The Diabetic Ocular Environment Facilitates the Development of Endogenous Bacterial Endophthalmitis

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PURPOSE. We tested the hypothesis that changes in the diabetic ocular environment facilitate the development of endogenous bacterial endophthalmitis (EBE).

METHODS. C57BL/6J mice were rendered diabetic with streptozotocin (STZ) for 1, 3, or 5 months' duration. Diabetic and age-matched nondiabetic mice were tail vein-injected with 10^8 CFU of *Klebsiella pneumoniae*, a common cause of EBE in diabetics. After either 2 or 4 days postinfection, the EBE incidence was assessed by electroretinography, histology, bacterial counts, and myeloperoxidase ELISAs. Blood-retinal barrier (BRB) permeability in uninfected diabetic mice also was determined.

RESULTS. No cases of EBE were observed among the 1-month diabetic group. Extending the time from diabetes induction to 3 months resulted in a 23.8% EBE incidence after 2 days, and a 22% incidence after 4 days. The incidence of EBE increased to 27% in the 5-month diabetic group. Infected eyes had an average 8.01×10^2 and 6.19×10^4 CFU/eye for the 3- and 5-month diabetic groups, respectively. There was no significant difference in BRB permeability between control and 1-month uninfected diabetic mice. However, 3- and 5-month diabetic mice had significantly greater BRB permeability than control mice. These results suggested that increasing the time from STZ diabetes induction to 3 and 5 months resulted in an ocular environment more conducive to the development of EBE.

CONCLUSIONS. These results demonstrated a correlation between an increase in BRB permeability and an increase in EBE incidence, supporting the hypothesis that diabetic ocular changes contribute to the development of EBE. (*Invest Ophthalmol Vis Sci.* 2012;53:7426-7431) DOI:10.1167/iov.12-10661

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Endogenous bacterial endophthalmitis (EBE) is a potentially blinding form of intraocular infection resulting from hematogenous spread of bacteria from a focus of infection into the eye. A number of conditions predispose individuals to EBE, including intravenous drug abuse and treatment with immunosuppressive agents. However, the most common predisposing risk factor is diabetes.¹⁻⁶ During the course of diabetes, permeability changes occur in the blood-retinal barrier (BRB). Of particular interest is the role that the diabetic ocular environment may have in the migration of bacteria from the vasculature into the eye, resulting in EBE. At present, the mechanisms underlying movement of bacteria across the BRB and into the eye from the bloodstream are not well understood.

One of the most common causes of EBE in diabetics is the opportunistic pathogen *Klebsiella pneumoniae*, a Gram-negative bacterium found in the environment and a constituent of the gastrointestinal tract microbiota.¹⁻⁶ *K. pneumoniae* is a frequent cause of community-acquired and healthcare-associated infections, which include pneumonia, bacteremia, urinary tract, and wound infections.⁷⁻¹⁰ *K. pneumoniae* is the most prevalent bacteria isolated from pyogenic liver abscesses in the United States, Trinidad, and Asian Pacific regions,¹¹⁻¹⁶ with diabetes as the major underlying condition associated with these abscesses.¹⁷⁻²³ Ten percent of pyogenic *K. pneumoniae* liver abscess cases are complicated by EBE.⁸ The visual outcome in patients with *K. pneumoniae* EBE is uniformly poor, ranging from hand motion visualization to evisceration or enucleation of the eye.¹⁻³ The prevalence of cases of *K. pneumoniae* EBE where diabetes is a predisposing risk factor led to the hypothesis that changes that occur within the ocular environment during diabetes contribute to the development of *K. pneumoniae* EBE. To test this hypothesis, we developed a streptozotocin (STZ)-induced diabetic mouse model of *K. pneumoniae* EBE. In this report, we demonstrated that the incidence of *K. pneumoniae* EBE was greater in STZ-induced diabetic mice than in control mice, and that the degree of BRB permeability may have facilitated a higher incidence of EBE. The results demonstrated a correlation between the duration of diabetes and the incidence of *K. pneumoniae* EBE, and also suggested that increases in BRB permeability may contribute to the enhanced incidence of *K. pneumoniae* EBE.

METHODS

Animals

Six-week-old C57BL/6J mice were acquired from the Jackson Laboratory and used in accordance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice were allowed to acclimate to the environment for two weeks before the establishment of diabetes. Mice were anesthetized with an intramuscular injection of 85 mg ketamine/kg and 14 mg xylazine/kg before tail-vein injections and electroretinography.

STZ Induction of Diabetes

To establish diabetes, 8-week-old male C57BL/6J mice were given a series of 2 STZ injections at 100 mg/kg one week apart.²⁴ STZ (Sigma-Aldrich, St. Louis, MO) was solubilized in freshly prepared 10 mM citrate buffer pH 4.5 and immediately injected intraperitoneally. Blood glucose levels were measured two weeks following the second STZ injection. All mice demonstrated blood glucose levels greater than or equal to 300 mg/dl. Mice were allowed to proceed for either 1, 3, or 5 months, and were the same age at the time of infection. Control animals were administered citrate buffer pH 4.5 only. Before injections with *K. pneumoniae* or Evans Blue dye, blood glucose levels were measured again to ensure a diabetic state.

Endogenous Bacterial Endophthalmitis Model

A clinical hypermucoviscosity (HMV) negative *K. pneumoniae* endophthalmitis isolate²⁵ was grown for 18 hours in brain heart infusion media (BHI; Difco Laboratories, Detroit, MI), and subcultured in prewarmed BHI to logarithmic phase. Bacteria then were centrifuged and resuspended in PBS. To establish endogenous endophthalmitis infections, all groups of mice were tail vein injected with 10⁸ CFU in 100 μ L PBS. At 2 days postinfection, some eyes were harvested for bacterial quantitation as described below. At 4 days postinfection, retinal function of both eyes was assessed by electroretinography. Both eyes from each mouse were harvested for bacterial and polymorphonuclear leukocyte (PMN) quantitation or histology.

Electroretinography

To determine the degree of retinal function 4 days after mice were infected with *K. pneumoniae*, mice were dark adapted for at least 6 hours and then anesthetized as described above. Pupils were dilated with 10% topical phenylephrine (Akorn, Inc., Lake Forest, IL) and gold-wire electrodes were placed over both corneas with a drop of GONAK (Hypromellose Ophthalmic Demulscen Solution 2.5%; Akorn, Inc.) to ensure maximum conductance. Scotopic ERGs were recorded for both eyes using a UTAS-E3000 Electrodiagnostic System (LKC Technologies, Inc., Gaithersburg, MD). The A-wave amplitude was measured from the prestimulus baseline to the A-wave trough, and the B-wave amplitude was measured from the A-wave trough to the B-wave peak. Percent ERG retention was calculated relative to mean baseline ERGs recorded from 6 eyes per group before infection.^{26,27}

Bacterial Quantitation

For each group of infected mice, eyes were selected at random for bacterial quantitation. Briefly, the left and right eyes from each mouse selected were enucleated, placed into separate tubes containing 400 μ L of sterile PBS and 1.0 mm sterile glass beads (Biospec Products, Inc., Bartlesville, OK), and homogenized for 60 seconds at 5,000 revolutions per minute (rpm) in a Mini-BeadBeater (Biospect Products, Inc.). Eye homogenates were diluted serially and plated in triplicate on brain heart infusion (BHI) agar and the CFU per eye determined.^{26,27}

PMN Quantitation

PMN leukocyte infiltration into eyes was assessed indirectly by measurement of myeloperoxidase (MPO) concentrations in whole eye homogenates. Eyes were enucleated and homogenized with glass beads in PBS as described for bacterial quantitation. Homogenates were diluted 1:4 and analyzed for MPO by ELISA according to manufacturer's instructions (Mouse MPO ELISA Test Kit; Cell Sciences, Canton, MA).^{27,28}

Histology

Because homogenization of eyes for bacterial quantitation precludes subsequent histologic analysis on eyes that are confirmed culture positive, eyes from each group were selected randomly and fixed in Excalibur's Alcoholic Z-Fix for 24 hours, and then embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E), and tissue Gram stain. Livers were fixed in 10% neutral buffered formalin for 24 hours, embedded in paraffin, and sections stained with hematoxylin and eosin.^{26,27}

Vascular Permeability

Albumin leakage from blood vessels into the retina was measured to quantify vascular permeability using a modified Evans blue protocol.²⁹ Mice were anesthetized as described above and 15 mg Evans Blue dye (Sigma-Aldrich) per kg were injected into the tail vein. After two hours, mice were perfused with 1% paraformaldehyde in citrate buffer (pH 4.2) for 1 minute, and retinas harvested and placed in 150 μ L formamide. Retinas were incubated at 70°C for 18 hours to extract the dye and centrifuged at 70,000 rpm (TLA 100.3 rotor; Beckman Coulter, Fullerton, CA) for 20 minutes. The optical density at 620 nanometers (OD₆₂₀) of 100 μ L of the supernatants was measured and the concentration of Evans blue was calculated from a standard curve of Evans Blue in formamide. The pellets then were solubilized in 1 mL of 0.2% SDS in PBS and the protein concentrations measured using a BCA protein assay. The concentration of Evans Blue in each sample then was normalized to the total protein per sample. Results were expressed in micrograms of Evans blue/mg total protein content.

Statistics

All values represent the mean $\pm$ SD of the number of eyes per group indicated in the figure legends for each assay. Two-tailed, two-sample *t*-tests were used for statistical comparisons between groups. A *P* value of ≤ 0.05 was considered significant.

RESULTS

Diabetes and Incidence of *K. pneumoniae* EBE

To test the hypothesis that changes in the diabetic ocular environment facilitated the development of *K. pneumoniae* EBE, we used a mouse model of STZ-induced diabetes.²⁴ Male C57BL/6J mice were injected with 100 mg/kg STZ and diabetes was allowed to progress for one month. One-month diabetic and age-matched sham injected control mice were tail vein-injected with 10⁸ CFU of a clinical ocular isolate of *K. pneumoniae*. After 2 days postinfection, the incidence of EBE was evaluated. No difference was observed in the incidence of EBE between the one-month diabetic and control mice (Table 1). We surmised that the changes in the architecture of the eye observed during diabetes progression had not yet occurred after one month, even though the mean blood glucose level of these mice was >400 mg/dl. We sought to determine whether extending the time from diabetes induction to 3 months might facilitate the changes necessary for EBE development. After 2 days postinfection, we observed a 23.8% incidence of infection among 3-month diabetic mice, while no cases of EBE were observed in control mice (Table 1).

To determine whether extending the time from diabetes induction to 5 months might result in greater incidence of EBE, we repeated the above-described experiments and included a 5-month diabetic group. Mice in all groups were age-matched at the time of infection and had similar blood glucose levels of >300 mg/dl. As shown in Table 2, the incidence of *K. pneumoniae* EBE among 1- and 3-month groups was similar to that observed previously, even though infection was allowed to

TABLE 1. Incidence of *K. pneumoniae* EBE at 2 Days Postinfection in STZ-Induced Diabetic or Age Matched Controls (1 Month or 3 Months in Duration) Infected with HMV– *K. pneumoniae*

Duration of Diabetes	1 Month	1 Month	3 Months	3 Months
Group	Diabetic	Control	Diabetic	Control
Total mice	15	10	25	25
<i>N</i> surviving after 2 days postinfection	15	10	21	25
Mice with EBE	0	0	5	0
% Infected of survivors	0	0	23.8	0

proceed for 4 days. We observed no cases of EBE among 1-month diabetic mice, but a 22% incidence among 3-month diabetic mice. Of interest was the slight increase in incidence observed in the 5-month STZ group to 27%, suggesting that increasing the time from STZ diabetes induction to 5 months resulted in an ocular environment more conducive to the development of EBE. The mean CFU per eye was 8.01×10^2 ($s = 1.08 \times 10^3$) for the 3-month and 6.19×10^4 ($s = 1.30 \times 10^5$) for the 5-month diabetic groups.

Retinal Function and Inflammation

To determine whether retinal function had been affected during infection, mice were subjected to electroretinography 4 days after infection with *K. pneumoniae*. No changes from preinfection baseline retinal function were observed among the 1-month diabetic mice. We also did not observe significant decreases in the percent A- or B-wave amplitudes retained among the animals in the 3- or 5-month diabetic groups that were culture confirmed with EBE (data not shown). These results are not surprising given the low number of CFU of this HMV– strain detected in the eyes of infected mice after 4 days. Previous studies of this HMV– strain in a direct injection model of endophthalmitis demonstrated that to observe significant retinal function decline, orders of magnitude higher concentrations of bacteria in the eye are necessary.^{25,28} Moreover, we detected essentially no infiltration of PMNs as measured indirectly by MPO concentrations in culture-confirmed, infected 3- or 5-month diabetic eyes. No significant differences were observed in the concentration of MPO in the eyes of 3- or 5-month diabetic animals with EBE versus the eyes of 1-month diabetic eyes without EBE (data not shown). Concentrations of MPO ranged from 2.5 to 3.0 ng per eye for the 1-month diabetic eyes without EBE, and the 3- and 5-month diabetic mice with EBE. We previously have shown that between 9 and 21 hours after direct infection of the vitreous with *K. pneumoniae*, MPO concentrations ranged from 10 to 60 ng per eye²⁵ in comparison.

Correlation between EBE and Underlying Disease State

To determine if there was a correlation between the presence of liver abscesses and EBE among infected animals, histologic analysis of liver sections from representative animals from each

group was performed. Interestingly, no abscesses were observed among the 1-month diabetic animals. However, liver abscesses were observed in 5 of 7 mice in the 3-month diabetic group, and in 2 of 5 in the 5-month diabetic group that were analyzed (Fig. 1). Of the 5 animals in the 3-month group that had liver abscesses (Figs. 1B, 1C), 1 animal had culture-confirmed EBE, and both animals with liver abscesses (Figs. 1D, 1E) in the 5-month group had EBE. The other 3 animals in that group were confirmed to have EBE; however, no liver abscesses were observed. These results are not suggestive of a direct correlation between the presence of liver abscesses and EBE occurrence, but demonstrated that in this diabetic model, liver abscesses do occur, consistent with reports of other bacterial species.^{30,31}

Correlation between EBE and Retinal Vascular Permeability

Because a greater incidence of *K. pneumoniae* EBE was observed in the 3- and 5-month diabetic groups than in the 1-month diabetic group, we hypothesized that the diabetic retinal environment at 3 and 5 months facilitated a higher incidence of EBE. To determine the extent of BRB permeability in these groups of diabetic mice, an Evans Blue dye assay was used to measure albumin leakage into the retina.²⁹ There was no difference in BRB permeability between 1-month control and 1-month diabetic mice (Fig. 2, $P = 0.23$). However, 3-month diabetic mice had significantly greater BRB permeability than nondiabetic control mice (Fig. 2, $P = 0.02$), suggesting that changes in vascular permeability occurred in the eyes of 3-month diabetic mice. An even greater degree of vascular permeability was observed among the 5-month diabetic mice relative to the control animals (Fig. 2, $P = 0.000004$). Taken together, these results showed a correlation between an increase in BRB permeability and an increase in EBE incidence, supporting our initial hypothesis that changes in the diabetic eye contribute to acquisition of EBE.

DISCUSSION

EBE results from bacteria seeding the bloodstream from a distant focus of infection and migrating across the BRB into the posterior segment of the eye. The often poor visual outcome and potential for bilateral blindness make EBE one of the most

TABLE 2. Incidence of *K. pneumoniae* EBE at 4 Days Postinfection in STZ-Induced Diabetic Mice (1 Month, 3 Months, and 5 Months in Duration) Infected with an HMV– *K. pneumoniae*

Duration of Diabetes	1 Month	3 Months	5 Months
<i>N</i> of mice infected with <i>K. pneumoniae</i>	27	35	32
<i>N</i> surviving after 4 days postinfection	24	32	30
Mice with EBE	0	7	8
% Infected of survivors	0	22	27
Mean CFU/eye	0	$8.01 \times 10^2 (\pm 1.08 \times 10^3)$	$6.19 \times 10^4 (\pm 1.30 \times 10^5)$

FIGURE 1. Histologic sections of livers from diabetic mice with culture-confirmed EBE. Representative 5 μ m sections from a 1-month diabetic mouse without EBE (A), the one 3-month diabetic mouse (B, C), and the two 5-month diabetic mice with culture-confirmed EBE (D, E) are shown. Arrows indicated location of abscesses. H&E, magnification 10 $\times$ (A, B, D, E) and 20 $\times$ (C).

destructive and devastating infections of the eye. Approximately 41% of cases of EBE result in a final visual acuity of count fingers or better, 26% of infected eyes lose all useful vision, and 29% required surgical removal.¹⁻³ *K. pneumoniae* causes the majority of Gram-negative cases of EBE.¹⁻⁶ *K. pneumoniae* has become the leading cause of pyogenic liver abscess in patients in Taiwan^{10,32} and there are increasing numbers of reports of *K. pneumoniae* EBE resulting from septicemia following pyogenic liver abscesses in diabetics.^{7-10,32,33} *K. pneumoniae* liver abscesses also are being reported with increased frequency in North America.³⁴

Diabetes is the primary risk factor for contracting bacterial EBE.¹⁻⁶ Diabetes is a metabolic disease due to dysregulation of

glucose metabolism that compromises the immune system and predisposes the individual to an increased risk of infection. Diabetes also compromises the eye architecturally, resulting in changes that may enhance the ability of organisms to invade the eye during systemic infection. Among patients with diabetes, specifically type II diabetes, diabetic retinopathy is a serious and common complication that impacts the retinal vasculature, glia, and retinal neurons.^{35,36} During the early stages, death of pericytes and thickening of the basement membrane occur, leading to capillary leakage and occlusion. These changes ultimately result in macular edema, formation of new vessels, and neuroretinal degeneration.³⁷⁻⁴² Increases in VEGF and the cytokines IL-1 β , IL-6, IL-8, and TNF α in diabetic retinas lead to angiogenesis and a breakdown in the BRB, implying an association with inflammation.⁴³ Intercellular adhesion molecule 1 (ICAM-1) and CD18 are upregulated, and contribute to increased leukocyte adhesion to the endothelial wall, leading to endothelial cell injury and death.⁴⁴⁻⁴⁷ Elevated expression of matrix metalloproteases in the retina also may facilitate increases in BRB permeability by degradation of tight junction complexes.⁴⁸ RPE and vascular endothelium, barrier cells of the BRB, also undergo changes in hyperglycemic environments in vitro and in vivo, including increases in VEGF that may contribute to vascular permeability.^{49,50} It currently is unknown whether these changes are linked to the development of EBE, and the pathogenic mechanisms underlying the migration of bacteria toward and into the eye, inflammation, and vision loss have not been addressed to our knowledge.

In our work, we observed a 22% to 27% incidence of EBE among 3- and 5-month diabetic mice, but no incidence of EBE among control, nondiabetic, and 1-month diabetic mice. Additionally, we demonstrated that increases in vascular permeability correlated with time from diabetes induction. While these infections were low-grade and did not entirely mimic human disease in terms of PMN infiltration and functional loss,^{25,28} this model provides a solid framework to study and gain a better understanding of the role that diabetes

FIGURE 2. Duration from diabetes induction correlates with vascular permeability. Albumin leakage from blood vessels into the retina was measured to quantify vascular permeability using a modified Evans blue protocol. The concentration of Evans blue was calculated from a standard curve and normalized to the total protein per sample. Results were expressed in micrograms of Evans blue per milligrams of total protein content. Bars: mean $\pm$ SD for $N \geq 5$ animals for all groups. A two-tailed *t*-test was used to assess significance between group (control versus 1 month $P = 0.23$, *control versus 3 month $P = 0.02$, **control versus 5 month $P = 0.000004$).

has in development of EBE, and of the interplay of host and bacterial factors that contribute to this devastating disease. In our model, we used an HMV[−] strain of *K. pneumoniae* to establish the model and provide baseline data for future comparisons with HMV⁺ *K. pneumoniae*. Our laboratory has demonstrated in a mouse model of experimentally-induced endophthalmitis, in which bacteria are introduced into the vitreous by direct injection, that the HMV phenotype contributes to pathogenesis. As reported by Wiskur et al., an HMV⁺ strain caused significantly greater inflammation and greater loss of visual function than an HMV[−] strain,²⁵ the same strain we used in our study. Further, the HMV⁺ strain was not readily cleared from the eye in this model.²⁵ More recently, our group used the experimentally-induced endophthalmitis model to compare directly a wild type HMV⁺ *K. pneumoniae* strain with an isogenic mutant defective in *magA*, the gene primarily responsible for the HMV phenotype.²⁸ The studies showed that mice infected with the *magA*[−] strain showed a significantly improved visual outcome relative to mice infected with the wildtype strain.²⁸ Both of these studies clearly established a role for the HMV phenotype in *K. pneumoniae* endophthalmitis. As this direct injection model circumvents systemic infection, it remains to be seen whether the HMV phenotype is important for crossing the BRB and establishing EBE. The HMV phenotype might enable bacteria to more readily cross the BRB, adhere to structures within the eye, and/or evade PMN clearance. Future studies in our laboratory will address this hypothesis.

To our knowledge, no studies have examined whether patients suffering from bacterial EBE had a history of diabetes-induced vision changes or diabetic retinopathy before infection. However, if barrier function of the BRB were altered and the immune response was unable to suppress transient bacteremia, bacteria could circumvent the immune response, reach the retinal vasculature, and gain access into the eye through a permeable BRB. While we did not observe EBE in nondiabetic mice, it might be possible that *K. pneumoniae* contributed directly to increasing vascular permeability. It has been shown that *Neisseria meningitidis*, especially unencapsulated strains, induce the upregulation of the vascular adhesion molecules CD62E, ICAM-1, and VCAM-1, leading to increased binding of leukocytes to the endothelium, and ultimately vascular damage and permeability.⁵¹ While it has not been shown that *K. pneumoniae* similarly induces upregulation of vascular adhesion molecules, we cannot rule out the possibility that *K. pneumoniae* contributes to the pathogenesis of EBE by this mechanism. We further acknowledge that systemic infection with *K. pneumoniae* might elicit increases in vascular permeability through the liberation of TNF α , as do other encapsulated organisms.^{52,53} However, as stated, we have not observed any incidence of EBE among control animals, so it is unlikely that systemic infection alone caused the permeability necessary for EBE development.

In addition to changes in BRB permeability during the course of diabetes, suppression of the innate immune system also contributes to an increased risk of infection, and potentially to EBE pathogenesis. PMNs are necessary for clearance of pathogens from the eye during acute bacterial endophthalmitis.^{25–29} However, PMNs from diabetic mice and humans display defective abilities to phagocytize and kill bacteria, including *Staphylococcus aureus* and *K. pneumoniae*.^{54,55} The possibility arises that the inability of PMNs to clear pathogens adequately from the bloodstream and the eye also may contribute to the development of EBE. However, this hypothesis has not been tested, and future studies will be necessary to address the role of the innate immune system in EBE pathogenesis.

To our knowledge, this is the first reported experimental model of endogenous bacterial endophthalmitis. Using this model, we have demonstrated a correlation between the duration of diabetes and the incidence of *K. pneumoniae* EBE. We also have shown that increases in retinal vascular permeability correlate with the time from diabetes induction. This model will prove useful in elucidating the mechanisms of EBE development and will allow the role of bacterial virulence traits to be dissected, as well as explore host factors and components of the immune system that are involved in EBE pathogenesis. Future studies will continue to explore the relationship between changes in the diabetic ocular environment and the development of EBE.

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References

1. Jackson TL, Eykyn SJ, Graham EM, Stanford MR. Endogenous bacterial endophthalmitis: a 17-year prospective series and review of 267 reported cases. *Surv Ophthalmol.* 2003;48:403–423.
2. Greenwald MJ, Wohl LG, Sell CH. Metastatic bacterial endophthalmitis: a contemporary reappraisal. *Surv Ophthalmol.* 1986;31:81–101.
3. Shamma HF. Endogenous *E. coli* endophthalmitis. *Surv Ophthalmol.* 1977;21:429–435.
4. Okada AA, Johnson RP, Liles WC, D'Amico DJ, Baker AS. Endogenous bacterial endophthalmitis. Report of a ten-year retrospective study. *Ophthalmology.* 1994;101:832–838.
5. Romero CF, Rai MK, Lowder CY, Adal KA. Endogenous endophthalmitis: case report and brief review. *Am Fam Physician.* 1999;60:510–514.
6. 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.
7. Fang CT, Chuang YP, Shun CT, Chang SC, Wang JT. A novel virulence gene in *Klebsiella pneumoniae* strains causing primary liver abscess and septic metastatic complications. *J Exp Med.* 2004;199:697–705.
8. Fung CP, Chang FY, Lee SC, et al. A global emerging disease of *Klebsiella pneumoniae* liver abscess: is serotype K1 an important factor for complicated endophthalmitis? *Gut.* 2002;50:420–424.
9. Chuang YP, Fang CT, Lai SY, Chang SC, Wang JT. Genetic determinants of capsular serotype K1 of *Klebsiella pneumoniae* causing primary pyogenic liver abscess. *J Infect Dis.* 2006;193:645–654.
10. Chang SC, Fang CT, Hsueh PR, Chen YC, Luh KT. *Klebsiella pneumoniae* isolates causing liver abscess in Taiwan. *Diagn Microbiol Infect Dis.* 2000;37:279–284.
11. Lederman ER, Crum NF. Pyogenic liver abscess with a focus on *Klebsiella pneumoniae* as a primary pathogen: an emerging disease with unique clinical characteristics. *Am J Gastroenterol.* 2005;100:322–331.
12. Rahimian J, Wilson T, Oram V, Holzman RS. Pyogenic liver abscess: recent trends in etiology and mortality. *Clin Infect Dis.* 2004;39:1654–1659.
13. Fang FC, Sandler N, Libby SJ. Liver abscess caused by *magA*⁺ *Klebsiella pneumoniae* in North America. *J Clin Microbiol.* 2005;43:991–992.
14. Lederman ER, Crum NF. *Klebsiella* liver abscess: a coast-to-coast phenomenon. *Clin Infect Dis.* 2005;41:273.
15. Ko WC, Paterson DL, Sagnimeni AJ, et al. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. *Emerg Infect Dis.* 2002;8:160–166.

16. Ohmori S, Shiraki K, Ito K, et al. Septic endophthalmitis and meningitis associated with *Klebsiella pneumoniae* liver abscess. *Hepato Res.* 2002;22:307–312.
17. Jardeleza MS, Chao-Chan C, Shalaby I, de la Cruz Z, Shen de F, Green WR. Clinicopathologic and ultrastructural study of endogenous *Klebsiella pneumoniae* endophthalmitis. *Retina.* 2005;25:657–662.
18. Scott IU, Matharoo N, Flynn HW Jr, Miller D. Endophthalmitis caused by *Klebsiella* species. *Am J Ophthalmol.* 2004;138:662–663.
19. Chen YJ, Kuo HK, Wu PC, et al. 10-year comparison of endogenous endophthalmitis outcomes: an east Asian experience with *Klebsiella pneumoniae* infection. *Retina.* 2004;24:383–390.
20. Giobbia M, Scotton PG, Carniato A, et al. Community-acquired *Klebsiella pneumoniae* bacteremia with meningitis and endophthalmitis in Italy. *Int J Infect Dis.* 2003;7:234–235.
21. Ayinala SR, Vulpe M, Azaz M, Cohen H, Donelson SS, Lee M. Pyogenic liver abscesses due to *Klebsiella pneumoniae* in a diabetic patient. *J Miss State Med Assoc.* 2001;42:67–70.
22. Saccante M. *Klebsiella pneumoniae* liver abscess, endophthalmitis, and meningitis in a man with newly recognized diabetes mellitus. *Clin Infect Dis.* 1999;29:1570–1571.
23. Liao HR, Lee HW, Leu HS, Lin BJ, Juang CJ. Endogenous *Klebsiella pneumoniae* endophthalmitis in diabetic patients. *Can J Ophthalmol.* 1992;27:143–147.
24. Rajala RV, Wiskur B, Tanito M, Callegan M, Rajala A. Diabetes reduces autophosphorylation of retinal insulin receptor and increases protein-tyrosine phosphatase-1B activity. *Invest Ophthalmol Vis Sci.* 2009;50:1033–1040.
25. Wiskur BJ, Hunt JJ, Callegan MC. Hypermucoviscosity as a virulence factor in experimental *Klebsiella pneumoniae* endophthalmitis. *Invest Ophthalmol Vis Sci.* 2008;49:4931–4938.
26. Ramadan RT, Ramirez R, Novosad BD, Callegan MC. Acute inflammation and loss of retinal architecture and function during experimental *Bacillus* endophthalmitis. *Curr Eye Res.* 2006;31:955–965.
27. Ramadan RT, Moyer AL, Callegan MC. A role for tumor necrosis factor- α in experimental *Bacillus cereus* endophthalmitis pathogenesis. *Invest Ophthalmol Vis Sci.* 2008;49:4482–4489.
28. Hunt JJ, Wang JT, Callegan MC. Contribution of mucoviscosity associated gene A (magA) to virulence in experimental *Klebsiella pneumoniae* endophthalmitis. *Invest Ophthalmol Vis Sci.* 2011;52:6860–6866.
29. Moyer AL, Ramadan RT, Novosad BD, Astley R, Callegan MC. *Bacillus cereus*-induced permeability of the blood-ocular barrier during experimental endophthalmitis. *Invest Ophthalmol Vis Sci.* 2009;50:3783–3793.
30. Nielsen OL, Iburg T, Aalback B, et al. A pig model of acute *Staphylococcus aureus* induced pyemia. *Acta Vet Scand.* 2009;51:14.
31. Carter PB. Pathogenicity of *Yersinia enterocolitica* for mice. *Infect Immun.* 1975;11:164–170.
32. Wang JH, Liu YC, Lee SS, et al. Primary liver abscess due to *Klebsiella pneumoniae* in Taiwan. *Clin Infect Dis.* 1998;26:1434–1438.
33. Chen YJ, Kuo HK, Wu PC, et al. 10-year comparison of endogenous endophthalmitis outcomes: an east Asian experience with *Klebsiella pneumoniae* infection. *Retina.* 2004;24:383–390.
34. Pope JV, Teich DL, Clardy P, McGillicuddy DC. *Klebsiella pneumoniae* liver abscess: an emerging problem in North America. *J Emerg Med.* 2011;41:103–105.
35. Engler C, Krogsaa B, Lund-Andersen H. Blood-retina barrier permeability and its relation to the progression of diabetic retinopathy in type 1 diabetics. An 8-year follow-up study. *Graefes Arch Clin Exp Ophthalmol.* 1991;229:442–446.
36. Aiello LP, Gardner TW, King GL, et al. Diabetic retinopathy. *Diabetes Care.* 1998;21:143–156.
37. Fong DS, Aiello L, Gardner TW, et al. American Diabetes Association: diabetic retinopathy. *Diabetes Care.* 2003;26:226–229.
38. Neely KA, Gardner TW. Ocular neovascularization: clarifying complex interactions. *Am J Pathol.* 1998;153:665–670.
39. Qaum T, Xu Q, Jousen AM, et al. VEGF-initiated blood-retinal barrier breakdown in early diabetes. *Invest Ophthalmol Vis Sci.* 2001;42:2408–2413.
40. Takeda M, Mori F, Yoshida A, et al. Constitutive nitric oxide synthase is associated with retinal vascular permeability in early diabetic rats. *Diabetologia.* 2001;44:1043–1050.
41. Asnaghi V, Gerhardinger C, Hoehn T, Adeboje A, Lorenzi M. A role for the polyol pathway in the early neuroretinal apoptosis and glial changes induced by diabetes in the rat. *Diabetes.* 2003;52:506–511.
42. Martin PM, Roon P, Van Ells TK, Ganapathy V, Smith SB. Death of retinal neurons in streptozotocin induced diabetic mice. *Invest Ophthalmol Vis Sci.* 2004;45:3330–3336.
43. Jo DH, Kim JH, Kim JH. How to overcome retinal neuropathy: the fight against angiogenesis-related blindness. *Arch Pharm Res.* 2010;33:1557–1565.
44. Schroder S, Palinski W, Schmid-Schonbein GW. Activated monocytes and granulocytes, capillary nonperfusion, and neovascularization in diabetic retinopathy. *Am J Pathol.* 1991;139:81–100.
45. Miyamoto K, Hiroshiba N, Tsujikawa A, Ogura Y. In vivo demonstration of increased leukocyte entrapment in retinal microcirculation of diabetic rats. *Invest Ophthalmol Vis Sci.* 1998;39:2190–2194.
46. Miyamoto K, Khosrof S, Bursell SE, et al. Prevention of leukostasis and vascular leakage in streptozotocin-induced diabetic retinopathy via intercellular adhesion molecule-1 inhibition. *Proc Natl Acad Sci U S A.* 1999;96:10836–10841.
47. Funatsu H, Yamashita H, Sakata K, et al. Vitreous levels of vascular endothelial growth factor and intercellular adhesion molecule 1 are related to diabetic macular edema. *Ophthalmology.* 2005;112:806–816.
48. Giebel SJ, Menicucci G, McGuire PG, Das A. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. *Lab Invest.* 2005;85:597–607.
49. Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE₂, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci.* 2006;47:1149–1160.
50. Losso JN, Truax RE, Richard G. trans-resveratrol inhibits hyperglycemia-induced inflammation and connexin downregulation in retinal pigment epithelial cells. *J Agric Food Chem.* 2010;58:8246–8252.
51. Dixon GL, Heyderman RS, Kotovicz K, et al. Endothelial adhesion molecule expression and its inhibition by recombinant bactericidal/permeability-increasing protein are influenced by the capsulation and lipooligosaccharide structure of *Neisseria meningitidis*. *Infect Immun.* 1999;67:5626–5633.
52. Simpson SQ, Singh R, Bice DE. Heat-killed pneumococci and pneumococcal capsular polysaccharides stimulate tumor necrosis factor- α production by murine macrophages. *Am J Respir Cell Mol Biol.* 1994;10:284–289.
53. Vallejo JG, Baker CJ, Edwards MS. Roles of the bacterial cell wall and capsule in induction of tumor necrosis factor α by type III group B streptococci. *Infect Immun.* 1996;64:5042–5046.
54. Lin J, Siu L, Fung C, et al. Impaired phagocytosis of capsular serotypes K1 or K2 *Klebsiella pneumoniae* in type 2 diabetes mellitus patients with poor glycemic control. *J Clin Endocrinol Metab.* 2006;91:3084–3087.
55. Park S, Rich J, Hanses F, Lee J. Defects in innate immunity predispose C57BL/6J-Leprdb/Leprdb mice to infection by *Staphylococcus aureus*. *Infect Immun.* 2009;77:1008–1014.