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Contribution of the pAD1-Encoded Cytolysin to the Severity of Experimental *Enterococcus faecalis* Endophthalmitis

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The contribution of the pAD1-encoded cytolysin to *Enterococcus faecalis* virulence in an experimental endophthalmitis model was studied by using isogenic strains differing only in the location of transposon Tn*917*. The course of experimental endophthalmitis in New Zealand White rabbits was evaluated by postoperative reduction in retinal neuroresponsiveness, thin-section histopathology, and transmission electron microscopy. Infections caused by cytolytic *E. faecalis* resulted in 99% loss of retinal function at postoperative day 3, while isogenic, noncytolytic strains produced reductions of only 74.2%. Light microscopy revealed near-total destruction of retinal architecture at 24 h postinfection with cytolytic *E. faecalis*, while noncytolytic strains produced few or no destructive changes. Transmission electron microscopy revealed tissue destruction in retinal layers as early as 6 h postinfection with cytolytic *E. faecalis*. In vivo and in vitro growth rates of cytolytic and noncytolytic *E. faecalis* showed similar kinetics. These data demonstrate the contribution of the pAD1-encoded cytolysin to both the course and the severity of experimental *E. faecalis* endophthalmitis.

Endophthalmitis is a sight-threatening condition that occurs as a complication of intraocular surgery or penetrating eye injury. The prognosis for recovery of useful vision is usually poor when the offending organism is a pathogen (25, 30, 41, 47). The production of extracellular virulence factors is believed to contribute to the severity of endophthalmitis; however, specific experimental models and direct evidence for this are lacking.

Enterococcus (*Streptococcus*) *faecalis* is among the most common bacteria associated with postoperative endophthalmitis (23, 27, 29), and these infections are frequently described as fulminant and destructive (1, 2, 9). *E. faecalis* has been reported to be the primary etiologic agent in endophthalmitis resulting from infected filtering blebs after glaucoma surgery (24, 31). Of the cases reported, most result in loss of useful vision, and enucleation is frequently required. Only about 15% of *E. faecalis* endophthalmitis cases result in a final visual acuity of 20/200 or better.

E. faecalis strains frequently harbor large, transmissible plasmids that encode a cytolysin effective in lysing both eukaryotic and gram-positive bacterial cells (3, 17, 22). The archetype for this plasmid class is the 59.6-kb conjugative plasmid pAD1, which has been extensively studied (5, 6, 16, 18). The cytolysin determinant (*cyl*) of pAD1 has been mapped by using transposon Tn*917* (46) mutagenesis and cloned and expressed in *Escherichia coli* (18). Recently, two genes of the *cyl* determinant have been described. *cylA* has been shown to be closely related to a class of serine proteases and is believed to play roles both in the activation of the cytolysin precursor (*cylL*) and in immunity of the host cell to its own cytolysin (42). *cylB* shows extensive homology to a class of bacterial transport proteins, and its expression is required for externalization of the cytolysin precursor (14). The exact location of *cylL* in the cytolysin determinant remains unclear.

The cytolytic phenotype has been found to correlate with strains causing human parenteral infections (17, 19), and cytolysin production was demonstrated to contribute to toxicity in a mouse intraperitoneal infection model (20). It has also been demonstrated that the presence of pAD1 contributes to the severity of experimental *E. faecalis* endophthalmitis (45). However, because of the large size of pAD1 (59.6 kb) and potential contributions by other plasmid-encoded factors, direct correlation between cytolysin expression and severity of infection was not possible. Therefore, the present study evaluates the contribution to virulence of the pAD1-encoded cytolysin by using isogenic strains of *E. faecalis* differing only in the location of transposon Tn*917* insertion into pAD1. Light microscopic examination of stained thin sections revealed more fulminant and destructive changes in gross retinal anatomy caused by the cytolysin-producing strain, changes which correlated with a reduction in retinal responsiveness to light stimulus. Transmission electron microscopy (TEM) revealed retinal damage at earlier times than detectable with light microscopy. Similar rates of growth in vivo between the cytolytic and noncytolytic strains further suggest a specific contribution by the cytolysin itself to the severity of the infection rather than secondary effects resulting from a potential growth advantage.

MATERIALS AND METHODS

Bacterial strains and media. The *E. faecalis* host strain used in this study was JH2SS (45) which harbored various Tn*917* insertional mutations of plasmid pAD1 (Table 1). Three noncytolytic mutants were selected which were defective in the three known functions of the cytolysin determinant. JH2SS(pAM771), JH2SS(pAM307), and JH2SS (pAM9055) were selected because they harbor Tn*917* insertions in *cylL*, *cylB*, and *cylA*, respectively (14, 18, 42). Locations of these Tn*917* insertions are depicted in Fig. 1. All strains were propagated in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Mich.) at 37°C without aeration. The medium was supplemented with streptomycin (500 μg/ml), spectinomycin (500 μg/ml), and erythromycin (10 μg/ml) unless otherwise specified. Before intravitreal

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TABLE 1. Bacterial strains used in this study[a]

Strain and plasmid content	Phenotype	Reference
JH2SS, pAD1::Tn*917* (pAM714)	Str^r Spc^r Em^r Cyl$^+$	16
JH2SS, pAD1::Tn*917* (pAM771)	Str^r Spc^r Em^r CylL$^-$ Cyl$^-$	18
JH2SS, pAD1::Tn*917* (pAM9055)	Str^r Spc^r Em^r CylA$^-$ Cyl$^-$	18
JH2SS, pAD1::Tn*917* (pAM307)	Str^r Spc^r Em^r CylB$^-$ Cyl$^-$	5

[a] Bacterial strains were generously provided by Don B. Clewell, University of Michigan, Ann Arbor. Str, streptomycin; Spc, spectinomycin; Em, erythromycin; Cyl, cytolytic (hemolysin/bacteriocin) activity; CylL, lytic moiety (exact location unknown; see Fig. 1); CylA, cytolysin activator (42); CylB, cytolysin transport protein (14).

inoculation, cells were washed twice by centrifugation at 5,000 × *g* for 10 min and resuspended in 1 volume of balanced salt solution (Dey Laboratories, Napa, Calif.). Enumeration of organisms and confirmation of cytolytic phenotype at the time of injection and after recovery from the vitreous was accomplished by plating on BHI blood agar which contained antibiotics as described above, washed human erythrocytes (5%, vol/vol), and Bacto-Agar (1.5%, wt/vol [Difco]).

Vertebrate animals. New Zealand White rabbits weighing 2 to 4 kg were used in this study. Animals were housed and cared for in accordance with Association for Research in Vision and Ophthalmology regulations at the Dean A. McGee Eye Institute animal care facility.

Intraocular injections. Rabbit eyes were dilated with topical 1% tropicamide and 2.5% phenylephrine hydrochloride before intravitreal injections. The animals then underwent general anesthesia with intramuscular ketamine (35 mg/kg of body weight) and xylazine (5 mg/kg of body weight). Once anesthetized, the animals were given topical ocular anesthesia (0.5% proparacaine hydrochloride), and the eyes were

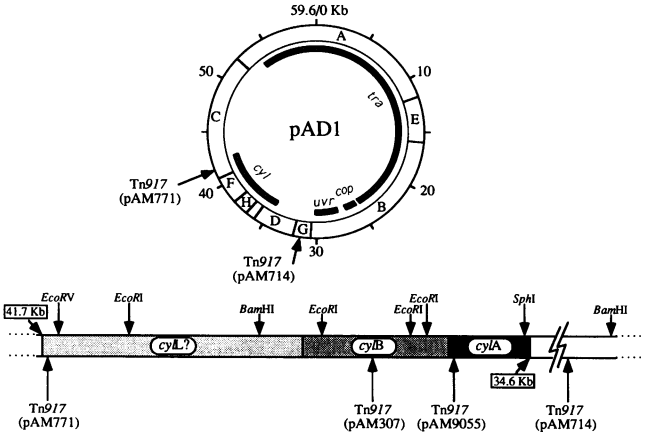


FIG. 1. Genetic and partial physical map of the pAD1 cytolysin determinant. Top: *Eco*RI restriction map of pAD1 (5, 18). Locations and three-letter abbreviations of the known components of pAD1 are depicted (6). *tra*, conjugative transfer functions (6); *cop*, copy control region (16); *uvr*, UV resistance (4). Bottom: Expanded view of cytolysin determinant. Restriction map of the *cyl* region is shown (18). Boxed numbers indicate approximate map locations of cytolysin boundaries. Locations of Tn*917* insertions used in this study are shown beneath the *cyl* determinant and are accompanied by their corresponding pAD1::Tn*917* designation (Table 1). *cylL*, lysin precursor component L (approximate location determined by transposon mutagenesis [18]); *cylB*, cytolysin transport function (14); *cylA*, cytolysin activator component A (42).

sterilely draped. Approximately 0.1 ml of anterior chamber fluid was aspirated with a tuberculin syringe to relieve intraocular pressure. A 25-gauge needle attached to a sterile disposable 1.0-ml syringe was then introduced through the pars plana approximately 3 mm from the limbus, and 0.1 ml (approximately 100 CFU) of *E. faecalis* suspension was slowly infused into the vitreous chamber. The eyes were immediately examined by indirect ophthalmoscopy to detect intraoperative complications.

Intraocular growth of *E. faecalis*. To determine differences in growth rates of cytolytic and noncytolytic *E. faecalis* strains, we injected approximately 100 organisms of each strain intravitreally into each of five eyes as described above, a means of infection found in previous studies to result in detectable endophthalmitis in all animals within 24 to 48 h (45). Simultaneously, 2 ml (approximate volume of rabbit vitreous [38]) of BHI broth was similarly inoculated to serve as an in vitro growth comparison. The inoculum level was confirmed by plate count. At 6, 12, 24, 48, and 72 h postinfection, animals were euthanized and the globes were enucleated. Eyes were rinsed with sterile balanced salt solution, and the vitreous was removed through a scleral incision. Vitreous mass was measured to the nearest 0.01 g. The vitreous was passed through a glass syringe fitted with an 18-gauge needle to homogenize it and reduce its viscosity and added to 5 ml of preweighed BHI broth and vigorously vortexed. The number of organisms in each sample was determined by plating duplicate serial 10-fold dilutions on BHI semisolid medium. After 24 h of incubation at 37°C, colonies were counted and concentrations were expressed as CFU per gram of vitreous.

ERG. Strains of *E. faecalis* JH2SS harboring Tn*917* insertional mutations of plasmid pAD1 were compared in their ability to affect neuroretinal responsiveness in infected eyes. Animals underwent general and topical ocular anesthesia as described above. After pharmacologic dilation and 30 min of dark adaptation, baseline B-wave amplitude was established by using scotopic bright flash electroretinography (ERG) (model CA-1000; Nicolet Instruments). After 72 h of recovery, approximately 100 CFU were injected intravitreally in either the left or right eye. The remaining eye served as either a surgical (balanced salt solution injection only) or absolute (no injection) control. ERG was performed at 24 and 72 h postoperatively. Percent loss of retinal function (B-wave amplitude) in these experiments was calculated as follows: [1 − (experimental B-wave amplitude/baseline B-wave amplitude)] × 100. Unpaired Student's *t* tests were used to compare ERG results. *P* values of < 0.05 were considered statistically significant.

Thin-section histopathology. Eyes infected with cytolytic *E. faecalis* JH2SS(pAM714) or noncytolytic JH2SS(pAM 771) were enucleated for histopathological analysis at 24, 48, and 72 h postinfection. Eyes were fixed in 10% formalin for 24 h and then sectioned and stained with hematoxylin and eosin (H&E) following standard procedure (43). Eyes were also enucleated at 6, 12, and 24 h postinfection and stained with H&E and Brown and Hopps modified tissue Gram stain following previously described methods (28). All histological staining was performed with an automated slide stainer (Histomatic 172; Fisher). Pathological interpretations were made with the investigator blinded as to the nature of the infecting organism.

TEM. Early retinal changes were assessed ultrastructurally by using TEM on eyes infected with *E. faecalis* JH2SS(pAM714) or JH2SS(pAM771). Eyes matched with those used for light microscopy were enucleated at 6 and 24

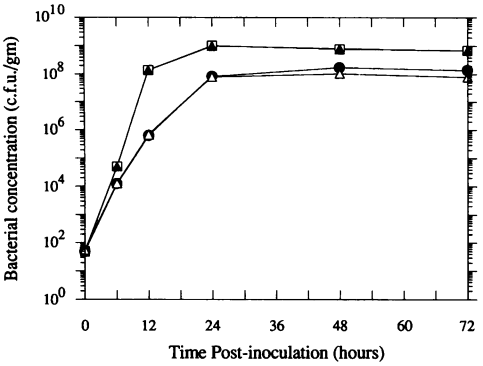


FIG. 2. In vivo growth rate of *E. faecalis*. Concentrations of organisms are expressed as CFU per gram of growth medium. In vivo intraocular growth rates: ●, JH2SS(pAM714); △, JH2SS (pAM771). In vitro (BHI broth) growth rates: ▲, JH2SS(pAM714); □, JH2SS(pAM771).

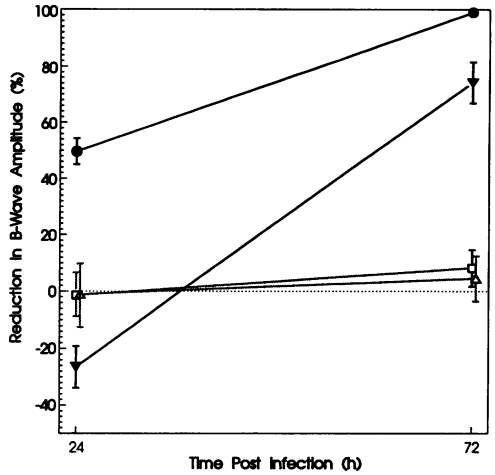


FIG. 3. B-wave amplitude reduction in *E. faecalis*-infected and control eyes. Eyes were infected with 100 CFU of cytolytic or noncytolytic *E. faecalis*. Loss of B-wave amplitude is expressed as percentage of preoperative B-wave amplitude (see Materials and Methods). ●, JH2SS(pAM714), $n = 5$; ▼, JH2SS(pAM771), JH2SS(pAM307), and JH2SS(pAM9055), $n = 7$; △, surgical controls (balanced salt solution injection, $n = 6$); □, absolute controls (no injection, $n = 4$). Error bars represent standard error of the mean.

h postinfection, and the cornea and iris were carefully removed with Wescott scissors to expose the lens and vitreous. The vitreous and lens were removed by using forceps and a suction pipette, with caution taken to avoid the retina. Evitreated eyes were fixed in 2% glutaraldehyde–2% paraformaldehyde–0.1 M sodium cacodylate buffer and allowed to stand at room temperature for 24 h. TEM-grade biochemicals were purchased from Polysciences Inc. unless otherwise specified. Retina sections were postfixed in 1% osmium tetroxide, washed in successive dehydrating concentrations of ethanol, and immersed in propylene oxide. Sections were then fixed in SPUR resin and baked at 65°C for 24 h. Cooled blocks were sectioned on an ultramicrotome (Ultracut; Reichert). Sections were placed on copper grids, stained with uranyl acetate and lead citrate, and viewed (Hitachi H5000; acceleration voltage, 75,000 V).

RESULTS

Intraocular growth of *E. faecalis*. The rates of in vivo growth of JH2SS(pAM714) and JH2SS(pAM771) are depicted in Fig. 2. The data reveal similar in vivo growth kinetics for both strains. A steady increase in numbers of organisms was seen through 24 h postinoculation, at which time growth rates reached a plateau and remained nearly constant for the duration of the experiment. Growth in BHI proceeded more rapidly than that in vitreous at earlier time points, reaching a final concentration approximately 10-fold that achieved in vivo.

ERG. Infections caused by JH2SS(pAM714) resulted in a significantly greater B-wave amplitude reduction of 99.0% ± 1.0% ($n = 5$) at postoperative day 3 than the reduction of 74.2% ± 7.4% observed for infections of noncytolytic JH2SS harboring pAD1 containing transposon insertions within the cytolysin determinant ($n = 7$, $P = 0.01$) (Fig. 3). Additionally, significant differences were observed at postoperative day 1 between cytolytic and noncytolytic strains ($P < 0.01$). Among the noncytolytic mutants tested [JH2SS(pAM771), $n = 3$; JH2SS(pAM307), $n = 2$; JH2SS(pAM9055), $n = 2$], there was no significant difference in B-wave reduction for any one type of mutant compared to the other types at days 1 and 3 ($P > 0.08$). While four of five eyes infected with JH2SS(pAM714) exhibited total loss of B-wave amplitude at postoperative day 3, all eyes infected with noncytolytic JH2SS retained residual neuroretinal function at day 3. B-wave amplitude reduction caused by JH2SS(pAM714) was significant compared with absolute ($n = 4$) and surgical ($n = 6$) controls at all time points ($P < 0.01$). Infections caused by noncytolytic organisms at postoperative day 1 resulted in a significant ($P = 0.027$) and reproducible supernormality in ERG response when compared with absolute and surgical controls. It has been hypothesized that this ERG supernormality may result from release of vasoactive amines from mast cells in the choroid near the photoreceptor layer (44); however, the exact physiological basis remains unknown. In contrast, a significant reduction in B-wave amplitude was observed in these animals by postoperative day 3 ($P < 0.03$). There were no significant differences between absolute and surgical control groups at any time point ($P > 0.5$).

Thin-section histopathology. Retinal sections stained with H&E at 48 and 72 h postinfection revealed histopathological changes similar to those previously observed with pAD1-containing and plasmid-free *E. faecalis* JH2SS (45). Briefly, eyes infected with JH2SS(pAM714) exhibited a more intense vitreitis as well a more marked infiltration of inflammatory cells into retinal layers at 48 and 72 h than eyes infected with JH2SS(pAM771). Furthermore, sections taken from JH2SS(pAM714)-infected eyes at 72 h showed near-total to total loss of all retinal architecture with massive infiltration of inflammatory cells into all retinal layers. These eyes exhibited no red retinal reflex upon slit lamp examination. In contrast, sections taken from JH2SS(pAM771)-infected eyes at 72 h demonstrated intact retinal layers as well as residual red reflex, even though the vitreous of these eyes demonstrated marked inflammation.

Thin sections stained at 6 and 12 h were essentially unremarkable regardless of the infecting organism (Fig. 4a and d; 6-h data not shown). Few inflammatory cells were observed, and these were concentrated near the optic disk. Brown and Hopps tissue Gram stain revealed few organisms distributed in the vitreous at 6 h postinfection. Organisms at 12 h postinfection were more abundant than those at 6 h; however, they were widely distributed and difficult to locate.

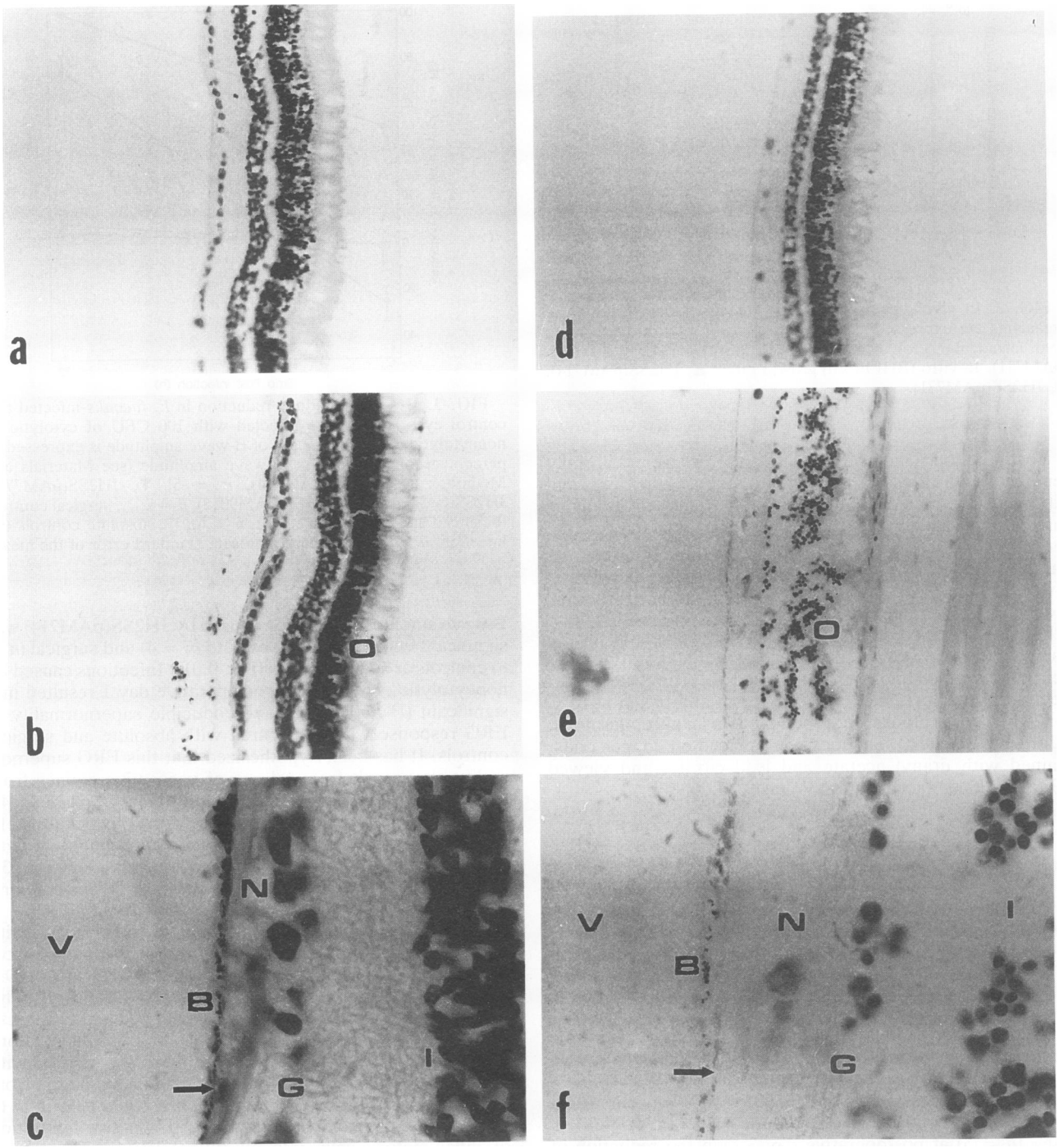


FIG. 4. Histological changes in rabbit retinas at 12 and 24 h postinfection (Brown and Hopps stain). (a to c) Retina from eyes infected with 100 CFU of *E. faecalis* JH2SS(pAM771). (a) 12 h postinfection (×25); (b) 24 h postinfection (×25); (c) 24 h postinfection (×100). (d to f) Retina from eyes infected with 100 CFU of *E. faecalis* JH2SS(pAM714). (d) 12 h postinfection (×25); (e) 24 h postinfection (×25); (f) 24 h postinfection (×100). B, bacterial cells; V, vitreous; G, ganglionic layer; N, nerve fiber layer; I, inner nuclear layer; O, outer nuclear layer. Arrow, inner limiting membrane of retina.

In contrast, differences in retinal fidelity between eyes infected with cytolytic JH2SS(pAM714) and noncytolytic JH2SS(pAM771) at 24 h postinfection were readily apparent at the light microscopy level. At 24 h postinoculation, sections of JH2SS(pAM771)-infected eyes, stained with H&E, revealed a marked inflammatory response near the posterior pole of the retina. However, no detectable changes in retinal structures or the internal limiting membrane of the retina were observed. Tissue Gram stain revealed numerous organisms lining the internal limiting membrane of the retina and vitreous sheets (Fig. 4c). In contrast to the 24-h results with noncytolytic JH2SS(pAM771), eyes infected with

JH2SS(pAM714) demonstrated a marked inflammatory response concentrated near the optic disk with near-complete dissolution of the internal limiting membrane as well as extensive vacuolization of the underlying nerve fiber layer. These sections also exhibited extensive cellular disorganization in the ganglionic, inner nuclear, and outer nuclear layers (Fig. 4f). Tissue Gram stain demonstrated organisms adhering to the remnants of the internal limiting membrane as well as vitreous sheets (Fig. 4f). Few, if any, organisms were found posterior to the retinal limiting membrane in either infection.

TEM. TEM results revealed detectable changes in retinal structures as early as 6 h postinoculation (Fig. 5). Sections from eyes infected with cytolytic JH2SS(pAM714) demonstrated an intact inner limiting membrane at 6 h postinfection. However, the underlying neural and ganglionic layers showed vacuolization and disruption of normal architecture (Fig. 5d). Similar sections from eyes infected with noncytolytic JH2SS(pAM771) showed a normal appearance with no disruption of retinal layers (Fig. 5a). As in stained thin sections, differences at 24 h postinfection were readily apparent. Eyes infected with noncytolytic JH2SS(pAM771) demonstrated normal retinal structures with little or no vacuolization as well as an intact retinal limiting membrane (Fig. 5b). In contrast, similar sections from eyes infected with cytolytic JH2SS(pAM714) showed extensive disorganization of all retinal layers (Fig. 5e). As a result of this extensive destruction, it was virtually impossible to identify retinal layers in these sections. Furthermore, the limiting membrane was not discernible in these sections.

TEM examination of the posterior retinal layers revealed the depth of destructive changes that occurred within the first 24 h of infection. Briefly, specimens from eyes infected with cytolytic JH2SS(pAM714) demonstrated disruption of the rod outer segment membranes, particularly at their junction with the pigmented epithelium, at 6 h postinoculation. There was also evidence of swelling of mitochondria and membrane disruption of the inner segments (data not shown). Similar specimens from JH2SS(pAM771)-infected eyes revealed no changes in these deep retinal layers. Specimens taken from JH2SS(pAM771)-infected eyes at 24 h showed almost no change in the functional retina (Fig. 5c). The rod outer segments exhibited very subtle swelling in the disk pattern. The inner segments, cell bodies, nuclear layer, and neural layer appeared to be intact. In contrast, TEM examination of specimens from JH2SS(pAM714)-infected eyes at 24 h revealed almost complete lysis of the functional retina. In many areas, there was complete disruption of the outer segments, inner segments, cell bodies, nuclear layer, and neural layer (Fig. 5f). Interestingly, it appeared that the pigmented epithelial layer remained undamaged throughout the course of infection (Fig. 5c and f).

DISCUSSION

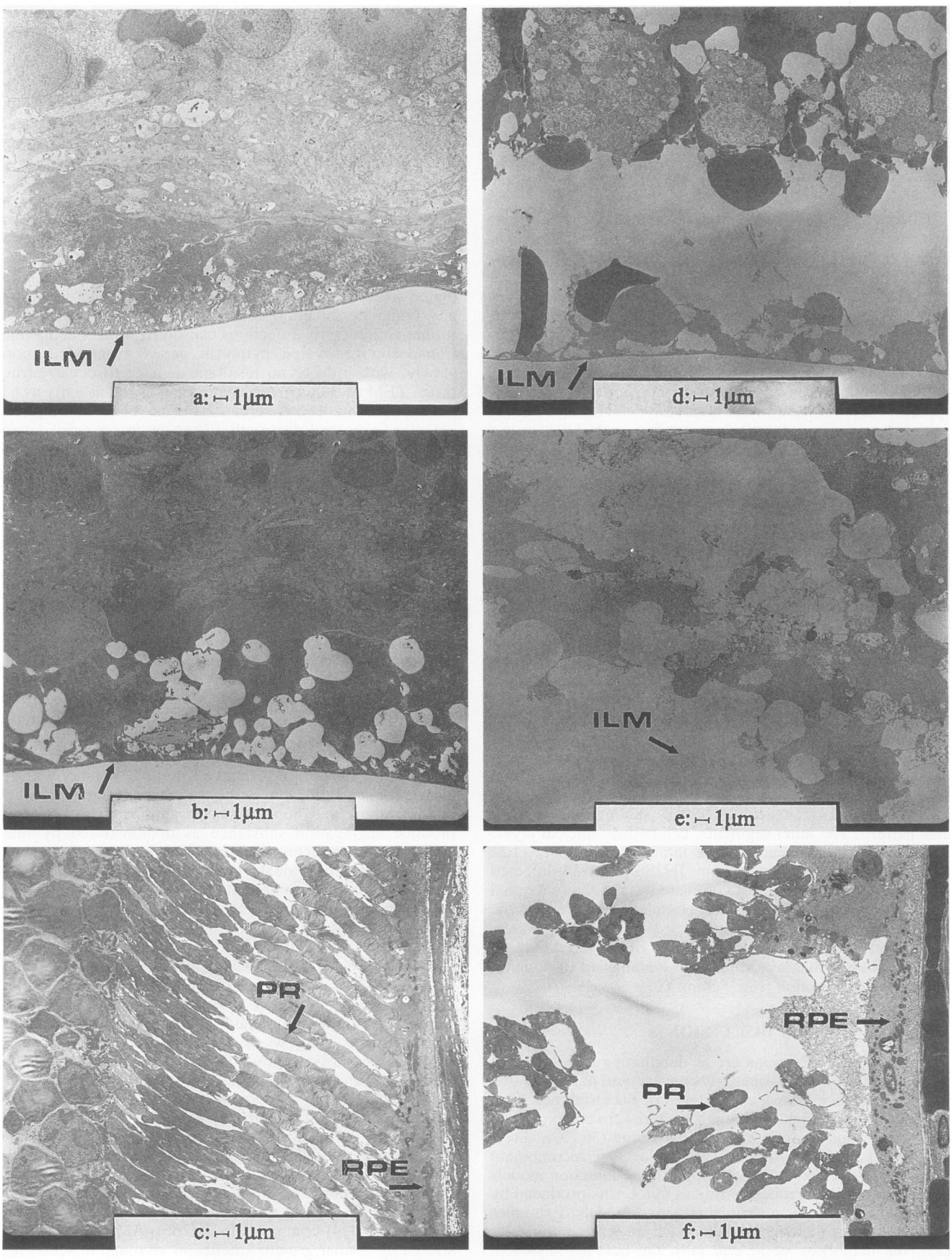
Few bacterial infections are as debilitating or proceed as rapidly as intraocular ones. The avascular nature of the intraocular space allows early stages of bacterial infections to proceed unchecked. Although bacterial toxins expressed during infection may damage retinal tissue (36, 37), direct evidence is lacking. Modern molecular and recombinant DNA techniques coupled with appropriate infection models have been used to probe the role of cytolysins produced by a number of bacteria in various infection models. Examples of such factors include the hemolysin of *Proteus mirabilis* (35), the hemolysin of *E. coli* (49), the beta-hemolysin of group B streptococcus (48), and listeriolysin of *Listeria monocytogenes* (12). In this study, a role for the pAD1-encoded cytolysin in determining the course and severity of experimental endophthalmitis is demonstrated.

Infections caused by cytolytic *E. faecalis* JH2SS(pAM 714) resulted in significantly greater loss of neuroretinal function when compared with the noncytolytic transposon insertional mutants JH2SS(pAM771), JH2SS(pAM307), and JH2SS(pAM9055). The observation of similar changes in B-wave amplitude resulting from infection with noncytolytic strains varying in the location of Tn*917* within the cytolysin determinant demonstrates that it is the active cytolysin rather than an individual cytolysin component that contributes to virulence in this model.

Thin-section histopathology demonstrated that eyes inoculated with cytolytic JH2SS(pAM714) are subject to a rapid and fulminant infection accompanied by near-total loss of retinal architecture. Infections caused by strains containing transposon-inactivated cytolysin genes progressed more slowly, with little or no resultant damage to ocular structures. TEM revealed changes in retinal layers as early as 6 h postinoculation. The observations of an intact limiting retinal membrane and destructive changes in posterior layers suggest that the cytolysin can penetrate and destroy cells located deep within the retina. JH2SS(pAM771)-infected eyes at 24 h postinfection were evaluated as structurally and functionally intact. In contrast, the damage produced by cytolytic JH2SS(pAM714) at 24 h postinfection resulted in a significantly dysfunctional eye.

Interestingly, both cytolytic JH2SS(pAM714) and noncytolytic JH2SS(pAM771) appeared to adhere to the internal limiting membrane of the retina. In addition to the cytolysin, pAD1 encodes a number of functions required for pheromone-responsive conjugation. The gene encoding aggregation substance, *asa1*, which is involved in mating pair formation, has been sequenced and found to contain two tetrapeptide motifs implicated in the binding of fibronectin to cell surfaces (13). This characteristic may aid in bacterial adherence, similar in fashion to the attachment of fibronectin to the retinal limiting membrane (26). Such an event would bring the cytolysin-producing cell in close contact with its target, concentrating cytolytic activity near the target. Since both cytolytic and noncytolytic strains used in this study produce aggregation substance as demonstrated by their ability to transfer in broth matings (data not shown), it would be interesting to evaluate endophthalmitis caused by cytolytic JH2SS possessing mutations in the aggregation substance gene to determine the potential contribution of specific adhesion. Alternatively, the fixation and staining processes used for thin-section histopathology may have selectively washed off organisms not in proximity to ocular structures, giving an artificial impression of specific adherence. The latter prospect is supported by the absence of bacteria in sections examined by TEM; eyes processed for TEM are extensively washed and fixed, and the vitreous is completely removed. Future studies using cryosectioning of whole eyes and specific immunostaining techniques will be useful in identifying the location of these organisms more precisely on or within retinal structures.

Although the results demonstrate the contribution of the pAD1-encoded cytolysin to *E. faecalis* virulence in endophthalmitis, the actual mechanism of damage to host tissue remains to be determined. The contrasting changes in retinal structures are not due to increased growth rate of cytolytic JH2SS(pAM714) compared to JH2SS(pAM771) as demonstrated by similar growth kinetics in vivo and in vitro. It is


ILM
a: ⊢1μm
ILM
d: ⊢1μm
ILM
b: ⊢1μm
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e: ⊢1μm
PR
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c: ⊢1μm
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PR
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FIG. 5. TEM. Contrasting retinal changes in eyes infected with 100 CFU of cytolytic *E. faecalis* JH2SS(pAM714) and noncytolytic JH2SS(pAM771). (a to c) Retinal sections from eyes infected with noncytolytic JH2SS(pAM771). (a) 6 h postinfection; (b) 24 h postinfection; (c) posterior retinal layers at 24 h postinfection. (d to f) Retinal sections from eyes infected with cytolytic JH2SS(pAM714). (d) 6 h postinfection; (e) 24 h postinfection; (f) posterior retinal layers at 24 h postinfection. ILM, retinal inner limiting membrane; PR, photoreceptor cell outer segments; RPE, retinal pigmented epithelium. Note that the inner limiting membrane is not discernible at 24 h postinfection with cytolytic JH2SS(pAM714) (e).

possible that the cytolysin induces an early and uncontrolled release of inflammatory mediators from damaged tissue or phagocytic cells, thereby causing an exaggerated inflammatory reaction and subsequent indirect tissue destruction. Indeed, the host inflammatory response has been shown to be important in the progression and severity of endophthalmitis (10, 11, 21, 29, 34).

Organisms isolated from more-fulminant intraocular infections are often associated with the production of extracellular enzymes and toxins. Intraocular infections caused by *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and many *Streptococcus* species are particularly damaging and often result in loss of all useful vision (7, 8, 37, 39, 40). In contrast, endophthalmitis caused by the commensal organism *Staphylococcus epidermidis* is treated with greater success and has been observed to resolve spontaneously with minimal permanent ocular damage (32, 33). The demonstrated contribution of the cytolysin to *E. faecalis* virulence in endophthalmitis, when coupled with the observed frequent association between the cytolytic phenotype and high-level gentamicin-kanamycin resistance in infection-derived isolates (15), may explain why *E. faecalis* endophthalmitis is frequently severe and refractory to treatment.

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