PCR-Based Evidence of Bacterial Involvement in Eyes with Suspected Intraocular Infection

Narciss Okhravi,[1] *Peter Adamson,*[1] *Nora Carroll,*[1] *Anthony Dunlop,*[1] *Melville M. Matheson,*[2] *Hamish M. A. Towler,*[3] *and Susan Lightman*[1]

Purpose. To assess the usefulness of polymerase chain reaction (PCR) in detection of bacteria in ocular samples.

Methods. Thirty-seven samples (aqueous and vitreous) were collected from 25 eyes showing typical symptoms and clinical signs of bacterial endophthalmitis. Ocular samples were also collected from 38 eyes that underwent routine surgery and from 15 eyes with intraocular inflammation due to nonbacterial causes. Panbacterial PCR was performed with a nested pair of 16S rRNA gene primers. Subsequent bacterial identification was completed for 18 paired samples (nine eyes) using restriction fragment length polymorphism (RFLP) and DNA sequencing.

Results. A 100% concordance was obtained between PCR and culture-positive samples. A PCR product was amplified from all 37 intraocular samples from eyes with suspected infection, whereas only 15 of 22 vitreous samples and 5 of 15 aqueous samples were culture positive. Culture-negative PCR-positive samples contained a preponderance of gram-negative bacterial sequences. Cloning and DNA analysis revealed 30 DNA sequences and included eight bacterial 16S rDNA, which currently remain unidentifiable. The presence of bacterial DNA was associated with an inflammatory response suggestive of infection and not colonization. All 15 samples from inflamed eyes with diverse uveitis diagnoses were PCR negative. The false-positive rate, due to contamination during sampling, was 5%.

Conclusions. Bacterial DNA was detected in all patients with typical clinical signs of endophthalmitis. Gram-negative organisms seem to play a much more important role in the pathogenesis of this disease than previously thought. PCR-based techniques have great value in the confirmation of the diagnosis of bacterial endophthalmitis especially in culture-negative eyes. (*Invest Ophthalmol Vis Sci.* 2000;41:3474–3479)

Ideally, infective endophthalmitis would be verified by culture in all cases. Currently, confirmation of the diagnosis of bacterial endophthalmitis is dependent on microbiologic isolation of organisms, but many cultures are negative (21%–63% in the published literature).[1–6] Several reasons have been postulated for this, including small sample size, sequestration of bacteria on solid surfaces (e.g., on intraocular lens, lens remnants, and lens capsule), prior use of antibiotics, and the fastidious nature of some of the organisms that cause intraocular infection.[1,7,8] These observations suggest that with a more sensitive and specific detection strategy, a microbiologic diagnosis may be obtained in more cases.[9]A sensitive and rapid diagnostic test would not only allow confident verification of the diagnosis (noninfective inflammation vs. infection) but also allow early commencement of specific and appropriate treatment.

Several investigators have reported the use of panbacterial polymerase chain reaction (PCR) in the analysis of ocular samples from clinical cases with suspected intraocular infection.[9–12] The main drawback of studies, using nested PCR for the detection of bacteria using panbacterial 16S rDNA gene primers, has been the coamplification of small amounts of the bacterial DNA that contaminate the purest commercial preparations of *Taq* DNA polymerase.[13,14] Several methods have been used to eradicate this, but none were found to be 100% effective in removing contaminating templates.[14–17] Our previous studies have demonstrated that the efficient elimination of this DNA from participation in the amplification reaction is reliably achieved by pretreatment of the polymerase with a restriction endonuclease, before first-round PCR amplification.[9,18–20]

Methods

The purpose of this study was to determine the ability of PCR to detect bacteria in samples collected from patients with presumed bacterial endophthalmitis. This study adhered to guidelines in the Declaration of Helsinki for research involving

From the Departments of [1]Clinical Ophthalmology and [2]Pathology, The Institute of Ophthalmology and University College London; and the [3]Department of Ophthalmology, Whipps Cross Hospital, Leytonstone, London, United Kingdom.

Supported by a Wellcome Vision Research Fellowship 045203 (NO), and locally organized research funds from Moorfields Eye Hospital Grants 221 and 271 (NO); and by Oclyx, Ltd., United Kingdom (NC).

Submitted for publication February 14, 2000; revised March 23, May 15, and June 2, 2000; accepted June 6, 2000.

Commercial relationships policy: C5 (NC); N (NO, PA, AD, MMM, HMAT, SL).

Corresponding author: Narciss Okhravi, Department of Clinical Ophthalmology, The Institute of Ophthalmology and University College London, Bath Street, London EC1V 9EL, UK.
nokhravi@hgmp.mrc.ac.uk

Investigative Ophthalmology & Visual Science, October 2000, Vol. 41, No. 11

human subjects. Thirty-seven ocular samples (aqueous and vitreous) were collected from 25 eyes of 25 patients with typical clinical symptoms and signs of endophthalmitis (clinical samples). Uninfected ocular samples were also collected from 38 eyes that underwent routine surgery (control samples) and 15 eyes with ocular inflammation secondary to nonbacterial causes (inflamed samples). For nine eyes for which data and paired samples were available, PCR was followed by digestion with restriction endonucleases, DNA sequencing, and/or cloning to identify the bacteria present.

Preoperative Cleaning Procedure

All eyes were sampled in an identical manner. When the patients arrived in the operating theater, 1 drop of 5% aqueous povidone iodine solution was instilled in the conjunctival sac after topical anesthesia. The eye and surrounding tissues were prepared for surgery, and once the lid speculum was in place 1 drop of 5% aqueous povidone iodine was instilled into the conjunctival sac for 5 minutes. The conjunctival sac was subsequently washed with 20 ml of sterile saline before sampling.

Samples from Clinical Cases of Endophthalmitis

Intraocular sampling was performed as previously described.[1,9] The sample for PCR (5–50 μl) was separated from that to be sent for microbiologic assessment at the time of sampling and handled under aseptic conditions. All samples were collected into microcentrifuge tubes and stored at −70°C until analysis.

Microbiology

Intraocular samples were examined by gram stain, and immediately cultured as previously described.[1] After isolation by culture, a biochemical identification system (API Analytab, New York, New York) was used. Organisms were subsequently stored on beads (Mast Diagnostics, Bootle, UK) at −70°C.

Aqueous and Vitreous Samples from Cases with No Evidence of Intraocular Infection

Samples were collected from patients who underwent routine cataract (aqueous samples only) and vitreoretinal surgery (vitreous samples only) to test the contamination rate at the time of sampling. Collection of normal and inflamed intraocular samples were also undertaken during planned surgical procedures in patients with no evidence of intraocular infection or inflammation or medical history of uveitis and/or diabetes mellitus (normal vitreous) or from patients with other causes of posterior segment inflammation not associated with bacterial infection (inflamed vitreous). Samples were aliquoted in a sterile manner and stored at −20°C.

DNA Extraction

For specificity testing, genomic bacterial DNA was extracted as previously described.[9] For direct PCR, bacteria were suspended in the distilled water component of the PCR mix and used directly in PCR reactions.

Protocol for Analysis of Clinical Samples

Five microliters of the ocular sample was added directly to the PCR reaction. If the PCR reaction was found to be negative, inhibition of the PCR reaction by the ocular sample was considered. The identity of PCR inhibitors in ocular samples is currently unknown. The only group that has addressed this issue to date studied the effect of vitreous from uninflamed eyes[21] (called normal vitreous in this article) for which no inhibitory effect has been noted by us.[9] Results in the analysis of clinical samples from patients with presumed bacterial endophthalmitis demonstrated variable inhibition of the PCR reaction. Fortunately, in all cases the dilution of ocular samples was sufficient to remove the effect of the inhibitors without sacrificing sensitivity. Therefore, if the PCR was found to be negative, the vitreous sample was diluted with sterile water to 50% (1:2), 20% (1:5), 10% (1:10), and 5% (1:20) and three experiments were run in parallel. The first was direct PCR of the diluted vitreous (using 5 μl of the sample at the required dilution), and the second and third involved spiking the dilute vitreous with approximately 600 and 1–5 live organisms, respectively, followed by direct PCR.

Experimental Conditions

Oligonucleotide primers, PCR reagents, cycling conditions, and restriction fragment length polymorphism (RFLP) analysis were identical with that previously described.[9] Before first-round PCR amplification, the polymerase (Replitherm *Taq*; Cambio, Cambridge, UK) was pretreated with *Alu*I restriction endonuclease (ratio of 3:1 units of *Taq*: *Alu*I; Promega, Southampton, UK) as reported previously.[9,18]

DNA Sequencing

DNA sequencing was performed on all PCR-positive samples. PCR fragments were directly cycle sequenced in both directions using an automated DNA sequencer (model 377 version 2.1.1, ABI Prism; ABI, Foster City, CA). Sequences were analyzed both manually and using database and software programs available through the HGMP computer center (http://www.hgmp.mrc.ac.uk), the National Collection of Biotechnical Information (NCBI) and the Ribosomal Database Project (RDP; http://www.cme.msu.edu/RDP/html/index.html) Web sites.

Cloning of PCR Products

Amplified DNA from PCR reactions were directly cloned into pCR II (Invitrogen, Leek, The Netherlands) to aid sequencing and to establish the identity of individual PCR products in samples with mixed populations of 16S rDNA that yielded combined patterns after RFLP analysis.

Protein Assay

Because high protein levels are expected in inflamed eyes and have been proposed to be a cause of PCR inhibition by intraocular samples,[21] a protein assay was undertaken. Each sample was diluted 1:4 and 1:200, assayed for protein using BCA reagent (Pierce, Chester, UK), and compared with bovine serum albumin standards (0–0.6 mg/ml).

Statistical Analysis

All statistical analysis was undertaken by computer (SPSS for Windows, ver. 6.0; SPSS, Chicago, IL).

Results

PCR Amplification of Clinical Samples

Ninety ocular samples from 78 eyes were tested using nested bacterial PCR. Thirty-seven samples (22 vitreous and 15 aque-

Table 1. Comparison of Culture and PCR Results for All Samples Tested

	Culture Positive		Culture Negative		
	Bacterial PCR Positive	Bacterial PCR Negative	Bacterial PCR Positive	Bacterial PCR Negative	Total
Clinical samples	20 (15 vitreous, 5 aqueous)	0	17 (7 vitreous, 10 aqueous)	0	37 samples from 25 eyes
Inflamed samples	0	0	0	15	15 samples from 15 eyes
Control samples	0	0	2	36	38 samples from 38 eyes
Total	20	0	19	51	90 samples from 78 eyes

Clinical samples were those collected from eyes with suspected intraocular infection and treated as bacterial endophthalmitis. Inflamed samples were collected from 15 eyes with ocular inflammation secondary to nonbacterial causes. Control samples were uninfected ocular samples collected from 38 eyes that underwent routine intraocular surgery. One of 19 control aqueous and 1 of 19 control vitreous samples were PCR positive but culture negative. These two patients did not show signs of postoperative inflammation up to 7 months after surgery, suggesting contamination at the time of sample collection.

ous) were collected from 25 eyes with unambiguous clinical signs of intraocular infection. Results of PCR analysis appear in Table 1. Of the vitreous and aqueous samples 15 (68%) of 22 and 5 (33%) of 15 samples were culture positive, respectively. PCR, however, detected the presence of bacterial DNA in 100% of samples from eyes with clinically suspected presence of infection. In only three eyes were both the aqueous and vitreous from the same eye culture positive.

PCR Amplification of 16S rDNA Genes from Control Ocular Samples

The sampling contamination rate using this preoperative cleaning procedure was 5% for both aqueous and vitreous sampling (Table 1). One each of 19 aqueous and 19 vitreous samples was PCR positive in the presence of adequate PCR reaction controls. None of the patients tested showed any clinical signs of infection or inflammation up to 7 months after surgery.

PCR Amplification of 16S rDNA Genes from Inflamed Ocular Samples

Fifteen intraocular samples from 15 eyes with diverse uveitis diagnoses were analyzed and repeatedly were negative for the presence of bacterial DNA using this nested PCR technique (Table 1).

Patient Data and Analysis of 18 Paired Clinical Samples from Nine Eyes

Eighteen paired samples (aqueous and vitreous) collected from nine patients with typical signs of bacterial endophthalmitis were analyzed in much greater detail.

Visual acuity at the occurrence of initial examination varied from hand movements to no perception of light. Endophthalmitis developed in two patients within 24 hours of cataract surgery (patients 2 and 9), and a further four in the first 5 days postoperatively (patients 3, 4, 6, and 7). Disease developed in one patient 20 days after glaucoma surgery (patient 8), in another 7 days after systemic symptoms secondary to gram-negative septicemia (patient 1), and in another secondary to keratitis (patient 5). The average age at occurrence of disease was 63 years (range, 39–88 years), and five of the nine patients were men.

An infective organism was identified by culture in 5 (55%) of 9 eyes. Culture-positive intraocular samples included 5 (55%) of 9 vitreous samples and 1 (9%) of 9 aqueous samples. The most commonly cultured organisms were coagulase-negative staphylococci (Table 2).

All intraocular samples from patients with presumed bacterial endophthalmitis were found to be PCR positive (Table 2). The level of PCR inhibition was found to be variable, with aqueous samples requiring dilution more often than vitreous samples. Dilution of the sample was required in the analysis of eight intraocular samples (five aqueous and three vitreous). Fifty-five percent of samples (10/18) did not require dilution and therefore required only one PCR run (6/9 undiluted vitreous and 4/9 undiluted aqueous). Thirty percent required two runs, and in 10%, three or four runs were necessary to obtain a result. In six of the nine patients, one or both intraocular samples had to be diluted.

The results from culture-positive samples demonstrated 100% concordance with PCR-RFLP sequencing results in all (5/5) vitreous but not in the one culture-positive aqueous sample (Table 2, sample 6). Four aqueous (samples 6, 8, 14, and 16) and three vitreous (samples 3, 11, and 13) samples were cloned because some ambiguity existed in the identity of the pathogen (Table 2, column 8). Nineteen cloned sequences were obtained from these seven ocular samples from six patients. These included 10 16S rDNA sequences from proteobacteria, 1 *Propionibacterium acnes* rDNA sequence, and 8 unidentifiable bacterial 16S rDNA sequences.

Protein Assay

A protein assay was performed on 17 of 18 paired samples. In addition, two samples from patients with culture-verified bacterial endophthalmitis collected at a later date, seven control samples, and two inflamed vitreous samples were also assayed. Results indicated that in the samples from patients with presumed bacterial endophthalmitis, protein levels varied from 2.5 to 130 mg/ml. Analysis of uninflamed PCR-negative vitreous and aqueous samples yielded results in the range of 0 to 0.8 mg/ml ($n = 7$). Inflamed PCR-negative vitreous samples yielded a protein assay measurement of 3.0 mg/ml ($n = 2$). Comparison between control PCR-negative ocular samples ($n = 7$; mean, 0.34 mg/ml), and PCR-positive samples from patients with bacterial endophthalmitis ($n = 19$; mean, 15.9 mg/ml) indicated a difference that was statistically significant (independent samples *t*-test assuming unequal variance, $P = 0.03$).

TABLE 2. RFLP, DNA Sequencing, and Cloning Results for Intraocular Samples from Patients with Presumed Bacterial Endophthalmitis

Sample Number	Patient Number	Sample	Dilution (%)*	PCR	RFLP	Sequencing	Proceed to Clone	Identification of Bacterial rDNA Isolated	Culture Results
1	1	Vit	None	+	EC/SM†	*Escherichia coli*		*E. coli*	*E. coli*
2	1	Aq	50	+	CNStaph‡	CNStaph		CNStaph	NG§
3	2	PM¶-Vit	10	+	Mixed pattern of infection (?)	Poor sequence quality	√	Two alpha proteobacteria: *Afipia* spp, *Methylobacterium* spp	NG
4	2	Aq	50	+	*Bacillus cereus*	*B. cereus*		*B. cereus*	NG
5	3	Vit	None	+	CNStaph	CNStaph		CNStaph	CNStaph
6	3	Aq	20	+	CNStaph (?)	Poor sequence quality	√	Three sequences: Gamma proteobacterium/ *Comamonas* spp, unidentified bacterial rDNA (2 species)	CNStaph
7	4	Vit	None	+	CNStaph	CNStaph		CNStaph	CNStaph
8	4	PM-Aq	None	+	Mixed pattern of infection (?)	Poor sequence quality	√	Unidentified bacterial rDNA (2 species)	NG
9	5	Vit	None	+	*Pseudomonas aeruginosa*	*Pseudomonas* spp		*Pseudomonas* spp	*Pseudomonas* spp
10	5	Aq	None	+	*P. aeruginosa*	*Pseudomonas* spp		*Pseudomonas* spp	NG
11	6	Vit	50	+	EC/SM†	Poor sequence quality	√	6 sequences: *Proteus* spp, *Propionibacterium acnes,* alpha proteobacteria: 4 sequences	NG
12	6	Aq	50	+	Not determined#	*Aeromonas* spp		*Aeromonas* spp	NG
13	7	Vit	50	+	*Pseudomonas aeruginosa*	*Pseudomonas* spp but poor sequence quality	√	*Pseudomonas* spp	NG
14	7	Aq	None	+	*Proteus mirabilis*	*Staphylococcus epidermidis*	√	Unidentified bacterial rDNA: 3 sequences	NG
15	8	Vit	None	+	*Streptococcus faecalis*	*S. faecalis*		*S. faecalis*	NG
16	8	Aq	50	+	CNStaph	*Pseudomonas* spp	√	*Pseudomonas* spp unidentified bacterial 16S rDNA: 1 sequence	NG
17	9	Vit	None	+	*S. faecalis*	*S. faecalis*		*S. faecalis*	β-hemolytic streptococci group D$^{\alpha}$
18	9	Aq	None	+	*S. aureus*	*S. faecalis*		*S. faecalis*	Streptococci seen on smear but NG

* Sample dilution required before nested PCR amplification was no longer inhibited.
† EC/SM, the RFLP pattern obtained by *Escherichia coli* and *Serratia marcescens* is identical.
‡ CNStaph is coagulase negative staphylococci.
§ NG, no growth by culture.
¶ PM, small sample volume meant none was available for PCR prior to processing in the microbiology department.
# The RFLP pattern did not conform to any known standard patterns. α, β-hemolytic on blood agar; Vit, vitreous; Aq, aqueous.

For 17 samples it was possible to determine an association with either gram-positive or gram-negative bacteria (both PCR and culture results agreed). The mean protein level was found to be higher for samples associated with gram-negative organisms (n = 8; mean, 27.4 mg/ml) than gram-positive (n = 9; mean, 7.9 mg/ml), but this finding failed to reach statistical significance. Of note, for all samples that contained gram-negative sequences indicated by PCR, the protein levels in culture-positive samples were very similar to those in culture-negative samples. The same was true for all samples that contained gram-positive sequences as indicated by PCR.

For seven of eight eyes with bacterial endophthalmitis for which paired results were available, the protein level in the aqueous sample was found to be on average 57.6% higher than in the vitreous (range, 0%–166%). The mean protein levels were higher in the presence of hypopyon-fibrin but, due to the very small sample without these clinical findings, the results were not amenable to valid statistical analysis. No correlation was found between protein levels and culture or PCR result (positive or negative) of an undiluted sample. However, the mean protein level was higher in those patients who had a poor visual outcome of perception of light or worse at 6-month follow-up (n = 17; mean protein level for those with vision of perception of light [POL] or worse at 6 months, 36.6 mg/ml [n = 6] versus mean protein in samples from eyes with final visual acuity better than POL, 6.6 mg/ml [n = 11]; independent samples t-test assuming equal variance P = 0.05).

DISCUSSION

In this study we applied molecular biologic techniques to the detection and identification of bacteria in ocular samples. Bacterial DNA was successfully detected using PCR and identified using RFLP, DNA sequencing, and cloning techniques. This study has demonstrated that by using PCR-based techniques, bacterial DNA can be found in 100% of samples from patients with typical clinical signs of bacterial endophthalmitis, whereas routine microbiologic analysis yielded positive results in only 68% of eyes. Vitreous was shown to be the sample of choice for both PCR and microbiologic analysis. Although PCR techniques cannot recover organisms for subsequent analysis (e.g., antibiotic testing), the molecular diagnosis of antibiotic resistance is increasingly becoming a reality.[22] Also, in the

analysis of culture-negative samples it is only by the use of PCR that a microbiologic diagnosis can be obtained.

In our study of ocular samples, the presence of infection was associated with a higher sample protein level suggestive of a reactive immune response. The higher protein levels in samples from eyes with gram-negative infection reflected the greater inflammatory response triggered by these organisms and the greater degree of ocular inflammation seen clinically in these patients. The higher protein levels in infected eyes and the similarity of protein levels between culture-positive and culture-negative samples containing similar bacterial sequences suggests that the bacterial sequences identified by PCR are significant markers of infection and not indicators of colonization. The protein assay was not only useful in assessing the relevance of detected bacterial sequences but also demonstrated prognostic significance for visual outcome. Similar results have reported in the study by Druel et al.,[23] in which cerebrospinal fluid (CSF) obtained from all patients after craniotomy, who showed clinical signs of meningitis, was compared with CSF from patients who underwent craniotomy and had no clinical evidence of infection. PCR confirmed the clinical suspicion, and results indicated that the inflammatory markers in the CSF were similar in all PCR-positive patients (whether culture positive or negative) and greater than for all PCR-negative patients, suggesting a true correlation with clinical disease.[23]

Infection with multiple organisms is considered a rare finding in postsurgical endophthalmitis. Very few reported cases of infection with multiple organisms exist, with most publications reporting none. However, in a few publications, mixed bacterial infections have been reported with an incidence of 54% ($n = 13$),[24] 29% ($n = 47$),[25] 19% ($n = 36$),[26] and 3% ($n = 78$)[27] of culture-positive patients. Multiple bacterial 16S rRNA sequences have also been PCR amplified from individual samples collected from the joints of patients with arthritis,[28] from CSF,[23] from prostatic biopsy specimens,[29] and from the blood of patients with suspected septicemia.[30,31] Dickey et al.[32] have also reported 13 of 30 patients with positive cultures at the end of routine cataract surgery; three of the cultures grew multiple organisms. In this study, samples that yielded poor sequence data and mixed RFLP patterns were likely to contain mixtures of organisms and were likely to be from the anterior chamber of the eye. Multiple organisms were present in 6 (66%) of 9 eyes tested, only one of which was culture positive and only for one organism.

The percentage of false-positive results (i.e., cultures that were PCR-positive because of contamination at the time of sampling) was 5%. The collection protocol for PCR samples, however, was much stricter than that routinely used for collection of samples for microbiologic analysis. To the best of our knowledge, however, the number of samples that indicate contamination by culture is not published. Often, the growth of just a few colonies in vitro suggests contamination and not infection. Recently, however, several investigators have reported quantitative PCR data addressing this issue more closely. Quantitative PCR analysis of CSF samples from patients with suspected bacterial meningitis, for example, has demonstrated that the number of bacteria in culture-negative, PCR-positive samples is smaller than the number of bacteria in culture-positive, PCR-positive samples.[23] These samples were taken from patients with classic symptoms and signs of meningitis, and, especially important, with CSF laboratory test results indicative of infection (e.g., reduced CSF glucose). The results suggest that infected samples containing fewer bacteria are more likely to be culture negative. The number of organisms detected by PCR are far greater than that witnessed in our clinical microbiology laboratory for culture-positive samples, and although culture-negative samples contain fewer bacteria, these still numbered 1000 colony-forming units/ml.

Comparison of the inhibition caused by ocular samples and that caused by samples from other body sites has not been productive, because much larger sample volumes (200 to 500 μl) have been used in work reported on synovial fluid and CSF, and therefore concentration of the DNA before PCR amplification is necessary, a process that removes all PCR inhibitors.[30,31] Fortunately, in all cases, the effects of inhibitors were eliminated by dilution of the ocular sample. In this study, 45% of samples required some dilution, but given the high sensitivity of this approach, routine dilution of all samples should be considered, thereby not only reducing the overall number of runs required but also allowing sufficient sample for retesting should it be required.

In the present study, six of seven samples that were cloned revealed multiple sequences, five of which contained sequences that were unidentifiable using the sequence data currently available ($n = 8$ DNA sequences, five eyes). For the eight unidentifiable sequences, the possibility of chimeric amplification products was considered and excluded using the chimera-check program available through the RDP Web site and using the NIX program available through the HGMP Web site.[33] Because the sequence of the 16S rDNA amplified does not include the entire gene sequence, no attempt was made to assign unidentifiable sequences to phylogenetic trees.[34]

Analysis of cloned sequences from culture-negative samples has revealed the presence of eight proteobacteria. To date, *Comamonas* spp. have not been reported as a cause of intraocular infection. As well as from ocular samples, sequences bearing their closest similarity to gram-negative proteobacteria have been isolated from culture-negative prostate, CSF, and bladder samples, in each case in the presence of a host inflammatory response.[23,29,35] These results, obtained by four different groups of workers, seem to suggest that these sequences may be significant findings. Gram-negative proteobacteria seem to play a much more important role in the pathogenesis of this disease than was previously thought. Of note, in 50% of cases, gram-positive and gram-negative organisms were isolated from the same eye, justifying the current use of broad-spectrum antibiotics that cover both sets of organisms. Intraocular infection with gram-negative bacteria has traditionally been associated with a poor visual outcome.[36] In this series of nine eyes, however, the presence of gram-negative infection was not necessarily associated with a poor visual prognosis, suggesting that perhaps the infective load is a more important prognostic factor than the gram status of the bacterium present. Our results revealed the presence of gram-negative bacteria that require specific culture techniques for successful isolation.[37] Perhaps current culture techniques should be modified, keeping in mind the specific needs of these organisms.

Acknowledgments

The authors thank the Endophthalmitis Study Group at Moorfields Eye Hospital (John Dart, Linda Ficker, Sue Lightman, and Steve Tuft) and

Philip Luthert, Department of Pathology, The Institute of Ophthalmology, for their help and support.

References

1. Okhravi N, Towler HMA, Hykin P, Matheson MM, Lightman S. Assessment of a standard treatment protocol on visual outcome following presumed bacterial endophthalmitis. *Br J Ophthalmol.* 1997;81:719–725.
2. Han DP, Wisniewski SR, Wilson LA, et al. Spectrum and susceptibilities of microbiologic isolates in the Endophthalmitis Vitrectomy Study. *Am J Ophthalmol.* 1996;122:1–17.
3. Forster RK, Abbott RL, Gelender H. Management of infectious endophthalmitis. *Ophthalmology.* 1980;87:313–319.
4. Stern GA, Engel HM, Dreibe WT. The treatment of post-operative endophthalmitis: results of differing approaches to treatment. *Ophthalmology.* 1989;96:62–67.
5. Verbraeken H, Rysselaere M. Bacteriological study of 92 cases of proven infectious endophthalmitis treated with pars plana vitrectomy. *Ophthalmologica.* 1991;203:17–23.
6. Heaven CJ, Mann PJ, Boase DL. Endophthalmitis following extracapsular cataract surgery: a review of 32 cases. *Br J Ophthalmol.* 1992;76:419–423.
7. Kalicharan D, Jongebloed WL, Los LI, Worst JGF. (An)aerobic bacteria found in the secondary-cataract material. A SEM/TEM study. *Doc Ophthalmol.* 1992;82:125–133.
8. Busin M, Cusumano A, Spitznas M. Intraocular lens removal from eyes with chronic low-grade endophthalmitis. *J Cataract Refract Surg.* 1995;21:679–684.
9. Okhravi N, Adamson P, Matheson MM, Towler HMA, Lightman S. PCR/RFLP based detection and speciation of bacteria causing endophthalmitis. *Invest Ophthalmol Vis Sci* 2000;41:1438–1447.
10. Therese KL, Anand AR, Madhavan HN. Polymerase chain reaction in the diagnosis of bacterial endophthalmitis. *Br J Ophthalmol.* 1998;82:1078–1082.
11. Knox CM, Cevallos V, Margolis TP, Dean D. Identification of bacterial pathogens in patients with endophthalmitis by 16S ribosomal DNA typing. *Am J Ophthalmol.* 1999;128:511–512.
12. Lohmann CP, Heeb M, Linde H-J, Gabel V-P, Reischl U. Diagnosis of infectious endophthalmitis after cataract surgery by polymerase chain reaction. *J Cataract Refract Surg.* 1998;24:821–826.
13. Hughes MS, Beck LA, Skuce RA. Identification and elimination of DNA sequences in Taq DNA polymerase. *J Clin Microbiol.* 1994; 32:2007–2008.
14. Rand KH, Houck H. Taq polymerase contains bacterial DNA of unknown origin. *Mol Cell Probes.* 1990;4:445–450.
15. Meier A, Persing DH, Finken M, Bottger EC. Elimination of contaminating DNA within polymerase chain reaction reagents: implications for a general approach to detection of uncultured pathogens. *J Clin Microbiol.* 1993;31:646–652.
16. Sarkar G, Sommer SS. Removal of DNA contamination in polymerase chain reaction reagents by ultraviolet irradiation. *Methods Enzymol.* 1993;218:381–388.
17. Hykin PG, Tobal K, McIntyre G, Matheson MM, Towler HM, Lightman SL. The diagnosis of delayed post-operative endophthalmitis by polymerase chain reaction of bacterial DNA in vitreous samples. *J Med Microbiol.* 1994;40:408–415.
18. Carroll N, Adamson P, Okhravi N. Elimination of contaminating bacterial DNA in Taq polymerase. *J Clin Microbiol.* 1999;37:3402–3404.
19. Okhravi N, Adamson P, Lightman S. Polymerase chain reaction in the diagnosis of bacterial endophthalmitis (letter). *Br J Ophthalmol.* 1999;83:378–380.
20. Carroll NM, Jaeger EEM, Choudhury S, et al. Detection of and discrimination between Gram positive/Gram negative bacteria in intraocular samples using nested PCR. *J Clin Microbiol* 2000;38: 1753–1757.
21. Wiedbrauk DL, Werner JC, Drevon AM. Inhibition of PCR by aqueous and vitreous fluids. *J Clin Microbiol.* 1995;33:2643–2646.
22. Li Z, Deguchi T, Yasuda M, et al. Alteration in the GyrA subunit of DNA gyrase and the ParC subunit of DNA topoisomerase IV in quinolone-resistant clinical isolates of *Staphylococcus epidermidis. Antimicrob Agents Chemother.* 1998;42:3293–3295.
23. Druel B, Vandenesch F, Greenland T, et al. Aseptic meningitis after neurosurgery: a demonstration of bacterial involvement. *Clin Microbiol Infect.* 1996;1:230–234.
24. Aaberg TM Jr, Flynn HW Jr, Murray TG. Intraocular ceftazidime as an alternative to the aminoglycosides in the treatment of endophthalmitis (letter). *Arch Ophthalmol.* 1994;112:18–19.
25. Hassan IJ, MacGowan AP, Cook SD. Endophthalmitis at the Bristol Eye Hospital: an 11-year review of 47 patients. *J Hosp Infect.* 1992;22:271–278.
26. Puliafito CA, Baker AS, Haafd J, Foster CS. Infectious endophthalmitis. *Ophthalmology.* 1982;89:921–929.
27. Shrader SK, Band JD, Lauter CB, Murphy P. The clinical spectrum of endophthalmitis: incidence, predisposing factors, and features influencing outcome. *J Infect Dis.* 1990;162:115–120.
28. Wilbrink B, van der Heijden IM, Schouls LM, et al. Detection of bacterial DNA in joint samples from patients with undifferentiated arthritis and reactive arthritis, using polymerase chain reaction with universal 16S ribosomal RNA primers. *Arthritis Rheum.* 1998;41:535–543.
29. Riley DE, Berger RE, Miner DC, Krieger JN. Diverse and related 16s rRNA-encoding DNA sequences in the prostate tissues of men with chronic prostatitis. *J Clin Microbiol.* 1998;36:1646–1652.
30. Hoshina S, Machida K. Diagnostic microbiology of DNA in septicemia (in Japanese). *Rinsho Byori* 1996;44:314–321.
31. Ley BE, Linton CJ, Bennett DM, Jalal H, Foot AB, Millar MR. Detection of bacteraemia in patients with fever and neutropenia using 16s rRNA gene amplification by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis.* 1998;17:247–253.
32. Dickey JB, Thompson KD, Jay WM. Anterior chamber aspirate cultures after uncomplicated cataract surgery (see comments). *Am J Ophthalmol.* 1991;112:278–282.
33. Wang GC, Wang Y. The frequency of chimeric molecules as a consequence of PCR co-amplification of 16S rRNA genes from different bacterial species. *Microbiology.* 1996;142:1107–1114.
34. Ludwig W, Schleifer KH. Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiol Rev.* 1994;15:155–173.
35. Domingue GJ, Ghoniem GM, Bost KL, Fermin C, Human LG. Dormant microbes in interstitial cystitis. *J Urol.* 1995;153:1321–1326.
36. Irvine WD, Flynn HW, Miller D, Pflugfelder SC. Endophthalmitis caused by Gram negative organisms. *Arch Ophthalmol.* 1992;110: 1450–1454.
37. Howard B, ed. *Clinical and Pathogenic Microbiology*. 2nd ed. St Louis: Mosby-Year, 1994.