

Immunology of *Staphylococcal* Biofilm Infections in the Eye: New Tools to Study Biofilm Endophthalmitis

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ABSTRACT

Endophthalmitis is an important disease of the eye that is most frequently caused by postoperative and post-traumatic introduction of bacteria into the posterior segment of the eye. In the case of severe infections, visual acuity is greatly damaged or completely lost. Much work has focused on the ability of planktonic bacteria to cause infection and ocular damage while little work has focused on chronic infections in endophthalmitis mediated by the formation of bacterial biofilms on the surface of the lens. This review focuses on the interaction of *Staphylococcus aureus* and *Staphylococcus epidermidis* lens-associated biofilms in endophthalmitis. Additionally, this review highlights some relevant biofilm-immune system interactions and outlines a new *in vivo* mouse model to explore biofilm-related infections in endophthalmitis.

INTRODUCTION

ENDOPHTHALMITIS IS A BACTERIAL INFECTION of the posterior of the eye that frequently results in loss of visual acuity by causing irreversible damage to important components that collectively define the eye. Most infections are caused by physical introduction of bacteria into the eye either through surgery or trauma, although bacteria from an infectious nidus elsewhere in the body occasionally gain access to the eye through the systemic circulation. Numerous studies have investigated the interaction of planktonic bacteria that cause endophthalmitis and a clearer picture of the pathology of the disease in regard to the immunology of host–planktonic bacterial interactions is now emerging. In comparison, there has been little work published on what role biofilms play in endophthalmitis. This review will discuss endophthalmitis in the context of staphylococcal biofilms, the immunology behind these infections, and outline a new *in vivo* mouse model that will help define both the immunology of the biofilm infection as well as help elucidate the role of immune privilege in endophthalmitis.

BACKGROUND OF PLANKTONIC *STAPHYLOCOCCAL* ENDOPHTHALMITIS

Bacterial endophthalmitis is a disease caused by bacteria that often threatens visual acuity (Giese and Mondino, 2001; Cal-

legan *et al.*, 2002). Considerable work has been done in regard to the epidemiology, pathology, and host–pathogen interactions that occur in endophthalmitis, and thus, only a few issues will be covered here. For a more extensive review of planktonic endophthalmitis, see two recent reviews (Giese and Mondino, 2001; Callegan *et al.*, 2002). By far, coagulase-negative *Staphylococcus epidermidis* is the most frequent etiological agent of endophthalmitis (Callegan *et al.*, 1999). *Staphylococcus aureus* follows as the second most common causative agent in postoperative endophthalmitis, and *Bacillus cereus* as the second most common cause in posttraumatic endophthalmitis (Affeldt *et al.*, 1987; Schemmer and Driebe, 1987; Thompson *et al.*, 1993; David *et al.*, 1994; Han *et al.*, 1996; Callegan *et al.*, 1999; Aaberg *et al.*, 1998). Patient outcome is largely dependent on the etiological agent. For example, *B. cereus* and *S. aureus* are typically associated with a massive inflammatory response and a particularly unfavorable visual prognosis (Aaberg *et al.*, 1998; David *et al.*, 1994; Schemmer and Driebe, 1987).

Protein toxins produced by these organisms have been postulated as critical factors in the pathogenesis of endophthalmitis caused by the latter two organisms. In *S. aureus* infections, a number of pore-forming toxins have been demonstrated as virulence factors in endophthalmitis, because abrogation of toxin production decreased the severity of the disease (Booth *et al.*, 1995, 1997). However, these studies also demonstrated the presence of an inflammatory response even with attenuation of the pore-forming toxins, suggesting that other factors also play a role in the

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pathology of the disease. Indeed, clinical isolates of *S. aureus* have been shown to secrete a variety of inflammatory factors that likely play a role in pathogenesis and the inflammatory response of the host (Humphreys *et al.*, 1989; O'Reilly *et al.*, 1990; Johnson *et al.*, 1991; Coia *et al.*, 1992).

Two major regulatory systems exist in *S. aureus* that are responsible for the production of the aforementioned toxins, the *agr* and *sar* gene loci (Cheung and Projan, 1994a; Heinrichs *et al.*, 1996; Morfeldt *et al.*, 1996; Lindsay and Foster, 1999). Accessory gene regulator (*agr*) has been shown to suppress post-exponential phase expression of cell surface binding proteins as well as control expression of secreted toxins (Recsei *et al.*, 1986; Balaban and Novick, 1995). Multiple studies have demonstrated that attenuation of the *agr* locus results in a decrease in pathogenesis (Abdelnour *et al.*, 1993; Cheung *et al.*, 1994; Gillaspay *et al.*, 1995; Kielian *et al.*, 2001). In one article, Booth *et al.* demonstrated that an *agr*-deficient mutant was less virulent than the parent strain in a rabbit endophthalmitis model (Booth *et al.*, 1995). Staphylococcal accessory regulator (*sar*) is also involved in the regulation of protein toxin synthesis and potential virulence (Cheung *et al.*, 1992; Cheung and Projan, 1994a, 1994b; Nilsson *et al.*, 1996; Booth *et al.*, 1997). These two regulatory systems are interconnected, and *sar*-encoded proteins are thought to interact with the *agr* locus during mid to late exponential phase to regulate the expression of RNAIII, the *agr* effector molecule (Cheung and Projan, 1994a; Heinrichs *et al.*, 1996). This correlation has been confirmed in endophthalmitis (Booth *et al.*, 1997; Callegan *et al.*, 1999). Additionally, *agr*/*sar*-mutants had decreased binding ability and diminished inflammation in the eye in a rabbit model of endophthalmitis (Cheung *et al.*, 1994; Giese *et al.*, 1999). Because *sar* and RNAIII homologs have been identified in *S. epidermidis*, it is likely that a similar regulatory mechanism is present during *S. epidermidis* pathogenesis, although there are no overt analogous extracellular virulence factors in this organism (Fluckiger *et al.*, 1998; Van Wamel *et al.*, 1998).

BACKGROUND OF BIOFILM STAPHYLOCOCCAL ENDOPHTHALMITIS

Most of what is known about endophthalmitis has been obtained from studies on the pathogen in the planktonic phase. However, recent work has demonstrated that biofilms may also play a large role in disease, especially in postoperative and trauma-induced endophthalmitis (Elder *et al.*, 1995; Warheker *et al.*, 1998; Okhravi *et al.*, 2000; Baird *et al.*, 2001). A number of our studies, as well as others, have demonstrated that the biofilm mode of growth offers a wide range of protection that their respective planktonic counterparts do not share (Costerton *et al.*, 1987, 1995, 1999; Jensen *et al.*, 1992; Stewart, 1996; Costerton, 2001; Stewart and Costerton, 2001). In general, staphylococcal biofilms, like other microbial communities, are more resistant to killing by antibiotics and the weapons of the host's immune system (Costerton *et al.*, 1999; Hoiby *et al.*, 2001; Stewart and Costerton, 2001). However, there have been few studies addressing staphylococcal biofilm formation in endophthalmitis, the ability of ocular isolates to form biofilms, and the molecular mechanisms of virulence in ocular-related biofilms. Nonetheless, interesting nonstaphylococcal examples have been reported.

For instance, postoperative intraocular infection with the less frequent endophthalmitis pathogen, *Propionibacterium acnes*, often displays clinical features of a typical biofilm-related infection. Visible plaques can be observed in the posterior capsule (the remnants of the capsular bag that is left after removal of its opacified lens contents during cataract surgery) that do not usually elicit a strong immune response (Roussel *et al.*, 1987). Moreover, *P. acnes* persists chronically in this particular niche, and often does not cause symptoms for months and sometimes years (Chien *et al.*, 1992; Clark *et al.*, 1999). Once an immune response ensues, which can be provoked by laser-induced destruction of the opacified posterior capsule and presumable disruption of the biofilm, it is not capable of clearing the infectious organisms (Carlson and Koch, 1988). Therefore, intraocular antibiotic treatment at high doses has to be repeatedly performed (Shaarawy *et al.*, 1995). Frequently, even this treatment regimen is not successful, and surgical removal of the posterior capsule is the only way to rid the eye of the biofilm infection (Clark *et al.*, 1999). All these clinical features are hallmarks of what occur in biofilm-related infections.

As for the more common pathogens *S. epidermidis* and *S. aureus*, there is increasing interest in their ability to adhere to the artificial lens and subsequently form biofilms. There are numerous studies on adherence of *S. epidermidis* to different lens materials and electron microscopic, and histological studies of artificial lenses from endophthalmitis patients have confirmed the occurrence of biofilms *in vivo* (Jansen *et al.*, 1991; Wenkel *et al.*, 1993). Although there is only one case report with a histopathologically confirmed demonstration of biofilm formation of *S. aureus* in endophthalmitis, this may represent a crucial initial stage in the infection that is quickly masked by a massive inflammatory response and planktonic dissemination of the organism (Seedor *et al.*, 1990). Further studies are certainly needed to elucidate the frequency of biofilm infections in endophthalmitis.

One interesting recent study by George O'Toole and colleagues (Zegans *et al.*, 2001 MPEIR meeting abstr.) reported that many ocular isolates of *Pseudomonas aeruginosa* readily formed biofilms and also demonstrated an increased resistance to the antibiotic ciprofloxacin when grown in biofilms. Additionally, they concluded that robust biofilm formation might actually be a characteristic of bacterial ocular isolates, lending support to the idea that biofilm-related endophthalmitis may be under diagnosed and under-reported. Further supporting the importance of biofilms in eye disease, Gilmore and colleagues have also demonstrated that *S. aureus* has the ability to form biofilms, including biofilms associated with the lens (Fig. 1). As with most medically relevant biofilm studies, O'Toole and his colleagues reiterated the common theme that a more detailed understanding of the mechanisms of biofilm resistance and biofilm growth must be obtained to develop novel prevention and treatment strategies. Our studies demonstrating leukocyte penetration of biofilms also suggest that other mechanisms likely exist that help the biofilm bacteria resist clearance (Leid *et al.*, 2002, submitted).

GENERAL IMMUNE SYSTEM RESPONSE TO STAPHYLOCOCCAL INFECTIONS

The mammalian immune system must respond to invading pathogens to maintain overall health and well-being. Depend-

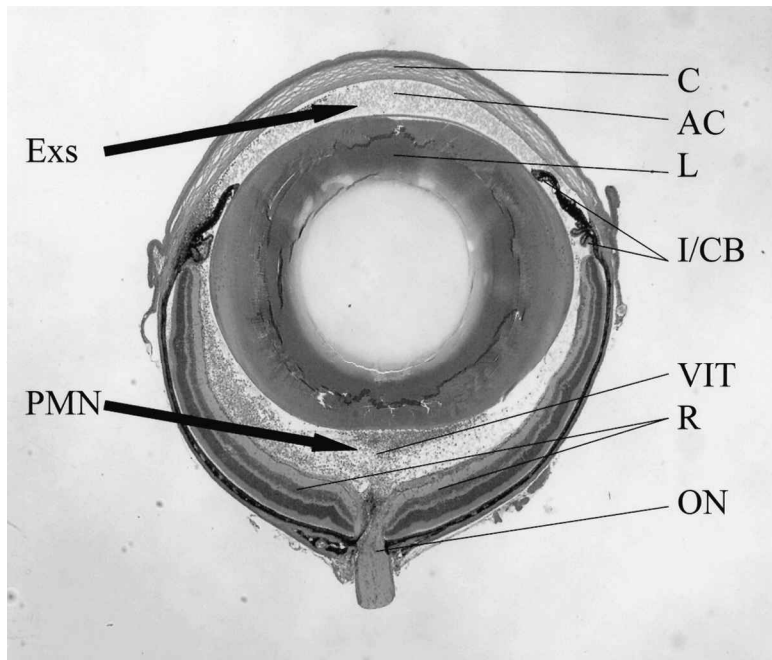


FIG. 1. Mouse eye 48 h after injection of 500 *S. aureus* RN 6390 CFU into the midvitreal cavity (VIT). Arrows point to fibrinous exudate (Exs) in the anterior chamber (AC), and granulocytes (PMN) in the (C = cornea, I/CB = iris and ciliary body, L = lens, ON = optic nerve, R = retina).

ing upon the specific pathogen(s), the response can be quite different, and can be especially important in the ability of the immune system to fight off infection. For staphylococcal infections, as with infections mediated by other bacterial species, a general paradigm of immune response exists (Fig. 2). As *S. aureus* starts to colonize a surface, a local concentration of the agr-inducing peptide occurs, macrophages and fibroblasts recognize formylated peptides and lipoteichoic acids, and C-reactive protein (prototypic acute phase response protein) binds and activates important complement components in the blood. These initial reactions set off a cascade of other interactions including cytokine production from neutrophils, macrophages, natural killer cells, T cells and endothelial cells, activation of mast cells and prostaglandin production, as well as the generation of C3a, C3b, and C5a. During this time frame, specific leukocyte recruitment also occurs. Defensins, small peptides that form ion-permeable channels in bacterial and mammalian cell membranes, are also secreted from activated macrophages, and have been shown to be effective against planktonic staphylococcal infections (Welling *et al.*, 1998; Cole *et al.*, 2001). Of the cytokine milieu, *S. aureus* predominantly triggers a Th1-type response with a large upregulation of IL-1, IL-12, INF- γ , and TNF- α (Assenmacher *et al.*, 1998; Sinha *et al.*, 1999). We have also seen similar responses to *S. aureus* biofilms (Leid *et al.*, 2002 submitted). In its most simplistic description, the immune response is arranged to activate the phagocytes of the immune system that, in turn, can activate adaptive immune components resulting in pathogen clearing under normal circumstances. As the immune response continues, IFN- γ and IL-12 return to baseline levels and the cell-mediated response diminishes. One interesting aspect of staphylococcal infections is

that there remains a sustained level of IL-1, TNF- α , and IL-6 (Meduri *et al.*, 1999).

INTRAOCULAR HOST DEFENSE

The eye appears to be endowed with powerful innate immune mechanisms that protect it from intraocular bacterial infection, because bacterial contamination of the anterior chamber is alarmingly common while endophthalmitis is comparably rare (Ariyasu *et al.*, 1993; Egger *et al.*, 1994; Aaberg *et al.*, 1997, 1998). As outlined above, the complement system and defensins are likely important players (Giese *et al.*, 1994; Haynes *et al.*, 2000).

However, because a clear visual axis is crucial to a clear retinal image, the eye possesses effective means that inhibit a fulminant immune response from occurring. These mechanisms collectively constitute "immune privilege" of the eye, and consist of anatomical features, such as the avascularity of the vitreous and barriers between the systemic circulation and the intraocular space, which limit access of macromolecules, pathogenic organisms, and host cells (Streilein, 1999). Additionally, certain mediators, such as transforming growth factor β , α -melanocyte stimulating hormone, and vasoactive intestinal peptide constitute a particular immunologic milieu and appear to modulate the activity of immune cells once they gain access into the eye (D'Orazio and Niederkorn, 1998). The importance of this phenomenon has been demonstrated for the adaptive immune response to corneal transplants and in the setting of experimental uveitis, but it is likely that it also influences the inflammatory response during the innate immune reaction to

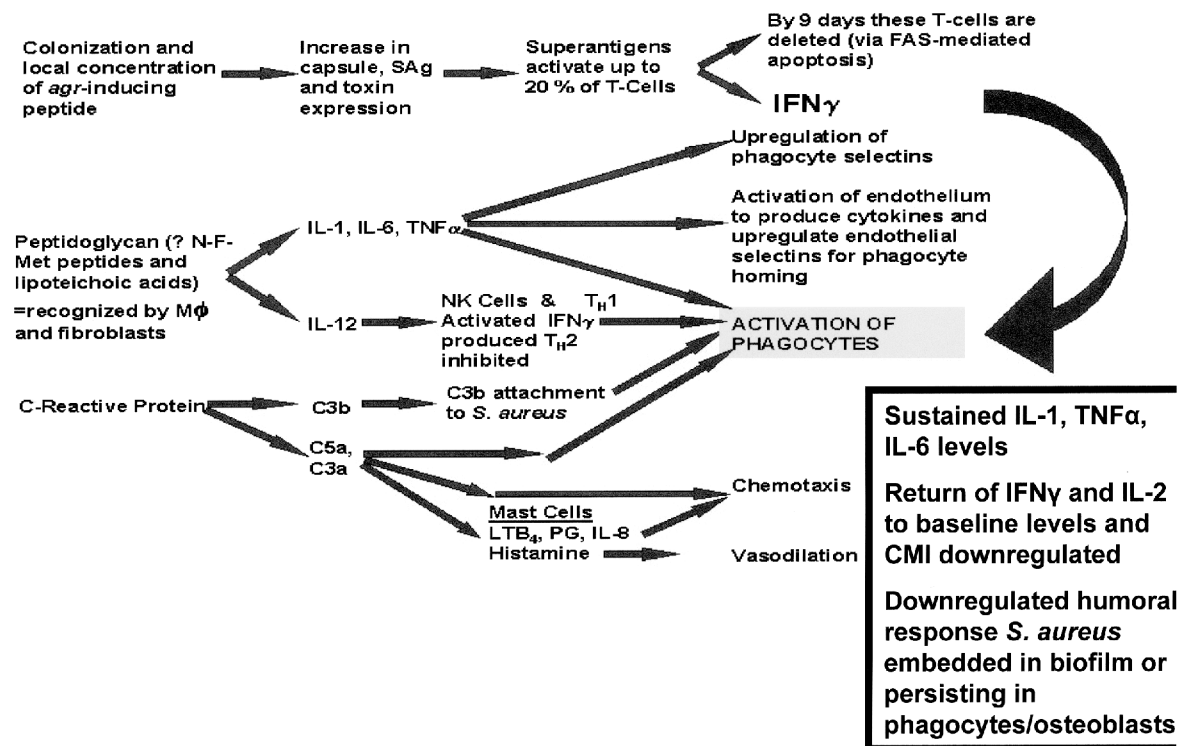


FIG. 2. General diagram of immune response to staphylococcal infection.

endophthalmitis (D'Orazio and Niederkorn, 1998; Niederkorn, 1999; Mo and Streilein, 2001). Studies to elucidate the role of immune privilege in intraocular bacterial infection have thus far been impossible, mainly due to the lack of a murine model (see below).

After having overcome the initial innate defense mechanisms, there appears to be a period of immunological silence, in which the micro-organisms can grow largely uninhibited by the immune system. Thus, the niche for bacterial biofilm formation may be enhanced under these conditions. Upon reaching a certain, poorly understood threshold, an immune response occurs with production of cytokines and the recruitment of inflammatory cells along with subsequent amplification of the original cytokine response the kinetics and magnitude of which seem to depend mainly on the nature of the micro-organism (Giese *et al.*, 1998, 2001). *S. aureus* and the Streptococci provoke a massive immune response within hours to days, and often lead to the destruction of the eye, whereas *P. acnes* endophthalmitis is notorious for its initially indolent course and flareups after months and years (Aldave *et al.*, 1999; Clark *et al.*, 1999; Samson and Foster, 2000; Giese and Mondino, 2001; Callegan *et al.*, 2002). In *S. aureus* endophthalmitis, a predominantly neutrophilic infiltrate can fill the whole eye and completely obstruct visualization of the retina, whereas *P. acnes* establishes itself in localized plaques in the posterior capsule where it appears to be able to persist unrecognized by the immune system for long periods of time. Later in the infection, lymphocytes enter the eye and, besides the production of antibody in extraocular sites, there are indications for local antibody production and follicle formation in the ciliary body and

choroids in the rabbit model (Engstrom *et al.*, 1991; Pleyer *et al.*, 1992).

Whereas the role of certain toxins in the pathogenesis of endophthalmitis has been firmly established, the contribution of the inflammatory response to ocular damage has not been demonstrated conclusively, as is the case in a related central nervous system infection, pneumococcal meningitis (Braun *et al.*, 1999). There are, however, experimental indications (Giese *et al.*, Abstr. Assoc. Res. Vis. Ophthalmol., 2000), and it is generally accepted to be important and accordingly treated by many clinicians with intraocular steroids injected along with antibiotics.

PARADIGMS OF BIOFILM RESISTANCE TO KILLING BY ANTIBIOTICS

By adopting a sessile mode of life, biofilm-embedded microbes enjoy a number of advantages over their planktonic counterparts. One advantage is the ability of the polymeric matrix to capture and concentrate a number of environmental nutrients, such as carbon, nitrogen, and phosphate. It has been previously demonstrated that nutrient concentration enables the survival of microbes in environments that would not support planktonic growth (Beveridge *et al.*, 1997).

Another advantage to the biofilm mode of life is to inhibit a number of removal strategies, such as antimicrobial and antifouling agent treatment, shear stress, host phagocytic clearance, and host oxygen radical defenses. The first mechanism by which inherent resistance to antimicrobial factors is me-

diated is through very low metabolic levels and drastically downregulated rates of cell division (e.g., small colony variants) of the deeply embedded microbes (Brown *et al.*, 1988). Therefore, clearance strategies that depend upon robust and actively dividing microbes (such as the β -lactam antibiotic family) are often ineffective (Tuomanen *et al.*, 1986). Second, biofilm structures demonstrate a physical barrier to penetration of the antimicrobial agents (Stewart, 1996). It has been previously shown that the polymeric matrix that forms the majority of biofilms retards the inward diffusion of a number of antimicrobial compounds (Stewart, 1996; Anderl *et al.*, 2000; Xu *et al.*, 2000). Also, the reactive oxidants produced by the host immune response may be deactivated in the outer layers of the biofilm faster than they can diffuse into the lower layers (De Beer *et al.*, 1994; Stewart, 1994). Third, a number of studies have shown that the gene expression within biofilms is altered due to the physical action of attachment (Davies *et al.*, 1993; Dalton and March, 1998; Mah and O'Toole, 2001; Pratten *et al.*, 2001; Sauer and Camper, 2001; Sauer *et al.*, 2002). The change in gene expression is a biologically programmed response to attachment and not due to nutrient deprivation. However, the link between antimicrobial resistance and altered gene expression has yet to be fully elucidated.

The last advantage to the biofilm mode of life is the potential for dispersion via detachment. Microcolonies can exist in discrete mushroom-shaped structures. These microcolonies may detach under the direction of mechanical fluid shear or through a genetically programmed response in which microbial lyase specific for the polymeric biofilm substrate is activated and mediates the detachment process (Boyd *et al.*, 1993; Boyd and Chakrabarty, 1994; Sutherland, 1995; Sutherland and Kennedy, 1996; Stoodley *et al.*, 2001a, 2001b). An example of programmed detachment has been demonstrated in the non-pathogenic, photosynthetic bacterium *Rhodobacter sphaeroides* (Puskas *et al.*, 1997). In this species, an acyl-homoserine lactone signaling molecule produced by the *cer* (community escape response) quorum sensing system is required for dispersal of cells from the modular communities. Once detached, the microcolony is protected because microbes are still embedded within the polymeric matrix. Under the direction of fluid flow, the microcolony travels to other regions of the host or water system to attach and promote biofilm formation on new surfaces. Preliminary studies of detached biofilms have demonstrated that these clumps are also resistant to antibiotic killing, thus making the potential for additional foci of infection much greater.

HUMAN LEUKOCYTE RESPONSE TO STAPHYLOCOCCAL BIOFILMS IN VITRO

As described above, when the immune system is functioning properly, clearance of a variety of pathogens occurs, sometimes without any overt signs (e.g., fever) that the pathogen was present. Thus, the immune system is extraordinarily well adept at clearing planktonic organisms from the body. Bacterial biofilms offer a much different pathology. A variety of mechanisms have been proposed for the ability of biofilms to resist attack (Costerton *et al.*, 1999; Hoiby *et al.*, 2001; Lewis, 2001;

Mah and O'Toole, 2001). In general, bacterial communities limit the ability of leukocytes to engulf and kill the pathogens by living as a large community protected by a glycocalyx coat. There is also a great deal of evidence that biofilms are much less immunogenic than their planktonic counterparts (Costerton, 1991; Hoiby *et al.*, 2001; Jensen *et al.*, 1990, 1992; Lewis, 2001). However, studies have demonstrated that leukocytes do respond to biofilms in variety of ways. For example, leukocytes have been shown to produce cytokines in response to *Staphylococcus epidermidis* biofilms (Dasgupta, 1996; Rozalska *et al.*, 1996). Phagocytic cells have been shown to produce and secrete superoxide and generate toxic compounds in response to biofilms, although at lower levels than when responding to planktonic bacteria (Jensen *et al.*, 1992; Yasuda *et al.*, 1994; Moran *et al.*, 1998; Janatova, 2000). Overall, the patterns described above with antibiotic resistance also hold true for immune responses against biofilms, that is, leukocytes are unable to effectively resolve biofilm infections.

It has been hypothesized that one of the major mechanisms of resistance is impairment of penetration of leukocytes into the biofilm to elicit appropriate effector responses (Costerton *et al.*, 1999; Dickinson and Bisno, 1989; Hoiby *et al.*, 2001; Kharazmi, 1991). Recently, we have begun to take another look at the interactions of human leukocytes with staphylococcal biofilms under conditions that mimic physiologic shear as well as under static conditions. Some interesting results have emerged, including the finding that under shear forces similar to those found *in vivo*, leukocytes elicit specific cytokine responses against the *S. aureus* biofilms (Leid *et al.*, 2002, submitted). Additionally, it appears that the biofilm itself is not as impenetrable as originally thought. Clearly, the interactions between the bacterial biofilm and the circulating leukocytes are much more complex, and there is still much to be learned about their intricate interactions. When this observation is multiplied by the complex nature of immune reactions in the eye, it is clear that the ability of bacteria to form biofilms in the ocular cavity causes great concern.

This point only underscores the need to develop models where biofilm interactions with components of the immune system can be more efficiently studied. Additionally, it demonstrates that even though we are gaining an immense amount of knowledge about biofilms, there are still many important unanswered questions that need to be addressed, both in terms of biofilm development, formation, and detachment, as well as determining the molecular interactions between the immune system and the biofilm. One recent observation is that *Pseudomonas aeruginosa* biofilms incorporate a large amount of DNA into their structure early in their development and when biofilms are grown in the presence of DNase, biofilm formation is dramatically reduced (Whitchurch *et al.*, 2002). Although these new data are exciting, it may be that DNA plays a more vital role in *Pseudomonas* biofilm formation than in other species of biofilm. Additionally, as this was a technical comment and not a report, more experiments need to be run to fully understand and lend credence to these observations. Nonetheless, the importance of this finding highlights the fact that biofilms offer an extremely different niche than their planktonic counterparts. The more studies that are undertaken, the more we will begin to understand what makes biofilm infections so difficult to treat.

NEW *IN VIVO* MODEL TO STUDY BIOFILM ENDOPTHALMITIS

Of the methods to study bacterial biofilm infections, especially those of the eye, the development of appropriate *in vivo* models are by far the most important research tools for a variety of reasons. First, the eye is an immunologically privileged site that is challenging to mimic *in vitro*. Second, the eye itself is a complex structure that does not lend itself to simple *in vitro* models. Therefore, several animal models for endophthalmitis have been established. The most commonly used species is the rabbit, although guinea pigs and rats have been employed to address specific questions. However, with the ready availability of myriad of immunologic reagents, knockouts, and transgenics, the mouse becomes clearly the species of choice in pathogenesis research.

Engelbert and Gilmore therefore developed a murine model of endophthalmitis, that allows for a variety of techniques and analytical tools to be employed, which will hopefully yield information that will lead to the development of novel therapeutic strategies and a consequent improvement in treatment outcome. Briefly, bacteria such as *S. aureus* can be injected into the vitreous cavity and depending upon the inoculum, are either cleared or persist and form biofilms along the lens (Fig. 3). The presence of biofilms on the posterior surface of the lens capsule was observed about 72 h after injection of 5000 *S. aureus* RN 6390 CFU into the midvitreous cavity. The morphology of these biofilms bear a striking resemblance to the histopathological picture of a section of the lens capsule from a patient with *S. aureus* endophthalmitis (Seedor *et al.*, 1990). Despite some anatomical differences (mice with an intact lens versus a patient after cataract operation, formation of biofilm

on posterior surface versus anterior surface of the lens capsule) this model will help study relevant ocular pathogens in the biofilm mode with respect to their interaction with the intraocular host response in an *in vivo* setting. Importantly, visualization of the progression of the infection is easily accomplished and lends itself to the use of GFP- (or other FP) expressing bacteria so that fluorescent staining techniques can be utilized to either identify infiltrating leukocytes or determine the viability of both the bacteria and the associated cells of the eye. This latter point is important because collateral damage of healthy tissue is often a hallmark of biofilm infections (Costerton *et al.*, 1999). Additionally, this model allows for the tapping and sampling of the vitreous, such that infiltrating leukocytes can be easily quantitated by flow cytometry. Because not much is known about the hierarchy of responding leukocytes to biofilm infections, this technique will prove to be extremely powerful. Overall, this model provides the tools to observe the combination of factors that are involved in the complex infection that is endophthalmitis.

CONCLUDING REMARKS

The importance of biofilms in the pathogenesis of many infections is becoming increasingly clear. We are just starting to elucidate the role of biofilms in endophthalmitis and the interaction of biofilms and the host immune system in general. Studies in our labs, as well as many others, are demonstrating that more complex mechanisms of interaction exist between the host and the biofilm bacteria, yet we still have a limited understanding of what these mechanisms of interaction are. The novel murine model described above is one tool that will help in this

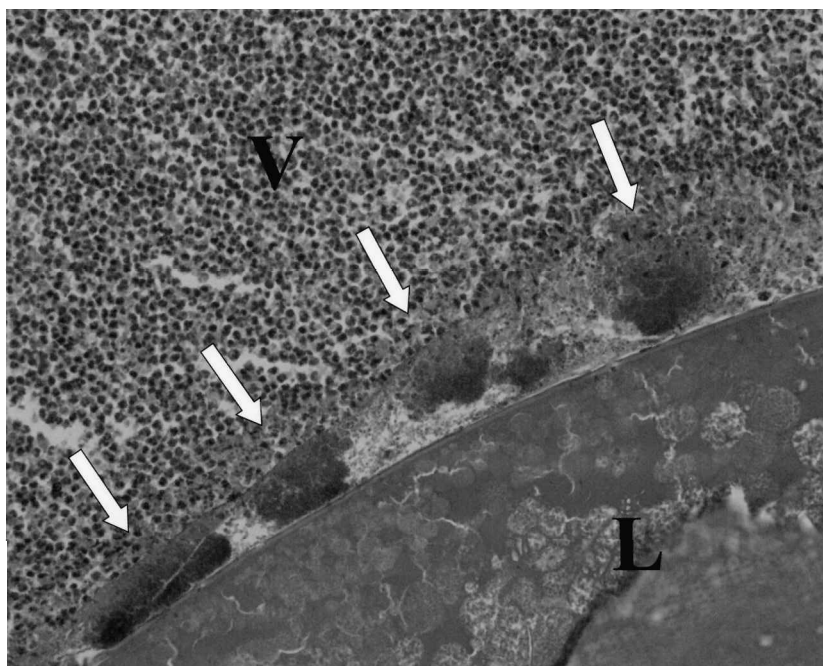


FIG. 3. Mouse eye 72 h after injection of 5000 *S. aureus* RN6390 CFU into the midvitreous cavity (V). Arrows point to the communities of *S. aureus* adjacent to the lens (L).

endeavor and will hopefully yield insights that can direct our efforts to develop new therapeutic strategies not only for endophthalmitis, but also other biofilm-related infections.

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