can also incorporate molecules into their cell wall to alter its composition and avoid host defenses. The addition of D-alanine or L-lysine to the cell wall of *S. aureus* alters the net charge, making it more positive and more resistant to many antimicrobial peptides of the innate immune system. As a result, cationic AMPs (CAMPs) are hindered by electrostatic repulsion. *P. aeruginosa* employs a similar mechanism of CAMPs repulsion by incorporating the cationic aminoarabinose into its lipid A matrix (Fedtke et al. 2004). This kind of modification is often seen in CF patients and is suggestive of specific lipid A structures associated with the disease. In addition to CAM resistance, this alteration leads to an increased recognition by TLR-4, contributing to progressive lung degeneration (Ernst et al. 2003).

Staining for C3 and IgG on 10-Day *S. epidermidis* Biofilm

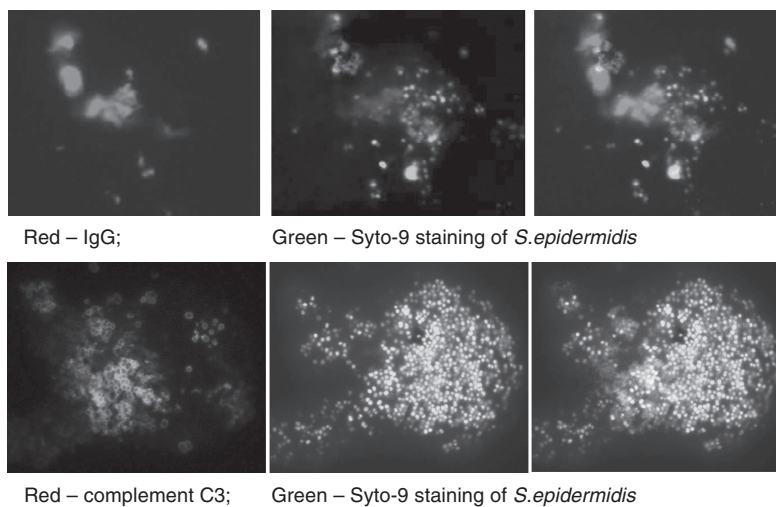


Fig. 1 Confocal micrographs of 10-Day-Old *Staphylococcus epidermidis* biofilms stained with Syto 9 (Green Fluorescence) and IgG (Red - top panel, specific for *S. epidermidis*) and Complement component C3 (Red - bottom panel). Notice that both antibody and complement bind to and opsonize the biofilm bacteria, although not at 100% efficiency

6 Cellular Mediators

Although many of the aforementioned molecules are released from cells of the human immune system, we have separated them above for clarity of their specific actions against biofilm-specific microorganisms. The final section of this chapter will detail cellular-specific mediators of the innate immune system and document their activity against biofilm microorganisms. Primarily, we focus on products secreted from activated neutrophils and macrophages.

S. mutans biofilms have been shown to absorb two-thirds the release of superoxide from neutrophils compared to planktonic bacteria. This may be accounted for by the presence of specific plasma proteins, such as albumin or immunoglobulins, which may hinder the attachment of cells to biofilms. These plasma proteins may block specific binding sites on neutrophils required for adhesion to bacteria and release of effective concentrations of microbicidal superoxide. The relatively high levels of plasma proteins in areas of gingival inflammation may affect neutrophil attachment, promoting cariogenic biofilm colonization (Shapira et al. 2000). What's more, extracellular polysaccharides produced by *S. mutans* inhibit neutrophilic detachment of biofilm bacteria (Steinberg et al. 1999). This might lead to prolonged PMN recruitment and accumulation due to frustrated phagocytosis. Evidence suggests that high neutrophil density is associated with reduced superoxide production as a protection mechanism for the host. Since superoxide is toxic to host cells, elevated concentrations could lead to neutrophil destruction and surrounding tissue damage (Shapira et al. 2000; Tanigawa et al. 1995).

Similar activity is seen with *P. aeruginosa* biofilms, as PMN oxidative burst responses are greatly reduced to only 25% of the response seen against planktonic bacteria (Jensen et al. 1990). Alginate production by *P. aeruginosa* biofilms protect against phagocytosis in the absence of specific host antibodies (Speert et al. 1986; van Oss 1978; Leid et al. 2005). This is not always the case, however, in the lungs of CF patients, as proteolytic enzymes from neutrophils may cleave immunoglobulins (Doring et al. 1985; Fick et al. 1985; Suter et al. 1984). Furthermore, alginate impedes chemotaxis, reducing PMN bactericidal potential and phagocytosis by macrophages (Simpson et al. 1988; Oliver and Weir 1990; Bayer et al. 1991; Stiver et al. 1988). Alginate may also act as a permeability barrier, trapping mediators and ions needed for an effective immune response (Jensen et al. 1992; Hoyle et al. 1990). Alginate further interferes with opsonization, induces proinflammatory cytokines, and suppresses lymphocyte transformation (Pedersen et al. 1992; Pedersen 1992). However, many of these studies were not done on alginate produced by biofilm microorganisms. Nonetheless, it is clear that *P. aeruginosa* biofilms result in defected neutrophil and monocyte killing (Leid et al. 2005; Jesaitis et al. 2003).

Initial colonization of CF lungs by *P. aeruginosa* can be eradicated by early and aggressive antibiotic therapy. However, once the bacteria convert to the mucoid form, characterized by alginate production, the organism can no longer be eliminated (Frederiksen et al. 1997). An experiment performed by Mathee et al. exposed non-mucoid *P. aeruginosa* to human PMNs and H₂O₂ and these scientists were

subsequently able to isolate mucoid variants. This suggested that mucoid conversion in the host is a defense mechanism of the bacteria in response to toxic oxygen byproducts. Therefore, H_2O_2 released by neutrophils, the predominant cells of inflammation in the CF lung, plays an important role in the conversion of *P. aeruginosa* to the incurable mucoid form (Mathee et al. 1999).

In general, *P. aeruginosa* biofilms show great resistance to toxic oxygen products such as H_2O_2 , converting it to O_2 through catalase and superoxide dismutase activity (Lu et al. 1998; Brown et al. 1995; Hassett et al. 1995). Biofilm alginate may serve as a sink for O_2 , H_2O_2 , O_2 and myeloperoxidase products as a result of neutrophil immobilization. During an infection, neutrophils accumulate on the biofilm extracellular matrix, releasing toxic oxygen species, degradation enzymes, defensins, and lipid inflammatory mediators. Immobilized neutrophils become exposed to their own oxidants, resulting in self-injury (Bass et al. 1977; Pietarinen-Runtti et al. 2000). Lactoferrin, released by neutrophils and discussed earlier, falls prey to the high concentration of proteases in these areas, suffering degradation and losing its anti-biofilm properties (Singh et al. 2002). In addition, the biofilm extracellular matrix helps to replenish neutrophil-depleted oxygen needed for bacterial metabolism. Quorum sensing in *P. aeruginosa* also leads to biofilm bacterial tolerance to H_2O_2 and neutrophil killing (Bjarnsholt et al. 2005). One product of the quorum sensing system in *P. aeruginosa* is rhamnolipids. This same group has recently shown that rhamnolipid production rapidly killed accumulating neutrophils (Jensen et al. 2007), although results with other strains of *P. aeruginosa* in our hands has not resulted in killing of neutrophils, even though rhamnolipids were produced. It is yet unclear what this discrepancy of results is the result of. However, it is clear that neutrophils, and their products, are incapable of killing biofilms of *P. aeruginosa*. In other words, it appears that neutrophils help to provide the biofilm with the nutrients it needs to survive, while inflicting self-injury and hindering host immune response (Jesaitis et al. 2003). Neutrophils also lend to the pathology of lung disorders, providing a source of DNA and actin polymers that contribute to the congestive viscosity of CF lung mucous (Kirchner et al. 1996; Khan et al. 1995).

We demonstrated the ability of human leukocytes to penetrate *S. aureus* biofilms under shear force, mimicking those conditions found in vivo (Leid et al. 2002). Our data indicated that neutrophil (and other leukocytes) access to biofilm bacteria was not hindered by the biofilm structure, suggesting that other properties of biofilms are responsible for incapacitating host immune response (Leid et al. 2002). Consequently, once neutrophils settle and/or penetrate biofilms, they become unable to migrate from their point of contact and their secreted products are quickly overcome by the biofilm microorganisms. Microscopic evidence suggests that neutrophils maintain a rounded morphology, characteristic of unstimulated cells. Despite this inactivated appearance, neutrophils preserve their capacity for respiratory burst, degranulation, and phagocytosis. However, they are unable to effectively clear the infection through bactericidal activities (Jesaitis et al. 2003). The observed rounded cell morphology might be explained by production of *P. aeruginosa* exotoxins (ExoS and ExoT) injected into the neutrophil by Type III machinery (Singh et al. 2002). ExoT is a rho GTPase that works to inhibit actin polymerization, thereby

affecting cytoskeletal remodeling (Kazmierczak et al. 2001; Krall et al. 2000; Pederson et al. 1999). The induction of ExoS is capable of stimulating TLR-2 and TLR-4 simultaneously, inducing an extensive and damaging proinflammatory response that includes neutrophils (Epelman et al. 2004).

A study by Mittal et al. (Mittal et al. 2004) found that biofilm cells were able to survive neutrophil killing in *P. aeruginosa* infection of the bladder. These biofilm cells later reached renal parenchyma despite high numbers of neutrophils present at the site of inflammation (Mittal et al. 2004). This may have been facilitated by the ability of *P. aeruginosa* to induce cell death of PMNs through Type III machinery. In addition to the induction of neutrophil necrosis, bacterial induction of apoptosis may influence the severity of infection. *P. aeruginosa* virulence factors – pyocyanin, exoenzyme S, and cell surface porins – have been shown to induce apoptosis of neutrophils and macrophages (Usher et al. 2002; Kaufman et al. 2000; Buommino et al. 1999). Quorum sensing molecules (homoserine lactones) may also be associated with this apoptotic process, further influencing the resilience of *P. aeruginosa* (Charlton et al. 2000). Thus, reduced oxidative potential, the presence of quorum sensing molecules, and induced apoptosis through cytotoxins may explain *P. aeruginosa*-associated UTI resistance to host defenses (Mittal et al. 2004).

Chronic CF lung infection is largely attenuated to the mucoid *P. aeruginosa* phenotype (Govan and Deretic 1996). However, small colony variants (SCV) have been isolated from the CF lung (Haussler et al. 1999). Some SCVs express hyperpiliation, increased twitching motility, and a preference for stationary growth, possibly contributing to biofilm formation (Haussler et al. 2003). In fact, recovery of SCVs may be correlated to poor lung conditions and use of inhalative antibiotics (Haussler et al. 1999). Furthermore, genes involved in the Type III secretion system are strongly upregulated in some SCVs. This resulted in increased cytotoxicity and enhanced virulence in a murine model respiratory tract infection (von Gotz et al. 2004). These variants may be the result of mutation and selection induced by the environmental conditions of the CF lung (Haussler et al. 2003). *S. aureus* SCVs in CF patients show similar resilience to phagocytosis and cationic peptides. In fact, positively charged antimicrobial peptides may actually select for SCVs. Consequently, AMPs are further hindered by the high salt concentration common in the CF lung (Sadowska et al. 2002).

As mentioned throughout this book, there is a tendency for implanted medical devices to become coated with fibrinogen and fibronectin host proteins, resulting in bacterial colonization of the device (Francois et al. 1998). Reduced blood flow to the area surrounding the implanted medical device is another consequence of implantation, compromising host defenses by impairing natural killer cells, lymphocytes, phagocytosis, superoxide activity, and in general, leukocyte inflammation (Roisman et al. 1983). The process of frustrated phagocytosis is also associated with indwelling medical devices (Roisman et al. 1983). This occurs when professional phagocytes undergo apoptosis in response to encountering a substrate too large to engulf and destroy. Reactive oxygen products and lysosomal enzymes are released, causing host tissue injury and poor local vascularity. The host is then susceptible to the development of biofilm and/or chronic infection (Santavirta et al. 1991, 1990;

Wang et al. 1997). Furthermore, heat released during PMMA bone cement polymerization may kill surrounding bone tissue creating a nonvascularized area. This would provide bacteria with an ideal growth environment, protected from circulating host defenses.

One preponderance of the collective amount of data relating to the innate immune system and biofilms is that neutrophils recovered from implant infection sites are highly activated yet unable to control the infection (Wagner et al. 2004). A loss of migratory ability is observed while cytotoxic potential is maintained, contributing to tissue destruction (Wagner et al. 2003). Because neutrophils release nitric oxide, cytotoxins, and other bactericidal agents that are toxic to host tissues, PMN response is limited in terms of time and space. This is usually achieved by macrophages, which infiltrate the infected area and clean up spent and apoptotic neutrophils (Ward and Lentsch 1999; Dallegri and Ottonello 1997; Kaplanski et al. 2003). Uptake by macrophages is necessary to prevent tissue damage from neutrophil release of cytotoxic and proteolytic mediators (Melnikoff et al. 1989; Savill 1997). This, however, is not the case in posttraumatic osteomyelitis, a device-associated infection resulting in progressive inflammatory disease. Colonization of the implant by a Staphylococci biofilm may require implant removal and even reconstructive surgery due to tissue destruction and osteolysis (Wagner et al. 2004). Here, macrophage infiltration is not observed and neutrophil mediated tissue destruction remains unrestrained (Wagner et al. 2003). CD16, the low-affinity receptor for IgG, may be a possible explanation in the observed neutrophil longevity as loss of CD16 is associated with cell apoptosis (Dransfield et al. 1994; Homburg et al. 1995). Neutrophils observed in posttraumatic osteomyelitis patients expressed elevated levels of CD16, which may explain why neutrophils remained viable at the infection site, escaping apoptosis. As a result, chronic inflammation and eventual osteolysis associated with posttraumatic osteomyelitis occurs. The exact mechanism of CD16 regulation in this setting is not yet understood (Wagner et al. 2003).

$\gamma\delta$ T-lymphocytes represent an additional cellular mediator in the innate immune response. Their role in biofilm defense certainly warrants further investigation. Elevated levels of these lymphocytes are commonly seen in the blood of CF patients (Perez-Payarols et al. 1994). In addition to possessing cytotoxic properties, $\gamma\delta$ T-cells also release cytokines that activate macrophages needed to clear infection sites of bacteria as well as apoptotic host defense cells (Julia et al. 1998). However, there have been no studies to date that specifically correlate $\gamma\delta$ T cells and biofilm persistence.

Toll-like receptors (TLR) are surface molecules on innate immune cells and are important structures in activating the host's immune system cascade. For example, monocytes and macrophages rely on TLR-4 to recognize bacterial LPS, releasing Interleukin-1 (IL-1) that promotes formation of β -defensin (Hoffmann 2003). Transcription of proinflammatory cytokines by TLR binding requires myeloid differentiation factor 88 (MyD88) (Takeda et al. 2003). Even non-TLR proinflammatory mediators, such as complement fragments, are unable to facilitate efficient neutrophil recruitment in MyD88 deficient mice (Skerrett et al. 2004). TLR-4 also induces NF- κ B activation, which can lead to downstream gene activation. This

pathway, however, is also reliant on functional NADPH oxidase (Sadikot et al. 2004). Production of superoxide in the respiratory burst is dependent on NADPH oxidase, as is H_2O_2 and OH ions (Babior 2004). Presence of reactive oxygen species liberates neutrophil elastase and cathepsin G from extracellular matrix, promoting host tissue injury (Reeves et al. 2002). Neutrophil elastase may cause a downregulation of TLR-4 in human bronchial epithelial cells (Devaney et al. 2003), resulting in a severely hindered innate immune system, predisposing the host to chronic infection (Hauber et al. 2005).

Upregulation of TLR-2 may occur through *P. aeruginosa* activation of TLR-4. TLR-4 is known to build tolerance against bacterial LPS, leading to hyporesponsiveness in instances of prolonged exposure (Medvedev et al. 2000). This indicates that TLR-4 is most active during the early phase of *P. aeruginosa* infection and that TLR-2 becomes more involved as the infection progresses (Power et al. 2004). TLR-2 expression works to reduce host signaling in response to adhesins during infection. This effect is troublesome in the CF patient. Mucins are constantly produced in the CF airway, resulting in excessive mucous secretion. Adhesins are needed to bind mucins; however, should the adhesins interact with the host, unblocked by TLR-2, an overexpression of calgranulins would result. In fact, excessive calgranulins are often observed in CF patients. Therefore, reduced TLR-2 expression may promote lung environment hostility and inflammation while hindering host defenses (Lorenz et al. 2004).

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