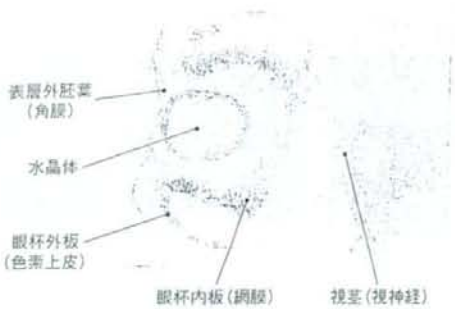

図 3 Pax6のモノクローナル抗体による発生ヒト眼(胎齢5週)の免疫染色[3]
発生初期では眼球のほぼ全体が染まる.

から出発した遺伝子が進化とともに眼形態形成の中心にいつづけて,角膜,虹彩,水晶体,網膜をつくるようになり,ついには視覚進化の頂点である黄斑を形成するに至ったことになる.

これまでにみつかった Pax6 の変異型と表現型には,遺伝子の変異が重篤なほど表現型も重症であるという法則がある.これは Pax6 に,①一対の対立遺伝子の両方が揃っていないと正常に機能しない(haploinsufficiency),②遺伝子障害の程度と表現型が相関する(dose dependent),という特徴があるためである.変異形式がストップコドン,フレームシフト,スプライシングエラーといったナンセンス変異では無虹彩のような眼球全体の形成不全を起こし,1 アミノ酸が置換した軽度なミスセンス変異では角膜,水晶体,網膜などで限局した形成不全を起こす.黄斑のみの形成不全がある孤立性黄斑低形成でみつかった変異はいずれもミスセンス変異である[4-6].

Pax6の選択的スプライスの働きと黄斑低形成の遺伝子変異

Pax6 遺伝子には,エクソン 5 とエクソン 6 の間に,14 のアミノ酸をコードする選択的スプライスのエクソン 5a が存在する.そして,これが読まれるか読まれないかによって,Pax6 蛋白は 14 アミノ酸が入るもの〔Pax6(+5a)〕と入らないもの〔Pax6(−5a)〕,2 種類のアイソフォームがつくられる[5].Pax6 蛋白では,転写因子として標的 DNA に接着する部位の paired domain があるが,14 アミノ酸はこのなかに存在する.paired domain はさらに N-terminal subdomain と C-terminal subdomain の 2 つに分かれ,異なるタイプの binding consensus をもつ標的 DNA を支配する.しかも生化学的検討によれば両 subdomain はたがいの働きを抑制しあっている.そして,エクソン 5a による 14 アミノ酸が入れば C-terminal が,入らなければ N-terminal が働くので,エクソン 5a は molecular switch の働きをもっている(図 1)[5].Pax6 の進化からみると,N-terminal subdomain は原始的動物にある基本的なもので,標的 DNA もいくつか判明している.一方,エクソン 5a は無脊椎動物では存在せず,脊椎動物に至って出現したので,C-terminal subdomain が働きはじめたのは進化的に比較的新しい.しかも,その機能はまったく不明で,標的遺伝子もみつかっていない.

これまでに発見された孤立性黄斑低形成の Pax6 ミスセンス変異はことごとく C-terminal subdomain あるいはエクソン 5a のなかに存在する[4,5].したがって,黄斑の形成にはこの C-terminal subdomain が関与していると推測された.

黄斑発生領域におけるPax6アイソフォームの発現

発生期の動物で時期別,眼組織別に mRNA を採取して cDNA を作成し,Pax6 の 2 つのアイソフォームを RT-PCR で検討すると,Pax6(−5a)は発生期全般にわたって広範な組織に発現する.しかし,Pax6(+5a)は発生期後半に後方網膜に強く発現することが示された.さらに免疫染色では,Pax6(−5a)に対する抗体では網膜は後方から前方まで均一に染まるのに対して,エクソン 5a がコードする 14 アミノ酸に対する抗体では黄斑領域を中心とする後極のみに染色がみられ,Pax6(+5a)は黄斑領域に限局して発現することが判明した(図 5)[7].

Pax6アイソフォームの網膜形成・分化に関する機能

Pax6 が黄斑形成に関与するならば,発生期の網膜に Pax6 を過剰に導入すると網膜の形成が進むはずである.しかし,過去の研究報告は逆の結果

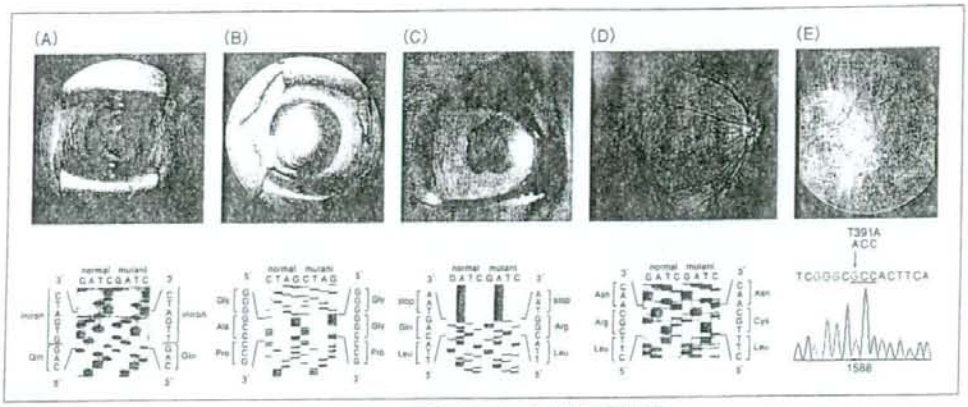

図 4　Pax6の変異がみつかった眼先天異常[4~6]
A：無虹彩，B：前眼部形成不全，C：瞳孔形成異常，D：黄斑低形成，E：視神経低形成．

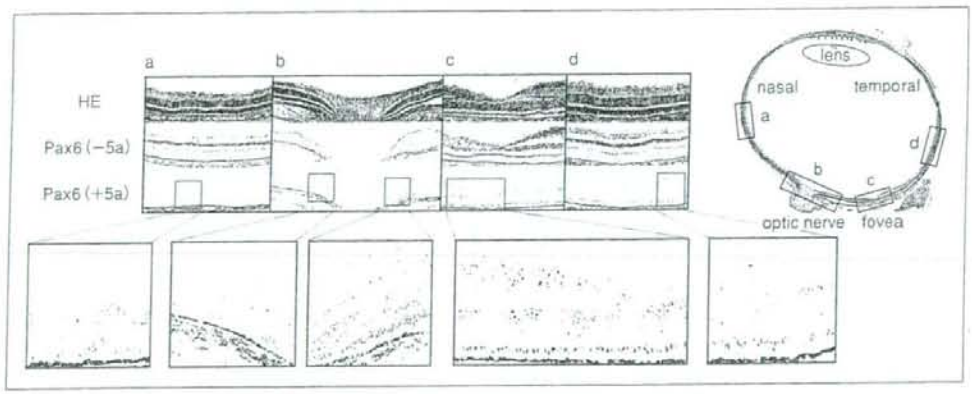

図 5　Pax6アイソフォームの発生期網膜における発現[7]
出生直後のマーモセット Pax6(−5a)に対する抗体では，網膜は後方から周辺部まで均一に染まるのに対して，エクソン 5a がコードする 14 アミノ酸に対する抗体では黄斑領域を中心とする後極のみに染色がみられる．Pax6(+5a)は黄斑領域に限局して発現することが示唆される．

を示していた．Pax6 の変異をもつマウスは小眼球になるが，一方で，Pax6 を過剰に導入したトランスジェニックマウスをつくっても小眼球が生ずる[8]．ここから Pax6 の発現量は少なくても多過ぎても正常に機能しないという考えが定着した．しかし，トランスジェニックマウスでは，導入した Pax6 が眼球だけでなく，中枢や視神経など多くの組織に発現する．小眼球は発生のわずかな均衡がくずれれば容易に起こるので，多くの組織に Pax6 が異常量発現すれば，組織間相互作用が障害され，結果として小眼球になることも考えられる．網膜への Pax6 の影響を知るためには網膜だけに遺伝子を導入しなければならない．そこでニワトリの発生期網膜に electroporation で Pax6 を直接導入した．Electroporation で導入した遺伝子は細胞質内で短期間発現するので，発生のような一時期に働く遺伝子の機能を観察する点では都合がよい．

発生初期(stage 12〜16)の網膜に，エクソン 5a を含まない Pax6 のアイソフォーム Pax6(−5a)を導入すると網膜が厚くなり，神経節細胞が増加し(図 6-B)，神経線維が硝子体腔に向かって増加した(図 6-C)．導入直後では神経芽細胞の分裂が亢

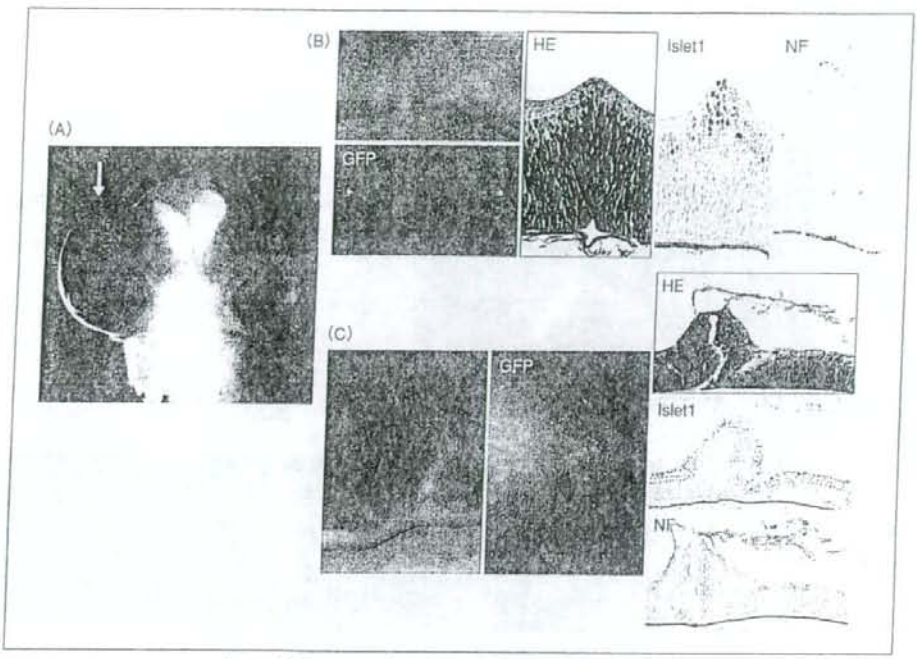

図 6　鶏胚へのPax6(−5a)導入による網膜の発育亢進[17]

2 日胚に導入，8 日胚の所見．

A：Pax6(−5a)を入れた右眼が大きくなる(矢印)．B：網膜が厚くなり，GFP で遺伝子の導入が確認され，組織所見では神経節細胞が増加している．C：網膜から硝子体腔へ線維構造が立ち上がり，組織所見では神経線維である．

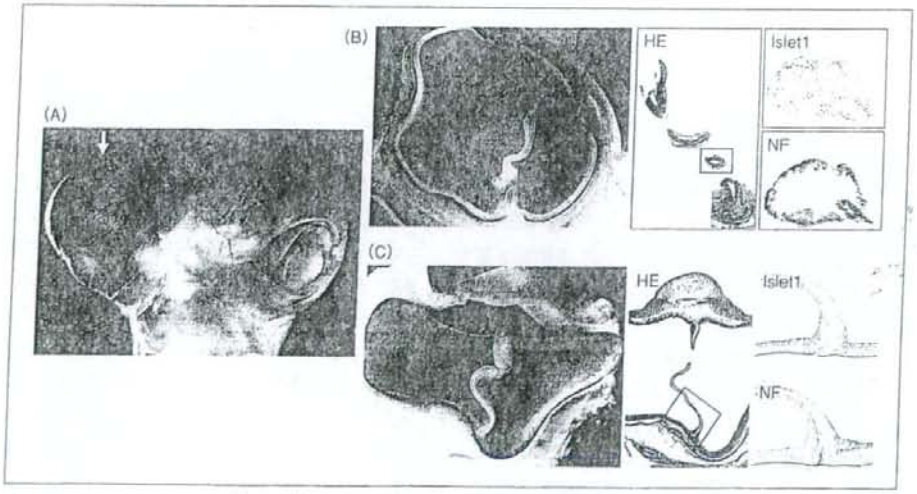

図 7　鶏胚へのPax6(+5a)導入による網膜の発育亢進[17]

2 日胚に導入，10 日胚の所見．

A：Pax6(+5a)を入れた右眼が極度に大きくなる(矢印)．B：網膜から茎状構造が立ち上がり，組織所見では管状の網膜で層構造はほぼ保たれている．C：網膜が水平に過剰発育して折りたたまれている．

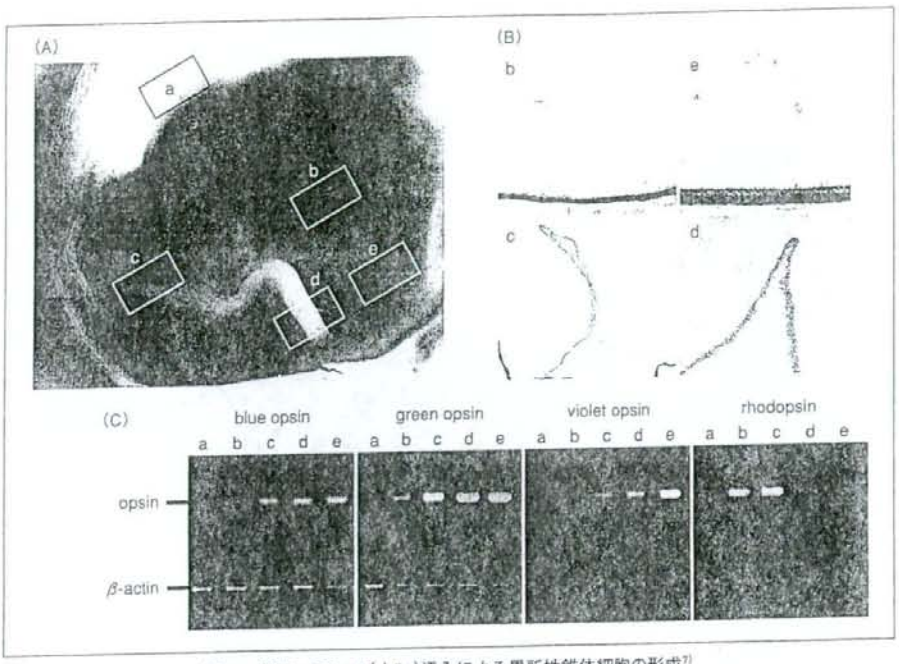

図 8　鶏胚へのPax6(+5a)導入による異所性錐体細胞の形成[7]

2 日胚に導入，19 日胚の所見．A：網膜が過剰に発育した襞を形成．この襞内では，周辺部(c)に本来は少ないオプシンをもつ錐体細胞が形成されている．B：免疫染色，C：RT-PCR．

進しており，Pax6 は網膜の成長を担っていると考えられた[7]．

つぎに，エクソン 5a を含む Pax6(+5a)を導入すると網膜から硝子体腔へ茎状構造が立ちあがった．これはすべて網膜であり，神経細胞と神経線維で構成されていた．茎状に伸びた組織では網膜が管状になっており，しかも視細胞から神経節細胞に至る層構造がほぼ形成されていた(図 7-B)．また，網膜が硝子体腔へ折りたたまれる所見もみられた(図 7-C)．網膜が水平方向へ過剰に発育し，抵抗の少ない硝子体腔へ伸展したと考えられる．

したがって，網膜を成長させる働きは Pax6(−5a)より Pax6(+5a)のほうがはるかに強いことが明らかになった．さらに，錐体視細胞が少なく杆体細胞がおもに存在する網膜周辺部に Pax6(+5a)を導入すると，異所性に錐体細胞の形成が観察された(図 8)[7]．この Pax6(+5a)による網膜の発育と錐体細胞の分化が硝子体腔へ突出せず網膜内の 1 カ所に集中すれば，黄斑になるのかもしれない．

Pax6 を入れた領域の眼球は拡大し，角膜と水晶体が対側へ偏位した．この作用は Pax6(−5a)より Pax6(+5a)のほうが強かった(図 6-A，図 7-A)[7]．眼球は発生初期には頭の横にあり，鼻側と耳側が同じ大きさであるが，発生が進むと眼球の位置が頭の横から顔の前へ向くとともに耳側が大きくなる．網膜の成長が進むと眼球が成長し，一方，網膜の形成不全では小眼球になることから，眼球の大きさには網膜の成長が関わっている．眼球の耳側が鼻側に比べて大きいのは Pax6(+5a)が耳側後極で強く発現して網膜の発育を進めるためであると考えられる．Pax6 にこのエクソン 5a が現れたのは脊椎動物になってからであるが，魚類で網膜の構造は急速に複雑化し，黄斑が生まれたのは，このエクソン 5a の追加が関与したことも示唆される．

Pax6などの眼形成遺伝子を用いた網膜の再生

Pax6/eyeless を異所導入すると昆虫ではほぼ完

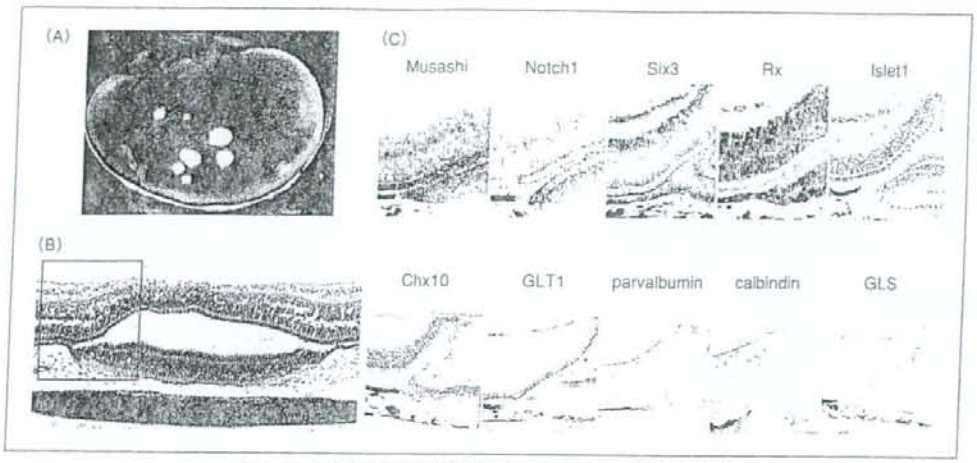

図 9　Pax6の導入による網膜色素上皮から網膜への分化転換[17]

3日胚に導入，14日胚の所見．遺伝子が導入された色素上皮は斑状に(A)，網膜へ分化転換している(B)．この異所網膜は *in situ* hybridization や免疫染色で網膜固有の遺伝子・蛋白発現がみられ(C)，ほぼ完全な層構造をもつが，層の方向は眼杯が折りたたまる向きに応じて本来の網膜と背合わせになっている．

全な複眼が形成される[2]．Pax6/eyeless 下流の eyes absent, sine oculis/Six, dachshund, Rx, teashirt の導入では小さい複眼が形成されるが[9-12]，哺乳類では，Pax を導入してもアフリカツメガエルの幼生で不完全な構造の異所眼が形成されるにすぎない[13]．また，Six6，Rx を発生期の脳や網膜色素上皮に導入すると，不完全ながら網膜組織が形成されるが[14-16]，Pax6 を導入するとかなり完全な層構造をもつ網膜を形成することができる(図 9)[17]．この網膜形成における Pax6(−5a)と Pax6(+5a)の働きの違いは現在検討中であるが，過去の研究からみて Pax6(+5a)のほうがより高度な網膜を形成できる可能性がある[7]．

近年，網膜色素変性症モデル動物や患者で，障害された黄斑部網膜下に胎児網膜を移植して視力が改善したことが報告された[18,19]．未熟な胎児網膜がレシピエント網膜内で分化し，シナプスを形成すると思われる．網膜色素変性症では視細胞以外の網膜構造はある程度温存されており，胎児網膜由来の視細胞のシナプスがつながったと推測される．自己の虹彩や色素上皮に網膜の形態形成遺伝子を導入あるいは発現誘導して網膜を再生できれば，やや不完全な構造であっても，このような網膜移植に利用できることが期待される．

おわりに

網膜の重症疾患で悩まされている患者や医師にとって，網膜を再生させて失われた視覚を復元させる医療は大きな夢である．しかし，構造が複雑な網膜をシナプスごと再構築することが難しく，中枢への神経投射を的確に復元しないかぎり有用な視力が得られない．できても，せいぜい光覚や手動弁の視力あるいは視野をすこし広げる程度と考えられていた．しかし，近年の再生医学研究によって視覚の復元への道はすこしずつ着実に進歩している．黄斑を形成する遺伝子システムを解明して再生医療に利用すれば，高度な視覚構造が復元できると期待される．

文献

1) Ton, C. T. T. et al. : *Cell*, **67** : 1059-1074, 1991.
2) Callaerts, P. et al. : *Annual. Rev. Neurosci.*, **20** : 483-532, 1997.
3) Nishina, S. et al. : *Br. J. Ophthalmol.*, **83** : 723-727, 1999.
4) Azuma, N. et al. : *Nat. Genet.*, **13** : 141-142, 1996.
5) Azuma, N. et al. : *Am. J. Hum. Genet.*, **65** : 656-663, 1999.
6) Azuma, N. et al. : *Am. J. Hum. Genet.*, **72** : 1565-1570, 2003.
7) Azuma, N. et al. : *Hum. Mol. Genet.*, **14** : 735-745, 2005.
8) Schedl, A. et al. : *Cell*, **86** : 71-82, 1996.

Contribution of secreted proteases to the pathogenesis of postoperative *Enterococcus faecalis* endophthalmitis

Takashi Suzuki, MD, PhD, Tomoyuki Wada, PhD, Seiko Kozai, MSc, Yasuyoshi Ike, MD, PhD, Michael S. Gilmore, PhD, Yuichi Ohashi, MD, PhD

PURPOSE: To determine how a secreted protease contributes to the pathogenesis of post-cataract endophthalmitis caused by *Enterococcus faecalis* using an aphakic rabbit endophthalmitis model.

SETTING: Department of Ophthalmology, Ehime University School of Medicine, Ehime, Japan.

METHODS: The pathogenesis of *E faecalis* OG1S (secreted protease-positive) and *E faecalis* OG1X (secreted protease-negative derivative of OG1S) was compared. After lens removal by phacoemulsification, either strain was inoculated into the lens bag. Changes in bacterial growth, electroretinography (ERG), and pathology of eyes were comparatively monitored throughout the course of the infection. Alternatively, culture fluid from either strain was injected into the vitreous body and ERG and pathology of the eyes were also examined.

RESULTS: The levels of growth in the anterior chamber and vitreous cavity were similar for both strains. However, infection with OG1S resulted in a significantly greater reduction in ERG b-wave amplitude than OG1X. Histological examination showed that the posterior lens capsules were severely affected in eyes infected with OG1S, and inflammatory cells and cocci were found in the anterior vitreous cavity 24 hours after the infection. By 48 hours, the retina architecture was profoundly affected in eyes infected with OG1S. In contrast, few pathological changes were noted in the posterior lens capsules and retina of eyes infected with OG1X. Culture fluid in which OG1S had grown decreased ERG b-wave amplitude and caused morphological changes of the posterior capsule and retina similar to those in the infected eye.

CONCLUSION: An extracellular protease plays a major role in the pathogenesis of *E faecalis*–induced postoperative endophthalmitis.

J Cataract Refract Surg 2008; 34:1776–1784 © 2008 ASCRS and ESCRS

Bacterial endophthalmitis is a sight-threatening complication of cataract surgery. Along with *Staphylococcus aureus*, *Enterococcus faecalis* endophthalmitis is often associated with significant vision loss.[1,2] A Japanese survey of postoperative endophthalmitis showed that *E faecalis* was the third most commonly isolated pathogen, accounting for 17% of culture-positive cases.[3] Because of the high level of antibiotic resistance in this genus, it is important to understand the pathogenesis of *E faecalis* endophthalmitis with a view toward developing new therapeutic approaches. Previous studies indicate that several enterococcal virulence factors contribute to the pathogenesis of *E faecalis* endophthalmitis; these include cytolysin, gelatinase, and serine protease (to the severity of *E faecalis* endophthalmitis).[4–9] Gelatinase and serine protease, secreted proteases regulated by a quorum-sensing system termed *fsr*, have been reported to be associated with the retinal destruction.[8,9] However, little is known about how these proteases might contribute to the pathogenesis of endophthalmitis after the anatomy of the eye is altered by cataract surgery. It is unknown where they colonize and how they translocate through the posterior capsule to cause ocular tissue damage. Bacteria have a chance to enter the anterior chamber through the surgical wound, and many studies[10–13] report contamination of the anterior chamber at the end of surgery to be as high as 5.7% to 21.1%. Thus, intraoperative or postoperative contamination of the anterior chamber seems to be the initial step of endophthalmitis. Rupture of the lens capsule is known to be a risk factor for endophthalmitis and

0886-3350/08/$—see front matter
doi:10.1016/j.jcrs.2008.06.033

suppurative posterior infection.[14] However, endophthalmitis has been reported in cases in which the posterior capsule was believed to remain intact after cataract surgery.[15,16] In these cases, the route of organism migration to the posterior segment is unknown.

The purpose of this study was to evaluate the pathogenesis of postoperative enterococcal endophthalmitis and determine the contribution of known secreted proteases to its progress. We used a rabbit model of *E faecalis* endophthalmitis with lensectomy. This model took a similar time course as that of clinical enterococcal endophthalmitis cases. Protease-positive and protease-negative *E faecalis* strains were used to examine the contribution of the secretory protease. Direct virulence of the secretory protease to the ocular tissues and visual function is also presented.

MATERIALS AND METHODS

Bacteria

The laboratory strains included *E faecalis* OG1S[17] (secreted protease-positive) and *E faecalis* OG1X, which is a secretory protease defective mutant originally derived by mutagenesis with nitrosoguanidine of OG1S.[18] The bacteria were grown in brain–heart infusion (BHI) (Difco Laboratories) broth for 18 hours at 37°C, washed twice with sterile physiological saline, and resuspended in sterile physiological saline. The concentration of bacteria in the suspension was determined spectrophotometrically and then adjusted to approximately 2×10^5 colony-forming units/mL (CFU/mL) with sterile physiological saline. The exact size of each inoculum was determined by the agar dilution method.

Preparation of Bacterium-Free Supernatants

Overnight cultures of *E faecalis* OG1S and OG1X in BHI broth were centrifuged and filter sterilized to obtain cell-free supernatants (MILLEX-GV, 0.22 µm pore, Nihon Millipore K.K.).

Bacteria Growth and Gelatinase Assay

An overnight culture of *E faecalis* was inoculated into BHI broth and cultivated at 37°C with gentle shaking. Bacteria growth in vitro was determined by an optical density at 660 nm (OD_{660}) and converted into CFUs by the following equation: Log_{10} CFU/mL = (Log_{10} OD_{660} + 8.7026)/1.0072. Gelatinase activity was measured using azo dye impregnated collagen (Azocoll, Calbiochem) as a substrate for gelatinase according to a protocol described previously.[19] Briefly, 40 µL of *E faecalis* culture supernatant was added to 0.8 mL of an Azocoll suspension, incubated for 4 hours with vigorous mixing (170 rpm), and centrifuged at 20<thin space>000 × *g* for 5 minutes; then, the OD_{540} of the supernatant was determined.

Animals

Male and female Japanese albino rabbits weighing 2.0 to 2.8 kg (Kitayama Labs Co., Ltd.) were maintained in accordance with Institutional Animal Care and Use Committee guidelines and the Association for Research in Vision and Ophthalmology Statement for Use of Laboratory Animals in Ophthalmic and Vision Research. Rabbits were anesthetized by an intramuscular injection with an equal mixture of 5% ketamine (Ketalar intramuscular 500 mg) and 2% xylazine (Selactar) at 1 mL/kg for all procedures. Rabbits were killed humanely with an overdose of pentobarbital sodium.

Experimental *Enterococcus Faecalis* Endophthalmitis After Lensectomy

The lensectomy was performed in both eyes as described previously.[20] Pupil dilation was achieved with a 1-drop mixture of tropicamide 0.5% and phenylephrine hydrochloride 0.5% (Mydrin-P). A clear corneal incision was created, and the lens was extracted by a phacoemulsification unit (Prestige, Advanced Medical Optics). After the incision was sutured, 0.1 mL of OG1S strain (5.08 to 5.45 $\log_{10}$ CFU/mL) or OG1X strain (4.49 to 4.86 $\log_{10}$ CFU/mL) was inoculated into the capsular bag using a blunt needle on a 1 mL tuberculin syringe. Contralateral eyes were injected with sterile physiological saline (surgical control).

Experimental Endophthalmitis with Bacterium-Free Supernatants

Bacterium-free supernatants were injected as described previously.[7] Just before injection, 0.1 mL of aqueous humor was aspirated to relieve intraocular pressure. The bacterium-free supernatants (0.1 mL) were injected into the midvitreous via a 30-gauge needle attached to a tuberculin syringe introduced through the pars plana approximately 3.0 mm from the limbus. Control eyes received sterile BHI broth.

Accepted for publication June 12, 2008.

Doctors Suzuki and Wada contributed equally as co-first authros.

From the Department of Ophthalmology (Suzuki, Ohashi), Ehime University School of Medicine, Shitsukawa, Toon-shi, Ehime, the Laboratory for Preclinical Development Research (Wada, Kozai), Senju Pharmaceutical Co., Ltd., Kobe, Hyogo, the Department of Microbiology and Laboratory of Bacterial Drug Resistance (Ike), Gunma University School of Medicine, Maebashi, Gunma, Japan; Schepens Eye Research Institute (Suzuki, Gilmore), Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA.

No author has a financial or proprietary interest in any material or method mentioned.

Supported in part by research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan.

Presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, USA, April 2008.

Corresponding author: Takashi Suzuki, MD, The Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, 20 Staniford Street, Boston, Massachusetts 02114, USA. E-mail: takashi.suzuki@schepens.harvard.edu.

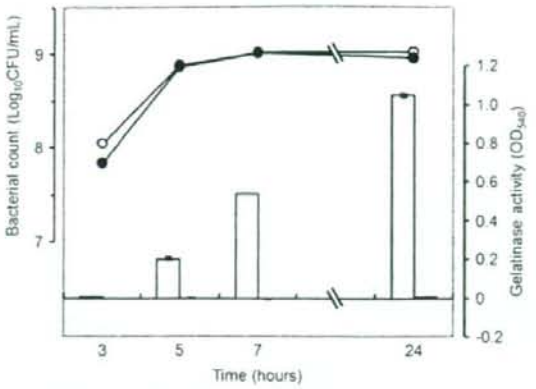

Figure 1. Time courses for cell growth and gelatinase activity of *E faecalis* OG1S (*open symbols*) and OG1X (*closed symbols*). Culture supernatant was collected at 3, 7, and 24 hours of cultivation and the colony count (*circles*) and gelatinase activity (*columns*) were determined. Data represent the mean ± standard error of the mean (SEM) of duplicate determinations (OD_{540} = optical density at 540 nm).

Slitlamp Examination

The eyes inoculated with living organisms were examined by Topcon SL-14 slitlamp biomicroscopy (Kougaku Kikai K.K.) 16, 24, 36, and 48 hours after inoculation. The intraocular inflammation was graded by a masked observer using the following scoring criteria: 0, normal; 1, small amount of fibrin on the pupil; 2, iris partially covered with fibrin and/or hypopyon; 3, iris covered with fibrin and/or hypopyon; 4, pupil not visible.

Electroretinography

Rabbits were dark-adapted for at least 30 minutes before electroretinography (ERG) recording. Under dim red light, monopolar ground and reference electrodes were placed in the ear and forehead, respectively. Bipolar contact lens electrodes were placed on both eyes after topical anesthesia of oxybuprocaine hydrochloride 0.4% (Benoxil Ophthalmic Solution 0.4%) was administered. A solution of hydroxyethylcellulose 1.5% (Scopisol 15) was used to ensure good contact between the contact lens electrode and cornea. Scotopic b-wave amplitude was recorded for each eye using flash ERG (LE-3000, Tomey Corp.). The percentage retinal function was defined as the rate of the b-wave amplitude in the experimental eye to the b-wave amplitude in the contralateral control eye.[7–9]

Bacterial Quantitation

Quantitation of *E faecalis* in ocular tissues was performed. The surface of the enucleated globe was cleaned with ethanol using a cotton swab and then rinsed with sterile physiological saline. The aqueous humor was collected with a 23-gauge needle. The cornea was removed aseptically, and the contents of the anterior chamber (fibrin and hypopyon) were collected and mixed with the aqueous humor. The iris and lens were dissected away, and the surface of the vitreous body was rinsed with sterile physiological saline. The vitreous was then collected. The volumes of the anterior chamber and vitreous body contents were adjusted to 3 mL and 5 mL, respectively, by the addition of sterile physiological saline. All specimens were then minced and vortexed. The bacterial CFUs were quantified by plating serial tenfold dilutions on BHI agar.

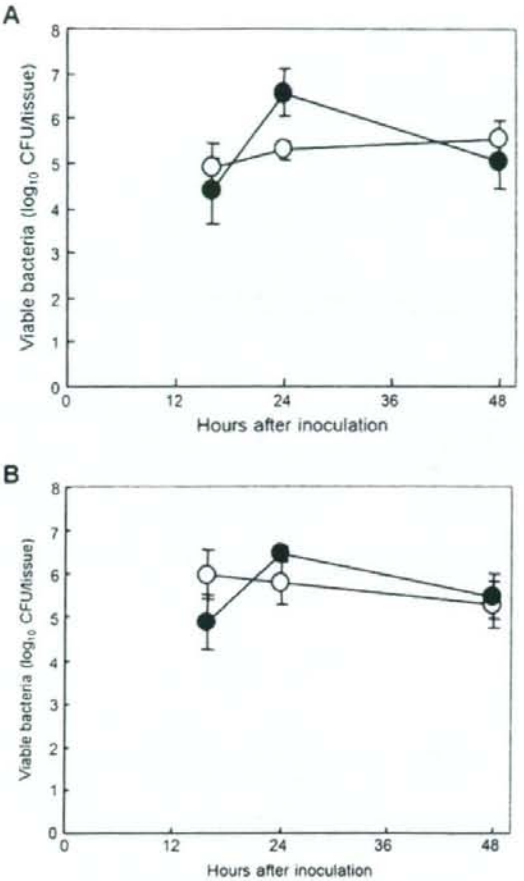

Figure 2. Number of viable organisms recovered from the aqueous humor (*A*) and vitreous (*B*). Eyes were inoculated with *E faecalis* OG1S (*open circle*) or OG1X (*closed circle*). Data represent the mean ± SEM (n = 4 to 6) (CFU = colony-forming units).

Histological Examination

Globes recovered for light microscopic analysis were fixed in phosphate-buffered 10% formalin solution for 24 hours. Eyes (4 per group) were sectioned and stained with hematoxylin-eosin.[7]

Statistical Analysis

The differences in infection courses between OG1S-infected eyes and OG1X-infected eyes were analyzed by Student *t* test at each time point. The Tukey-Kramer test was

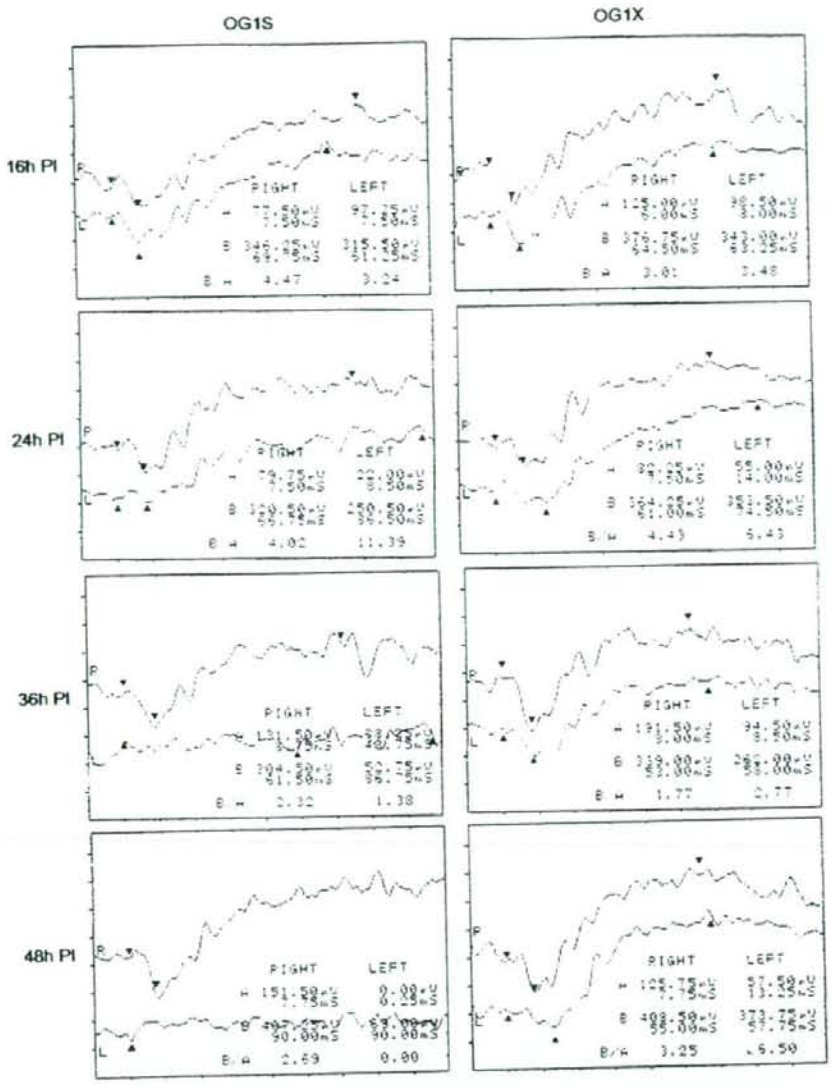

Figure 3. The whole ERG trace of eyes infected with OG1S or OG1X at each time point. Each pair of traces represents the responses recorded from the right, control eye (*upper trace*), and the left, experimental eye (*lower trace*). The ERG responses exhibited severe functional damage in the eye infected with OG1S 36 hours or 48 hours postinoculation (PI).

used for multiple comparisons. A P value less than 0.05 was considered significant.

RESULTS

Bacteria Growth and Gelatinase Activity

Figure 1 shows the time courses for cell growth and gelatinase activity of *E faecalis* cultures. The data showed similar in vitro growth patterns for OG1S and OG1X, while only OG1S showed gelatinase activity, which became apparent after the late-log phase of cell growth.

Experimental *Enterococcus Faecalis* Endophthalmitis

No significant differences were observed in anterior segment inflammation between the OG1S and OG1X inoculation groups over time (data not shown). There were no significant differences between the number of recovered *E faecalis* OG1S and OG1X from the anterior chamber and vitreous body at each time point ($P > .05$) (Figure 2).

The representative whole ERG trace of eyes infected with OG1S or OG1X at each time point is shown in Figure 3, and the result is summarized in Figure 4.

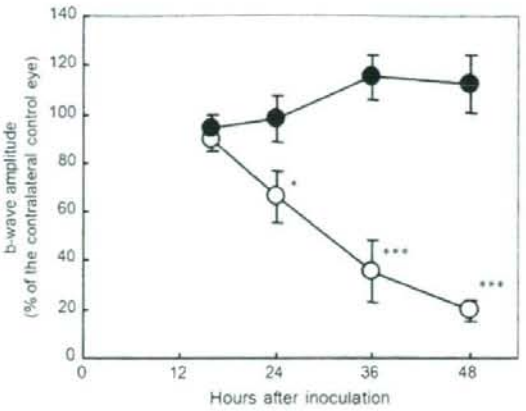

Figure 4. Retinal responsiveness of eyes infected with *E faecalis* OG1S and OG1X. Data represent the mean ± SEM (n = 8) (* = $P<.05$, *** = $P<.001$; both 2-sided Student *t* test).

Infection with OG1S resulted in a significantly greater reduction of b-wave amplitude than infection with the secretory protease-negative mutant OG1X from 24 to 48 hours postinoculation ($P \leq .0222$). *Enterococcus faecalis* OG1S-infected eyes had normal ERG responses at 16 hours, and retinal function declined steadily thereafter, resulting in 66% and 35% retention of retinal function at 24 hours and 36 hours, respectively. At 48 hours, all 8 eyes infected with OG1S had nearly flat ERGs. In contrast, b-wave amplitude tended to increase during the course of OG1X infection.

Histologically, all infected eyes had moderate inflammatory cell accumulation in the lens capsule, anterior chamber, iris, and ciliary body 16 hours postinoculation, while the structures of the lens capsule and retinal layers were intact. Inflammatory cells or cocci were not observed in the vitreous cavity. At 24 hours, eyes infected with OG1S had destruction of the lens capsule, migration of inflammatory cells, and cocci underneath the capsule (Figure 5). The retinal layers were apparently intact. In contrast, eyes infected with OG1X did not have destruction of the lens capsule, and the migration of inflammatory cells and cocci was limited to the anterior segment. At 48 hours, the eyes infected with OG1S had disruption of the retinal layers with massive infiltration of the vitreous cavity; inflammatory cells originated mainly from the iris–ciliary body and optic nerve head. The eyes infected with OG1X, in contrast, had only sparsely distributed inflammatory cells in the vitreous body. The retinas in these eyes had a normal appearance.

Virulence of Secreted Proteases

Figure 6 shows the effect of the bacterium-free supernatant on retinal function as measured by ERG. The retinal function in eyes intravitreously injected with culture supernatant of OG1S decreased by 27% compared with that in contralateral control eyes 24 hours postinoculation. The change was statistically significant compared with that in eyes injected with sterile BHI or culture supernatant of OG1X ($P<.0001$). In contrast, no significant change was detected in the retinal function in eyes injected with culture supernatant of OG1X.

Destruction of the photoreceptor outer segment layer, constriction of retinal vessels, and degradation of the lens capsule were the typical histological changes observed at 24 hours in all eyes injected with culture supernatant of OG1S (Figure 7). In contrast, eyes injected with culture supernatant of OG1X or sterile BHI retained normal appearances except for the presence of a few inflammatory cells.

DISCUSSION

Enterococcus faecalis, an important pathogen of endophthalmitis after cataract surgery, is associated with major loss of vision. An endophthalmitis vitrectomy study[1] found that endophthalmitis caused by *E faecalis* had the worst visual progress of all causes. Thus, the pathogenesis of *E faecalis* must be studied to treat and prevent endophthalmitis. The migration of the organism from the anterior segment to the posterior segment after cataract surgery must be elucidated. Rupture of the posterior capsule during surgery is associated with a significantly higher incidence of postoperative endophthalmitis because aqueous humor contaminants can readily access the vitreous cavity.[10–13] However, the development of postoperative endophthalmitis in patients who have surgery without major intraoperative complications is a mystery.[15,16] Some investigators report that cataract surgery without posterior capsule rupture does not allow substances in the aqueous humor to move into the vitreous cavity because the posterior capsule[21,22] and anterior vitreous membrane[23] form a firm barrier. Thus, little is known about the routes organisms take from the anterior chamber to the vitreous cavity. The histological analysis in this study showed that 2 processes might be involved in the migration of *E faecalis* to the posterior segment. One explanation is that organisms that cannot produce secretory protease go through the Zinn zonule to the peripheral vitreous body, and the other is that organisms that can produce secretory protease migrate to the central vitreous through damaged capsule along with the Zinn zonule. Whether the inflammatory cells or the organism destroyed the lens capsule is not known. Because the lens capsule was damaged after the supernatant of the secretory protease-positive strain was injected into

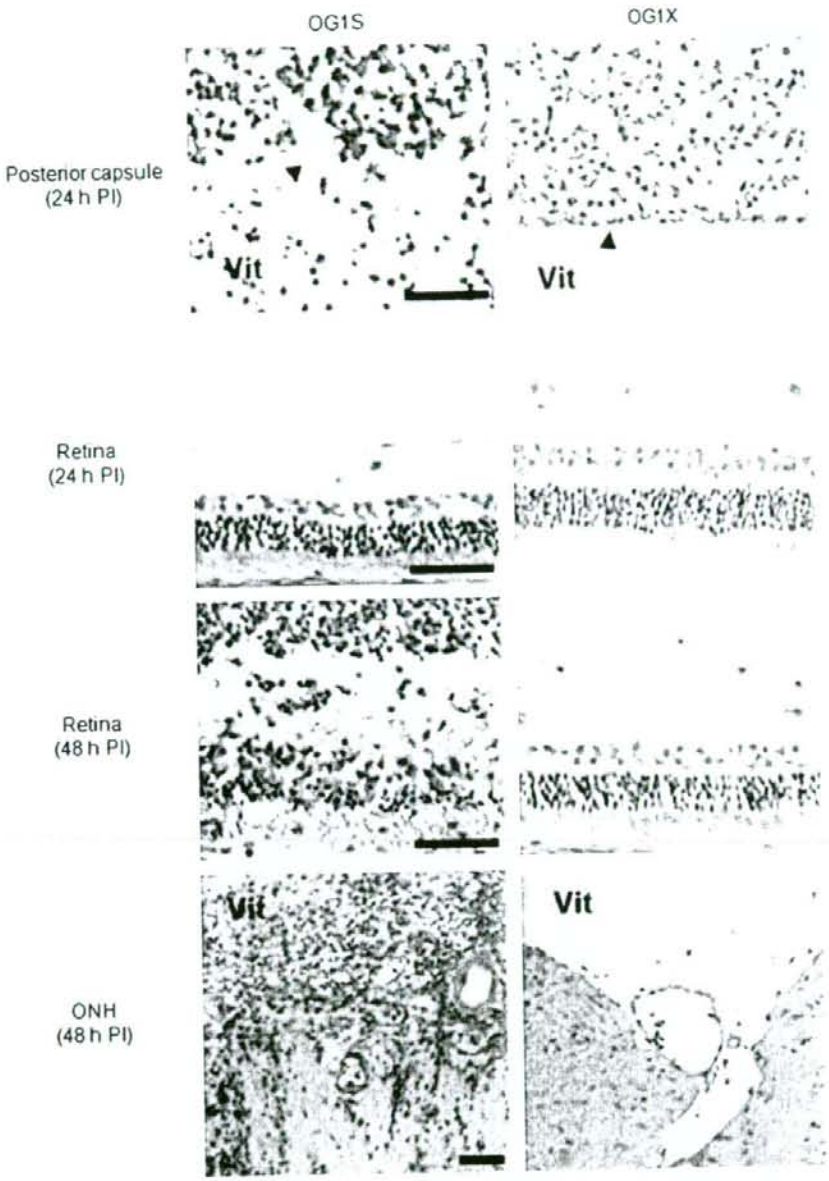

Figure 5. Histological analysis of experimental *E faecalis* endophthalmitis. Degradation of posterior lens capsule (*arrowhead*), destruction of photoreceptor segments, and destructive inflammatory cell infiltration from the optic nerve head (ONH) were observed in eyes inoculated with OG1S. Scale bar = 50 μm (PI = postinoculation; Vit = vitreous cavity).

the vitreous body, some protease might have lysed the lens capsule. These results imply that *E faecalis*, which produces secretory protease, could migrate to the vitreous body directly through a disrupted capsule and that secretory protease plays an important role in the migration of *E faecalis* from the anterior segment to the posterior segment. Although no significant differences were observed in the clinical inflammation of the anterior chamber between eyes inoculated with OG1S and eyes inoculated with OG1X, the histological study showed that eyes inoculated with OG1S had more rapid and massive inflammation of the whole vitreous cavity. Secretory protease might be an important factor for inducing inflammatory cells in the vitreous via not only tissue of the posterior segment, such as the ciliary body and retina, but also the anterior

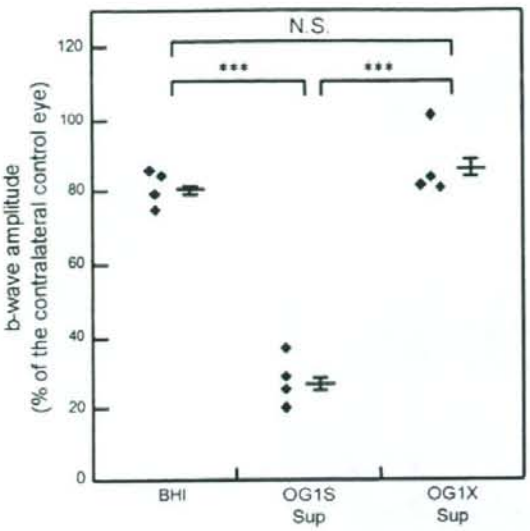

Figure 6. Retinal responsiveness of the eyes injected with culture supernatants of *E faecalis* OG1S and OG1X. Data represent the mean ± SEM and individual values (*** = $P < .001$, 2-sided Tukey-Kramer multiple comparison test; BHI = brain-heart infusion; N.S. = not significant; Sup = supernatant).

segment. The bacterial quantitation in the anterior chamber and vitreous body in eyes infected by OG1S was almost the same as in eyes infected by OG1X. However, massive inflammation in endophthalmitis due to OG1S seemed decrease the sensitivity of the bacterial quantitation, and in effect more OG1S than OG1X might have passed to the vitreous cavity. Furthermore, our results imply that the inside of the lens capsule might be a suitable place for organisms to colonize and increase regardless of secretory protease. This fact seems to be explained by the observation that aqueous humor in the lens capsule could not be exchanged quickly. Thus, cataract surgeons should be sure to irrigate the bag well to prevent the colonization of organisms and endophthalmitis.

Our data show that secretory protease contributes to the functional and morphological damage in the retina, as described previously.[8,9] The pathogenesis of the organism in endophthalmitis was initiated by injection of the organism into the vitreous body directly. Thus, these models provide a sensitive infection system in which organ function can be directly assessed (by ERG) and the effects of the infection can be monitored. However, little is known about whether the organisms can directly attach to retinal tissue before causing postoperative endophthalmitis. In addition, actual postoperative endophthalmitis might be influenced by an immune reaction of the anterior segment. Our model reflects actual postoperative endophthalmitis. The b-wave amplitude of eyes inoculated with OG1S significantly decreased 24 hours postinfection, although organisms and inflammatory cells were not recognized around retinal tissue at the same time. Furthermore, the b-wave amplitude in eyes inoculated with the supernatant of OG1S also decreased. Moreover, the histological analysis showed that the photoreceptors of the retina were not recognized and the retinal vessel was obstructed regardless of mild inflammatory cells. These phenomena imply that secretory protease directly damages retinal tissue such as vessels and photoreceptors without an inflammatory response. Secretory protease is proposed to have an important role in our model as well as the mouse peritonitis[24] and *Caenorhabditis elegans*[25] models of enterococcal infection. Along with these infections, gelatinase of secretory protease plays an important role in biofilm formation[26,27] and translocation of bacteria across intestinal cell layers.[28] However, debate continues on the exact mechanism for the virulence activity of secretory protease. We used the nitrosoguanidine-generated secretory negative (OG1X) and positive (OG1S) strain. Thus, OG1X loses not only gelatinase but also serine protease and little is known which secretory protease could cause retinal damage. Further study is needed to clarify the exact mechanism for the virulence of secretory protease in the retina.

In our animal model, it took 48 hours for the secretory protease-positive organism OG1S to induce a loss of retinal function completely. This was slower than in previous enterococcal endophthalmitis models,[8,9] likely due to the difference in inocula amount and strain or the barrier between the anterior and posterior segments. In addition, gelatinase production is regulated by a *fsr* quorum-sensing system encoded by the *fsr* locus[24,29] and gelatinase increases in proportion to time, as shown in the gelatinase assay in vitro. The *fsr* system in *E faecalis* controls biofilm formation[27,30] and other genes that are important for virulence[31] in addition to gelatinase and a serine protease. The *fsr* quorum-sensing system also mediates a cyclic peptide, gelatinase biosynthesis-activating pheromone (GBAP), and GBAP acts as an autoinducer.[19,32] Thus, the amount of secretory protease in the posterior segment might be small in the initial phase of bacterial contamination of the posterior segment and secretory protease increasing in the late phase might cause rapid retinal damage. The manner of secretory protease increase might influence the clinical manifestation. A previous study[8] found that deleting the *fsrB* gene significantly decreased virulence in an endophthalmitis model. If we could suppress the quorum-sensing system in the initial step of infection, retinal damage might be kept to a minimum. Our

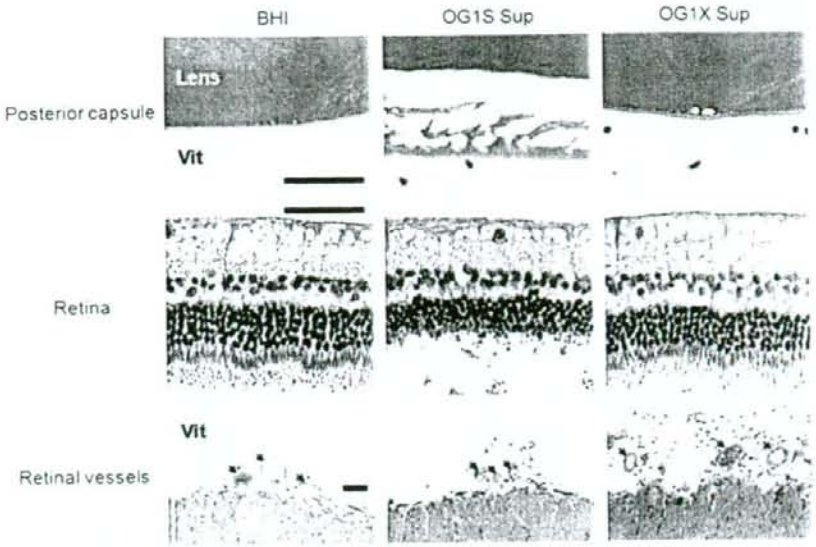

Figure 7. Histopathology of eyes intravitreally injected with culture supernatants (Sup) of *E faecalis* OG1S and OG1X. Control eyes received sterile BHI broth. Degradation of posterior lens capsule, destruction of photoreceptor segments, and constriction of retinal vessels (*arrow*) were observed in the eyes injected with OG1S supernatant (BHI = brain-heart infusion; NFL = retinal nerve fiber layer; Vit = vitreous).

endophthalmitis model will also be helpful in evaluating treatment.

In conclusion, our results show that secretory protease plays a major role in the virulence of *E faecalis* for migration from the anterior segment to the posterior segment and also contributes to retinal damage. The experimental endophthalmitis model using organisms inoculated into the lens capsule and anterior chamber after lensectomy is consistent with postoperative endophthalmitis and useful for evaluating prophylaxis and treatment.

REFERENCES

1. Endophthalmitis Vitrectomy Study Group. Microbiologic factors and visual outcome in the Endophthalmitis Vitrectomy Study. Am J Ophthalmol 1996; 122:830–846
2. Scott IU, Loo RH, Flynn HW Jr, Miller D. Endophthalmitis caused by Enterococcus faecalis; antibiotic selection and treatment outcomes. Ophthalmology 2003; 110:1573–1577
3. Hara J. [Relation between causative organism and inflammation onset in pseudophakic endophthalmitis following cataract surgery]. [Japanese] Atarashii Ganka 2003; 20:657–660
4. Stevens SX, Jensen HG, Jett BD, Gilmore MS. A hemolysin-encoding plasmid contributes to bacterial virulence in experimental Enterococcus faecalis endophthalmitis. Invest Ophthalmol Vis Sci 1992; 33:1650–1656. Available at: http://www.iovs.org/cgi/content/abstract/33/5/1650. Accessed June 25, 2008
5. Jett BD, Jensen HG, Nordquist RE, Gilmore MS. Contribution of the pAD1-encoded cytolysin to the severity of experimental Enterococcus faecalis endophthalmitis. Infect Immun 1992; 60:2445–2452
6. Jett BD, Atkuri RV, Gilmore MS. Enterococcus faecalis localization in experimental endophthalmitis: role of plasmid-encoded aggregation substance. Infect Immun 1998; 66: 843–848
7. Callegan MC, Booth MC, Jett BD, Gilmore MS. Pathogenesis of gram-positive bacterial endophthalmitis. Infect Immun 1999; 67:3348–3356
8. Mylonakis E, Engelbert M, Qin X, Sifri CD, Murray BE, Ausubel FM, Gilmore MS, Calderwood SB. The Enterococcus faecalis fsrB gene, a key component of the fsr quorum-sensing system, is associated with virulence in the rabbit endophthalmitis model. Infect Immun 2002; 70:4678–4681
9. Engelbert M, Mylonakis E, Ausubel FM, Calderwood SB, Gilmore MS. Contribution of gelatinase, serine protease, and fsr to the pathogenesis of Enterococcus faecalis endophthalmitis. Infect Immun 2004; 72:3628–3633
10. Sobaci G, Tuncer K, Taş A, Özyurt M, Bayer A, Kutlu U. The effect of intraoperative antibiotics in irrigating solutions on aqueous humor contamination and endophthalmitis after phacoemulsification surgery. Eur J Ophthalmol 2003; 13:773–778
11. John T, Sims M, Hoffmann C. Intraocular bacterial contamination during sutureless, small incision, single-port phacoemulsification. J Cataract Refract Surg 2000; 26:1786–1791
12. Manners TD, Chitkara DK, Marsh PJ, Stoddart MG. Anterior chamber aspirate cultures in small incision cataract surgery. Br J Ophthalmol 1995; 79:878–880
13. Tervo T, Ljungberg P, Kautiainen T, Puska P, Lehto I, Raivio I, Järvinen E, Kuusela P, Tarkkanen A. Prospective evaluation of external ocular microbial growth and aqueous humor contamination during cataract surgery. J Cataract Refract Surg 1999; 25:65–71
14. Wong TY, Chee S-P. The epidemiology of acute endophthalmitis after cataract surgery in an Asian population. Ophthalmology 2004; 111:699–705
15. Braun M, Jonas JB, Schönherr U, Naumann GOH. Ochrobactrum anthropi endophthalmitis after uncomplicated cataract surgery. Am J Ophthalmol 1996; 122:272–273
16. Jirásková N, Rozsíval P. Delayed-onset Pseudomonas stutzeri endophthalmitis after uncomplicated cataract surgery. J Cataract Refract Surg 1998; 24:866–867

17. Dunny GM, Brown BL, Clewell DB. Induced cell aggregation and mating in Streptococcus faecalis: evidence for a bacterial sex pheromone. Proc Natl Acad Sci USA 1978; 75:3479–3483. Available at: http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=392801&blobtype=pdf. Accessed June 26, 2008
18. Ike Y, Craig RA, White BA, Yagi Y, Clewell DB. Modification of Streptococcus faecalis sex pheromones after acquisition of plasmid DNA. Proc Natl Acad Sci USA 1983; 80:5369–5373. Available at: http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=384257&blobtype=pdf. Accessed June 26, 2008
19. Nakayama J, Cao Y, Horii T, Sakuda S, Akkermans AD, de Vos WM, Nagasawa H. Gelatinase biosynthesis-activating pheromone: a peptide lactone that mediates a quorum sensing in Enterococcus faecalis. Mol Microbiol 2001; 41:145–154. Available at: http://www.ingentaconnect.com/content/bsc/mole/2001/00000041/00000001/art02486. Accessed June 26, 2008
20. Kurosaka D, Kato K, Nagamoto T. Presence of α smooth muscle actin in lens epithelial cells of aphakic rabbit eyes. Br J Ophthalmol 1996; 80:906–910
21. Smith RT, Campbell CJ, Koester CJ, Trokel S, Anderson A. The barrier function in extracapsular cataract surgery. Ophthalmology 1990; 97:90–95
22. Ohrloff C, Schalnus R, Rothe R, Spitznas M. Role of the posterior capsule in the aqueous–vitreous barrier in aphakic and pseudophakic eyes. J Cataract Refract Surg 1990; 16:198–201
23. De Groot V, Hubert M, Van Best JA, Engelen S, Van Aelst S, Tassignon M-J. Lack of fluorophotometric evidence of aqueous–vitreous barrier disruption after posterior capsulorhexis. J Cataract Refract Surg 2003; 29:2330–2338
24. Qin X, Singh KV, Weinstock GM, Murray BE. Characterization of fsr, a regulator controlling expression of gelatinase and serine protease in Enterococcus faecalis OG1RF. J Bacteriol 2001; 183:3372–3382. Available at: http://jb.asm.org/cgi/reprint/183/11/3372. Accessed June 26, 2008
25. Garsin DA, Sifri CD, Mylonakis E, Qin X, Singh KV, Murray BE, Calderwood SB, Ausubel FM. A simple model host for identifying gram-positive virulence factors. Proc Natl Acad Sci USA 2001; 98:10892–10897. Available at: http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=58570&blobtype=pdf. Accessed June 26, 2008
26. Di Rosa R, Creti R, Venditti M, D'Amelio R, Arciola CR, Montanaro L, Baldassarri L. Relationship between biofilm formation, the enterococcal surface protein (Esp) and gelatinase in clinical isolates of Enterococcus faecalis and Enterococcus faecium. FEMS Microbiol Lett 2006; 256:145–150
27. Hancock LE, Perego M. The Enterococcus faecalis fsr two-component system controls biofilm development through production of gelatinase. J Bacteriol 2004; 186:5629–5639. Available at: http://jb.asm.org/cgi/reprint/186/17/5629. Accessed June 26, 2008
28. Zeng J, Teng F, Murray BE. Gelatinase is important for translocation of Enterococcus faecalis across polarized human enterocyte-like T84 cells. Infect Immun 2005; 73:1606–1612. Available at: http://iai.asm.org/cgi/reprint/73/3/1606. Accessed June 26, 2008
29. Qin X, Singh KV, Weinstock GM, Murray BE. Effects of Enterococcus faecalis fsr genes on production of gelatinase and a serine protease and virulence. Infect Immun 2000; 68:2579–2586. Available at: http://iai.asm.org/cgi/reprint/68/5/2579. Accessed June 26, 2008
30. Pillai SK, Sakoulas G, Eliopoulos GM, Moellering RC Jr, Murray BE, Inouye RT. Effects of glucose on fsr-mediated biofilm formation in Enterococcus faecalis. J Infect Dis 2004; 190:967–970. Available at: http://www.journals.uchicago.edu/doi/pdf/10.1086/423139. Accessed June 26, 2008
31. Bourgogne A, Hilsenbeck SG, Dunny GM, Murray BE. Comparison of OG1RF and an isogenic fsrB deletion mutant by transcriptional analysis: the Fsr system of Enterococcus faecalis is more than the activator of gelatinase and serine protease. J Bacteriol 2006; 188:2875–2884. Available at: http://jb.asm.org/cgi/reprint/188/8/2875. Accessed June 26, 2008
32. Nakayama J, Cao Y, Horii T, Sakuda S, Nagasawa H. Chemical synthesis and biological activity of the gelatinase biosynthesis-activating pheromone of Enterococcus faecalis and its analogs. Biosci Biotechnol Biochem 2001; 65:2322–2325. Available at: http://www.jstage.jst.go.jp/article/bbb/65/10/2322/_pdf. Accessed June 26, 2008

First author:
Dr Takashi Suzuki

Schepens Eye Research Institute, Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts, USA

to the atypical growth of the neovascularization. While NO is known to derive vasodilatation and up-regulates regional basal blood flow,[4] it also activates angiogenic cell migration and proliferation-inducing factors, including fibroblast growth factor 2 and vascular endothelial growth factor.[5] Because retinal angiogenesis is ongoing in premature infants, NO might have contributed to the atypical neovascularization near the optic disc in our patient.

In animal models of oxygen-induced retinopathy, neovascularization induced by obliteration of the immature capillaries also develops from the optic disc and posterior retina.[6] Because AP-ROP develops in the posterior retinal area, including zone I, this suggests that immature capillaries may be widely present, and neovascularization arises from the retina near the optic disc. Capillary nonperfusion in vascularized retinas has been identified in eyes with threshold ROP.[7] Thus, there might be a much wider area of nonperfusion in the posterior retina in eyes with AP-ROP, which should be studied using fluorescein angiography.

Acknowledgments. This work was supported by grants for research on sensory disorders from the Ministry of Health, Labour and Welfare, Japan.

Key Words: aggressive posterior retinopathy of prematurity, fibrovascular proliferation, photocoagulation, regrowth, vitrectomy

Miina Hiraoka, Sachiko Nishina, Atsuko Nakagawa, Kentaro Matsuoka, and Noriyuki Azuma
Department of Ophthalmology, National Center for Child Health and Development, Tokyo, Japan

Received: December 11, 2007 / Accepted: April 23, 2008
Correspondence to: Noriyuki Azuma, Department of Ophthalmology, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
e-mail: azuma-n@nccbd.go.jp

DOI 10.1007/s10384-008-0557-3

References

1. Foos RY. Retinopathy of prematurity. Pathologic correlation of clinical stages. Retina 1987;7:260–276.
2. International Committee for the Classification of Retinopathy of Prematurity. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol 2005;123:991–999.
3. Azuma N, Ishikawa K, Hama Y, Hiraoka M, Suzuki Y, Nishina S. Early vitreous surgery for aggressive posterior retinopathy of prematurity. Am J Ophthalmol 2006;142:636–643.
4. Gross SS, Wolin MS. Nitric oxide: pathophysiological mechanisms. Annu Rev Physiol 1995;57:737–769.
5. Ziche M, Morbidelli L. Nitric oxide and angiogenesis. J Neurooncol 2000;50:139–148.
6. McLeod DS, D'Anna SA, Lutty GA. Clinical and histopathological features of canine oxygen-induced proliferative retinopathy. Invest Ophthalmol Vis Sci 1998;39:1918–1932.
7. Schulenburg WE, Tsanaktsidis G. Variations in the morphology of retinopathy of prematurity in extremely low birthweight infants. Br J Ophthalmol 2004;88:1500–1503.

Eyeball Luxation in *Bacillus cereus*-Induced Panophthalmitis Following a Double-Penetrating Ocular Injury

Since it was first reported in 1952, posttraumatic *Bacillus cereus* panophthalmitis has been known as a relatively rare but very serious progressive and devastating ocular infection usually leading to enucleation.[1] The clinical features of this ocular infection include severe pain, chemosis, proptosis, and retinal hemorrhage.[2,3] In this report, we present a case that initially manifested as marked proptosis, then rapidly worsened into eyeball luxation at the time of surgery. To the best of our knowledge, ours is the first report of this unusual manifestation, which can be associated with double-penetrating ocular injury.

Case Report

A 30-year-old Japanese man visited our clinic with decreased vision and ocular pain. He recalled that about 14 h earlier his left eye might have been struck by a foreign body when he was trying to tie his shoestring while a truck was passing by. Unaware of the injury, he did not seek medical attention until the next morning, when he woke up with severe ocular pain. The patient's vision was reduced to uncertainty of light perception, the lids were swollen and ecchymotic, and the conjunctiva was severely chemotic (Fig. 1A). On slit-lamp examination the cornea was diffusely edematous, and a 3.5-mm corneal laceration was visible close to the temporal limbus. A severe fibrinous iritis and hypopyon were present, and fundus details could not be visualized. Computed tomography revealed the presence of an intraocular foreign body in the posterior vitreous (Fig. 1B). The patient received immediate anterior chamber and intravitreal injection with vancomycin (1 mg/0.1 ml) and imipenem (2 mg/0.1 ml). Within 2 h after admission, the proptosis of his left eye had rapidly worsened into eyeball luxation and complete ophthalmoplegia (Fig. 1C). At the time of surgery, leukocytosis and malaise were remarkable. His temperature rose to 38.7°C, and the white cell count was 21.1×10^9 and the C-reactive protein level was 10.30 mg/dl. Septicemia was suspected, and ocular enucleation was performed. Surgical findings revealed a scleral laceration 10 mm behind the temporal limbus, and a metallic foreign body measuring 4×2.5 mm was found in the prolapsed vitreous (Fig. 1D). Enucleation alone could not reduce the swollen orbital tissues, and partial tarsorrhaphy was performed to close the eyelids.

Postoperatively the patient became afebrile within 48 h, the swollen orbital tissues resolved, and he recovered uneventfully. Pathological examination of the enucleated eyeball demonstrated that there was intensive neutrophilic infiltration around the perforated sclera, and the sclera was found to be entirely melted (Fig. 2A and B). In addition, a

Jpn J Ophthalmol
Vol 52: 407–425, 2008

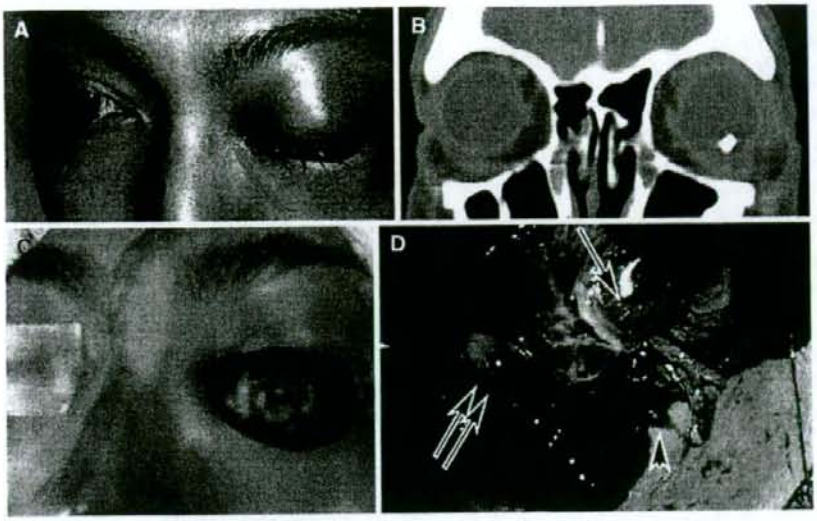

Figure 1. **A** The injured left eye was proptotic on the patient's visit. **B** Coronal view demonstrating a foreign body of high density in the left eye. **C** The proptosis progressed rapidly into eyeball luxation at the time of surgery. **D** Surgical view showing corneal wound (*arrow*) and posterior scleral laceration of double penetration (*double arrows*). A metallic foreign body was found in the prolapsed vitreal and uveal material at the scleral wound (*arrowhead*).

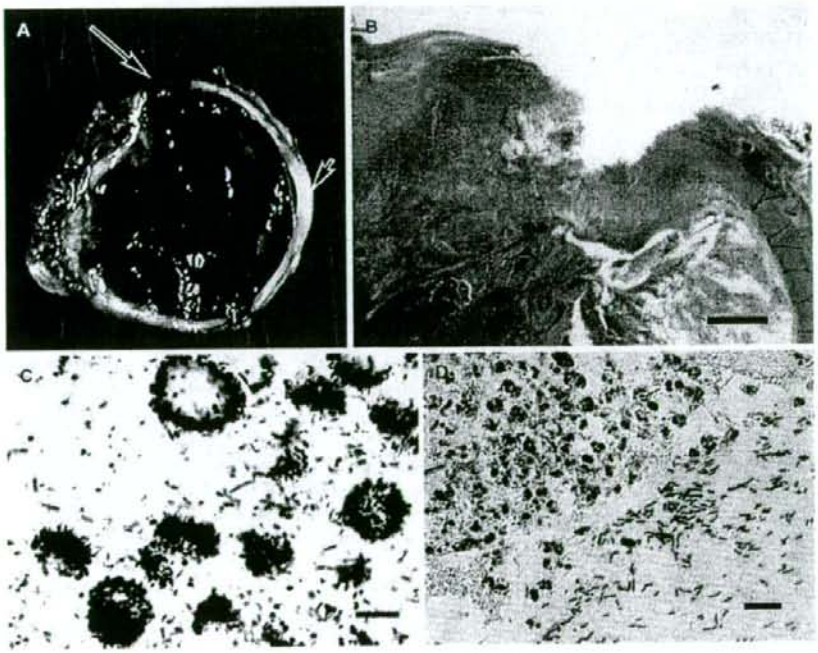

Figure 2. **A** Enucleated eyeball showing posterior scleral wound (*arrow*); corneal laceration is not seen in this section (*arrowhead*: cornea). **B** H&E staining demonstrating scleral melting with intensive neutrophilic infiltration close to the perforated sclera (bar = 1000 μm). **C** Vitreal samples inside the eyeball showing destroyed retinal and choroidal structures and abundant Gram positively stained rod-shaped *Bacillus cereus* (bar = 10 μm). **D** Orbital abscess outside the eyeball showing intensive infiltration of neutrophils and massive *Bacillus cereus* (bar = 10 μm).

purulent inflammation was noted both inside the eyeball with loss of retinal and choroidal structures (Fig. 2C) and outside the eyeball with orbital phlegmonous abscesses (Fig. 2D). Cultures of the orbital abscess and the vitreous sample both yielded the Gram-positive rod-shaped *Bacillus cereus*.

Comments

Bacillus cereus is well known for inducing endophthalmitis following traumatic injury, especially when a history of soil contamination involving a metallic foreign body is present.[5] Our patient was unusual in that the proptosis of his injured

eye progressed so rapidly that eyeball luxation presented at the time of surgery, precluding any possible therapies.

Besides the virulent nature of *Bacillus cereus*, other factors could have facilitated this eyeball luxation. First, a double-penetrating injury may have facilitated the infiltration of vitreal material into the retrobulbar tissues. The scleral laceration could have been caused biochemically by various bacterial proteases or mechanically by the foreign body itself. Second, exotoxins, such as phospholipase C, hemolysin BL, and proteases complicated by *Bacillus cereus* may have potentially inflicted direct tissue damage, swelling, and destruction of the orbital tissues;[4,5] hence, the whole eyeball may have been squeezed out of the lid, and, in a vicious circle, the eyeball luxation accelerated the necrosis of the ocular tissues. Third, the lens capsules were ruptured and phacoanaphylactic endophthalmitis may have been an additional factor promoting and worsening the inflammation.

This case emphasizes the severity of this devastating ocular infection. Rapid progress of proptosis warrants suspicion of double-penetrating injury and extremely urgent surgical intervention is crucial.

Acknowledgement. The authors are grateful to Dr. James M. Hill (LSU Eye Center), Dr. Micelle Callegan (Univ. of Oklahoma Health Science Center), Dr. Richard O'Callaghan and Dr. Mary E. Marquart (Univ. of Mississippi Medical Center) for their invaluable advice on this manuscript.

Key Words: *Bacillus cereus*, double-penetrating injury, luxation, panophthalmitis

Xiaodong Zheng[1,2], Toshio Kodama[1], and Yuichi Ohashi[2]
[1]Department of Ophthalmology, Matsuyama Red Cross Hospital, Ehime, Japan; [2]Department of Ophthalmology, Ehime University School of Medicine, Ehime, Japan

Received: January 21, 2008 / Accepted: April 23, 2008
Correspondence to: Xiaodong Zheng, Department of Ophthalmology, Matsuyama Red Cross Hospital, Matsuyama, Ehime 790-8524, Japan
e-mail: zhengdonjp@matsuyama.jrc.or.jp

DOI 10.1007/s10384-008-0567-1

References

1. Davenport R, Smith C. Panophthalmitis due to an organism of the *Bacillus cereus* group. Br J Ophthalmol 1952;36:389–392.
2. Drobniewski FA. *Bacillus cereus* and related species. Clin Microbiol Rev 1993;6:324–338.
3. David DB, Kirkby GR, Noble BA. Bacillus cereus endophthalmitis. Br J Ophthalmol 1994;78:577–580.
4. Beecher DJ, Olsen TW, Somers EB, et al. Evidence for contribution of tripartite hemolysin BL, phosphatidylcholine-preferring phospholipase C, and collagenase to virulence of *Bacillus cereus* endophthalmitis. Infect Immun 2000;68:5269–5276.
5. Callegan MC, Kane ST, Cochran DC, et al. Bacillus endophthalmitis: roles of bacterial toxins and motility during infection. Invest Opthalmol Vis Sci 2005;46:3233–3238.

A Case of Anterior Ischemic Optic Neuropathy Associated with Fabry's Disease

Fabry's disease is an X-linked recessive disorder associated with deficiencies of α-galactosidase A.[1] This deficiency promotes the deposition of the glycosphingolipid trihexosyl ceramide, particularly in vascular endothelial cells and smooth muscles. Progressive glycosphingolipid storage in the vascular endothelial cells is responsible for ischemic complications involving brain, kidneys, and heart.[2] The ocular complications are corneal opacities, cataracts, and conjunctival and retinal vessel tortuosity. We report a case of Fabry's disease in a male Japanese patient complicated by anterior ischemic optic neuropathy (AION).

Case Report

A 26-year-old man had experienced anhidrosis and skin lesions since childhood and was later diagnosed as having Fabry's disease. In 1995, he was referred to the Eye Clinic at Chiba University Hospital for detailed examinations of putative ocular complications. His best-corrected visual acuity was 1.0 OU. Mild opacities were seen in the cornea and lens, but no abnormalities were noted in the conjunctiva and retina.

Nine years later, the patient returned complaining of a sudden visual decrease in his right eye on the previous day. The visual acuity was hand motion OD and 1.0 OS. The direct light reaction of the pupil was sluggish, and the relative afferent pupillary defect was positive in the right eye. Fundus examination revealed pallor and swelling of the optic disc, dilatation and tortuosity of the retinal vessels, and retinal opacification, presumably along the course of the cilioretinal artery in the right eye (Fig. 1A). Fluorescein angiography revealed peripapillary choroidal nonperfusion and delayed filling of the temporal segment of the optic disc in the right eye (Fig. 1C). Retinal nonperfusion corresponding to the retinal opacification could be detected. The retinal vessels in the unaffected left eye were slightly dilated and tortuous (Fig. 1B). Goldmann kinetic perimetry revealed severe visual field loss with loss of fixation in the right eye (Fig. 2). During the physical examination, the patient had no complaint of headache, weight loss, or fever. He had no pain or swelling over the superficial temporal arteries. Laboratory examination showed an erythrocyte sedimentation rate of 25 mm in the first hour, C-reactive protein levels of 0.9 mg/dl, no leukocytosis, and negative antinuclear, antiphospholipid, and antineutrophil cytoplasmic antibodies. No abnormal brain lesions responsible for visual field defects were detected by magnetic resonance imaging.

On the basis of these observations and the results of the examinations, the patient was diagnosed as having nonarteritic AION. Because his parents desired steroid therapy,

Transcanalicular-Endonasal Semiconductor Diode Laser–Assisted Revision Surgery for Failed External Dacryocystorhinostomy

JUNJI NARIOKA AND YUICHI OHASHI

• PURPOSE: To report the results of transcanalicular-endonasal revision dacryocystorhinostomy (DCR) with a semiconductor diode laser in cases of failed external DCR.

• DESIGN: Prospective, nonrandomized, interventional case series.

• METHODS: Fifteen cases in 13 patients with failed external DCR underwent transcanalicular-endonasal DCR with a semiconductor diode laser. A functional successful outcome was defined as a patent nasolacrimal drainage system in nasolacrimal irrigation and a resolution of the symptomatic epiphora and/or mucoid discharge.

• RESULTS: The patients were followed for a mean postoperative period of 27.3 months (range, nine to 54 months). The mean duration of the surgery was 19.6 minutes. After the initial revision transcanalicular-endonasal DCR surgery, patency to irrigation was obtained in 12 cases (80%), and 15 cases (100%) after a second revision treatment. Three cases required repeated revision surgery, and three other cases were considered to be functional failures in spite of a patent lacrimal system after the final revision surgery. The overall functional success rate was 80% (12 cases) at the final examination (mean, 27.3 months after surgery), and there were no intraoperative and postoperative complications. The presence of a canalicular obstruction or granulation tissue was not significantly related to the success rates of the revision surgery. The length of time between the primary and revision surgery, gender, age, the duration of the first revision surgery, and the timing of stent removal were also not significantly related to the failed cases.

• CONCLUSION: Transcanalicular-endonasal DCR is a minimally invasive procedure and is recommended for patients as an alternative procedure for failed external DCR. (Am J Ophthalmol 2008;146:60–68. © 2008 by Elsevier Inc. All rights reserved.)

Accepted for publication Feb 28, 2008.

From the Department of Ophthalmology, Ehime University School of Medicine, Ehime, Japan.

Inquiries to Junji Narioka, Department of Ophthalmology, Ehime University School of Medicine, Shitsukawa, Toon, Ehime 791-0295, Japan; e-mail: nariokaj@m2.dion.ne.jp

EXTERNAL DACRYOCYSTORHINOSTOMY (DCR) IS A standard surgical procedure used to treat nasolacrimal duct obstructions (NLDOs),[1,2] although there are several alternative techniques such as endonasal DCR[3,4] and endocanalicular laser DCR.[5–7] The success rate of external DCR is higher than 90%,[1,3,4] but some cases require revision surgery with either an external, endonasal, or transcanalicular approach. A repeat external DCR is usually performed for the revision surgery with a reopening of the skin incision,[8–12] but some authors recommend an endonasal or transcanalicular approach if an adequate bony window had been achieved at the primary surgery. However, the success rates of these procedures range from 46% to 100% for the transcanalicular approach[4,13,14] and 0% to 100% for the endonasal approach.[11,13,15–20]

Although the rhinostomy created by the transcanalicular approach is smaller than that with the endonasal and external approaches,[21] it allows a direct application of the laser energy to the obstructed site.[22] The endonasal approach can create a larger rhinostomy,[8] but it is more difficult to identify the obstructed site correctly, and this procedure can occasionally cause serious collateral damage of orbital structures, resulting in exposed orbital fat tissue.[17,18,22–24] Therefore, we hypothesized that a combination of these two approaches will lead to higher success rates for revision surgery. We combined transcanalicular and endonasal approaches for revision DCR (transcanalicular-endonasal DCR).

A portable semiconductor diode laser system has been recently used in lacrimal and oculoplastic surgeries.[5,6,25] It has been used not only for the primary external DCR[25] but also for primary transcanalicular DCR.[5,6] However, the efficacy of the use of the diode laser for revision DCR has not been studied. Thus, the purpose of this study is to report the results of transcanalicular-endonasal DCR using a semiconductor diode laser for cases of failed external DCR.

METHODS

THIS WAS A PROSPECTIVE, NONRANDOMIZED, INTERVENTIONAL case study that was conducted on 13 consecutive adult patients between June 19, 2002 and December 4,

0002-9394/08/$34.00
doi:10.1016/j.ajo.2008.02.028

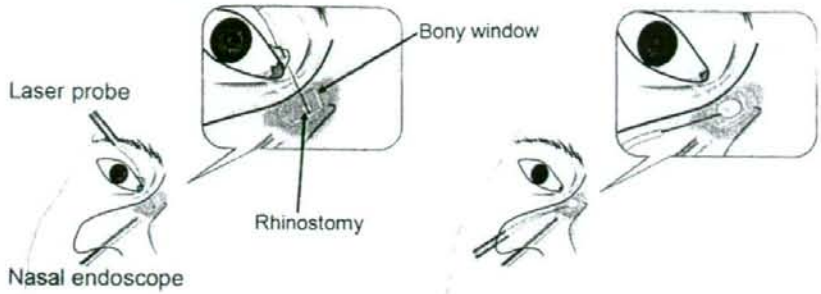

FIGURE 1. The drawings show our transcanalicular-endonasal approach for reopening a rhinostomy (Left) and endonasal approach for enlargement of the rhinostomy (Right). (Left) First step. The laser probe is inserted though the upper canaliculus and pushed to the obstruction. Application of the laser light reopens the rhinostomy. If the laser probe cannot be inserted to reopen the rhinostomy from the lower canaliculus, additional laser was applied though the lower canaliculus. (Right) Second step. The endonasal laser was used to enlarge the rhinostomy.

2006. All patients had undergone one unsuccessful external DCR by a single surgeon (J.N.) at one of three surgical centers (Matsuyama Red Cross Hospital, Saijo City Shuso Hospital, or Ehime University Hospital) before the transcanalicular-endonasal DCR. An informed consent was obtained from all subjects after the nature and possible consequences of the surgery were explained.

The indications for the primary DCR were epiphora and/or mucoid discharge caused by an NLDO, acute or chronic dacryocystitis, obstruction of the common canaliculus, and/or canalicular obstruction. Transcanalicular-endonasal DCR was performed in all cases of failed external DCR. After careful nasolacrimal probing and nasal endoscopic examination, a diagnosis of a failed DCR (that is, occlusion of the rhinostomy and/or canaliculus) was made if a recurrence of epiphora and/or mucoid discharge were detected, an elevation of the tear meniscus height was present, or a failure of nasolacrimal irrigation was evident. The canalicular obstruction was easily identified by the reflux of fluid through the same punctum during nasolacrimal irrigation.[26] If the reflux was from the opposite canaliculus, an obstruction of either the lateral aspect of the common canaliculus, the canalicular-lacrimal sac junction, or a lacrimal sac–nasal mucosa anastomosis was most likely present.[12,26]

• TRANSCANALICULAR-ENDONASAL REVISION DACRYOCYSTORHINOSTOMY SURGICAL TECHNIQUE: All surgeries were performed under local anesthesia by one surgeon (J.N.). Our transcanalicular-endonasal revision DCR was performed as follows. In the first step, the rhinostomy was reopened at the obstructed site under direct endoscopic view by a transcanalicular approach. In the second step, the rhinostomy was enlarged by an endonasal approach (Figure 1). A 4-mm-diameter rigid nasal endoscope with 30-degree side view (EN 6530, Machida Endoscope Co, Ltd, Tokyo, Japan) was used to examine the rhinostomy site and to control the tip of the laser probe under direct view throughout the procedure. The symptomatic eye was anesthetized topically with 0.4% oxybuprocain hydrochloride. A nasal spray containing 4% lidocaine with 1:100,000 solution of epinephrine was used to anesthetize the nasal cavity. The anterior ethmoidal nerve was blocked with 2% lidocaine, and a 2% lidocaine with epinephrine 1:100,000 solution was then injected into the nasal mucosa at the intended rhinostomy site and lateral nasal wall just superior and anterior of the middle turbinate under endoscopic view. A nasal pack soaked in 4% lidocaine with epinephrine 1:100,000 solution was inserted into the middle nasal meatus.

The settings on the semiconductor diode laser were 3 watt (W) and 0.5 second (Osada Medical Co, Ltd, Tokyo, Japan), and the laser energy was delivered by a 630-μm semi-rigid quartz fiber that tapered to a 600-μm tip, using the contact mode. In the first step, the lacrimal probe (0.64 mm in diameter) was inserted into the upper canaliculus and directed medially to search for the obstructed site. The site was identified by the pearl-like colored scar tissue covering the nasal mucosa (Figure 2, Top left). The lacrimal probe was removed and the quartz fiber tip was carefully inserted through the upper canaliculus into the nasal cavity. The laser energy was applied until the probe tip was recognized by nasal endoscopy. The appearance of the probe indicated that a small rhinostomy had been recreated.

Next, the laser probe was inserted from the lower canaliculus, and if the probe passed into the nasal cavity smoothly through the reopened rhinostomy, the probe was removed. But if the probe could not be inserted through the scar tissue, additional laser energy was applied through

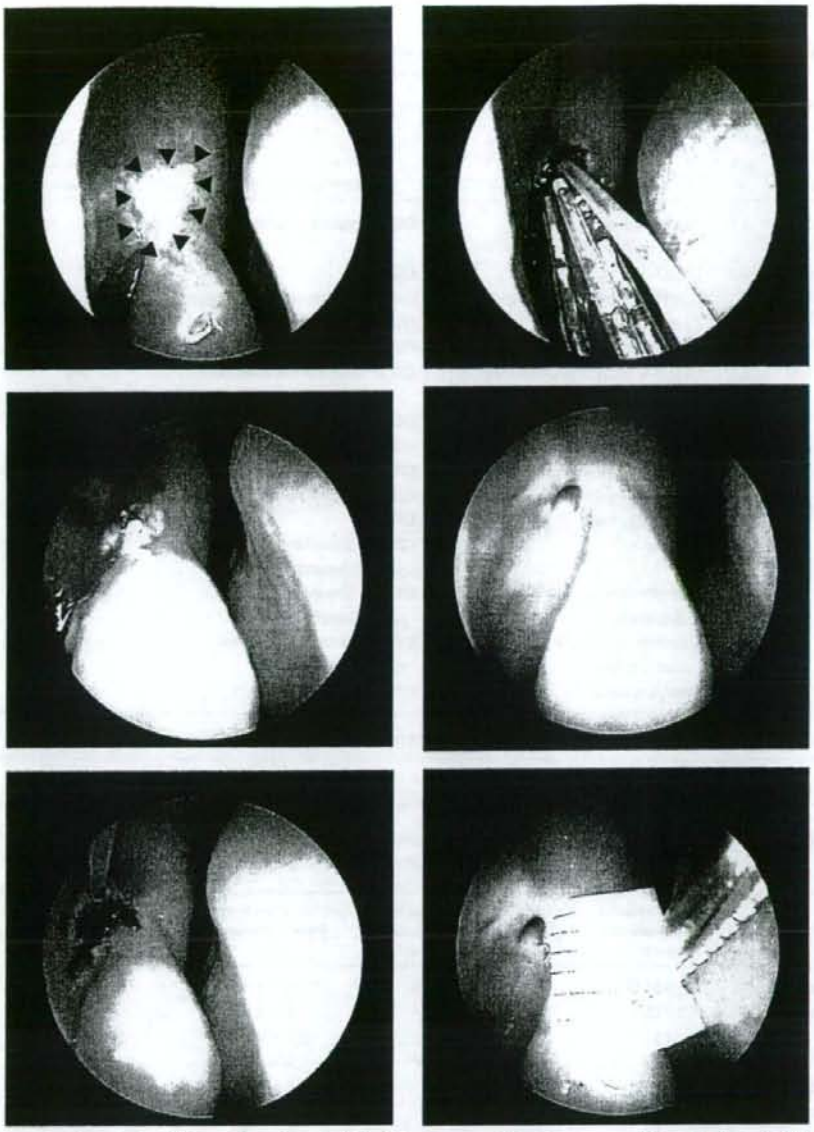

FIGURE 2. Endoscopic view of the right nasal cavity of a failed dacryocystorhinostomy case (Case 2). (Top left) Scar tissue closing the rhinostomy appears pearl-like in color (arrowhead). (Middle left) Transcanalicular approach for reopening of the rhinostomy. Glow of laser from the laser fiber optic. A small rhinostomy was created. (Bottom left) Endonasal approach for enlargement of the rhinostomy. The pearl-colored scar tissue around the initial reopened rhinostomy was carefully and completely dissected. (Top right) Silicone stents have been drawn through the rhinostomy. (Middle right) Endoscopic view of rhinostomy at six months after revision surgery. The rhinostomy moves with blinking. (Bottom right) Endoscopic view of rhinostomy at six months after revision surgery. A scaled Schirmer paper strip was used to measure the vertical size of the rhinostomy and the size was about 2 mm.

the lower canaliculus until the probe was recognized from the nasal cavity. Then we confirmed that the laser probe could be inserted smoothly into the nasal cavity through the reopened rhinostomy from both upper and lower canaliculi (Figure 2, Middle left).

In the second step, the endonasal approach was used to enlarge the rhinostomy and the rhinostomy was enlarged until an adequate-size rhinostomy was created. The pearl-colored scar tissue around the initially reopened rhinostomy was completely dissected, but the laser was not