Published in final edited form as:

Expert Rev Ophthalmol. 2010 April 1; 5(2): 129–134. doi:10.1586/eop.10.12.

Herpes simplex virus keratitis: histopathology and corneal allograft outcomes

Roni M Shtein, MD and

Department of Ophthalmology and Visual Sciences, University of Michigan, Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105, USA, Tel.: +1 734 763 5506, Fax: +1 734 936 2340, ronim@umich.edu

Victor M Elner, MD, PhD[†]

Department of Ophthalmology and Visual Sciences, Department of Pathology, University of Michigan, Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105, USA, Tel.: +1 734 764 4182, Fax: +1 734 936 3815, velner@umich.edu

Abstract

Molecular biologic studies have elucidated the roles of specific and innate immune responses in the pathogenesis of HSV keratitis. Recent evidence suggests that interference with innate immune mechanisms may reduce the severity of HSV keratitis and its complications. The agents targeting innate immune responses are particularly amenable to translational therapy for patients at risk of complications of HSV keratitis and poor corneal transplant outcomes. Histopathologic evidence of neovascularization or inflammation in tissue removed at the time of corneal transplantation predicts patients who are at risk for allograft rejection or failure. Such patients are prime candidates for close monitoring and intensive therapy. Newer imaging techniques, such as clinical confocal biomicroscopy, might be useful in identifying neovascularization and inflammation that place patients at high risk for poor allograft outcomes prior to corneal transplantation.

Keywords

allograft failure; allograft rejection; herpes simplex virus; histopathology; inflammation; keratitis; neovascularization; penetrating keratoplasty

Herpes simplex virus (HSV) is a leading infectious cause of severe corneal opacification and neovascularization. Although visual rehabilitation of eyes with HSV scarring can sometimes be performed with anterior lamellar keratoplasty, many cases still require full-thickness corneal transplantation surgery. Since eyes with a history of HSV are known to be ‘high risk’ for poor outcomes following penetrating keratoplasty (PKP), corneal surgeons routinely wait for clinical stability without signs of disease activity prior to surgical intervention. Recent evidence shows that even under these conditions, there may be subclinical inflammation that signifies

© 2010 Expert Reviews Ltd

[†]Author for correspondence Department of Ophthalmology and Visual Sciences, Department of Pathology, University of Michigan, Kellogg Eye Center, 1000 Wall Street, Ann Arbor, MI 48105, USA, Tel.: +1 734 764 4182, Fax: +1 734 936 3815, velner@umich.edu.

Financial & competing interests disclosure

The authors have received the following grants from the NIH/National Eye Institute: EY017885 (Roni Shtein), P30EY07003 (Roni Shtein and Victor Elner) and EY09441 (Victor Elner). Victor Elner has received the Senior Scientific Investigator award from Research to Prevent Blindness. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

continued host immune reactivity that could potentially affect allograft survival. Histopathologic evaluation of corneal tissue removed at the time of PKP can provide information about host corneal inflammation and neovascularization that can predict future allograft outcomes. This report will review some of the histopathologic changes seen in corneas with a history of HSV stromal keratitis, and the potential clinical utility of these findings.

Pathogenesis of HSV stromal keratitis

Herpes simplex virus stromal keratitis leads to severe inflammation within the corneal stroma, and subsequent corneal opacification and neovascularization. The pathogenesis of HSV stromal keratitis has been studied extensively in an effort to understand the interactions of the virus and host immune response that result in corneal scarring. Stromal inflammation in HSV keratitis is thought to be due to a combination of HSV-specific T-cell activation by viral antigens, cytokine-mediated T-cell activation, and autoimmunity due to components of HSV binding to host autoantigens [1].

Many corneal HSV studies have been performed in the murine model in which stromal inflammation is mediated primarily by CD4⁺ T cells. Following corneal HSV infection, viral antigens are engulfed by Langerhans cells and macrophages in the cornea, and presented to antigen-specific T cells. A variety of chemokines are expressed in the murine cornea at different phases of infection, orchestrating progressive infiltration of activated HSV-specific T cells into the cornea [2]. Th1 cells, primarily involved in stimulating cellular immunity, seem to predominate in the development of HSV stromal keratitis [3,4]. In humans, the inflammatory infiltrate in HSV stromal keratitis is known to be composed of a variety of inflammatory cells, including lymphocytes, neutrophils and mononuclear phagocytes.

Cultured human corneal cells have also been used to study the pathogenesis of HSV infection. The innate immune response is activated very quickly after HSV binding to corneal epithelial cells, with the subsequent release of a range of inflammatory cytokines [5]. Furthermore, it has been shown that the upregulated immune response in stromal keratocytes in culture is not only activated by viral particles, but also in response to neutralized HSV immune complexes [6], indicating a prolonged and multiphasic immune system response to HSV infection.

Herpes simplex virus is a ubiquitous infection, and a large proportion of the population have evidence of exposure to HSV antigens; however, not all exposed individuals develop clinical manifestations of HSV. Furthermore, not all individuals with corneal HSV infections develop stromal keratitis. In a murine model, HSV-infected corneas with and without stromal keratitis had almost identical numbers of CD4⁺ T cells; however, there were more neutrophils, and increased expression of the chemokine, MIP-1 and the cytokine, IL-6, in corneas with stromal keratitis [7]. The inflammatory cytokines, IL-1 and IL-6, are elevated in HSV stromal keratitis and play an important role in the inflammatory cascade activated by HSV infection [2,3]. Macrophage inflammatory proteins (MIPs) and monocyte chemoattractant protein (MCP)-1 attract leukocytes to the cornea, and are key molecules in the pathogenesis of HSV keratitis and the development of corneal opacity [3]. The central roles played by these inflammatory cytokines and chemokines indicate that the development of HSV stromal keratitis with its resultant corneal opacification and neovascularization may correlate more closely with nonspecific host immune responses than HSV-specific T-cell responses [7]. Thus, blocking the nonspecific innate immune mechanisms triggered early in HSV infection may be of critical importance to preventing blinding sequelae of disease.

Other nonspecific inflammatory components, such as COX2 and matrix metalloproteinases (MMPs), are integral to the development of HSV keratitis [8,9]. COX2 is an important nonspecific mediator of inflammation and corneal scarring that is produced both by uninfected stromal cells and inflammatory cells in the cornea early after HSV infection [8]. Inhibition of

COX2 in a mouse model leads to less neutrophil infiltration and lower VEGF levels, resulting in milder stromal keratitis and reduced corneal neovascularization [8]. MMP-9 is produced by infiltrating neutrophils and contributes to neovascularization in HSV keratitis [9]. The inhibition of MMP-9 results in reduced corneal neovascularization in a mouse model [9]. Both COX2 and MMPs are potential targets for therapy to prevent damaging sequelae from HSV stromal keratitis. There is also evidence that blocking the innate immune responses with IL-1 receptor antagonist or IL-10 can reduce HSV stromal keratitis [10,11].

Corneal inflammation and neovascularization are intertwined in the pathogenesis of HSV stromal keratitis. Angiogenic factors, such as VEGF, play important roles in the development of neovascularization and scarring in HSV keratitis [3]. The proinflammatory cytokine IL-6 is associated with the development of neovascularization in HSV stromal keratitis, at least partially due to the fact that it is secreted by HSV-infected cells, and can stimulate noninfected cells and inflammatory cells to produce VEGF [10]. The inhibition of VEGF has been shown to reduce the severity of HSV keratitis in a murine model [12]. Subconjunctival injections of anti-VEGF monoclonal antibody (bevacizumab) also reduce neutrophil infiltration, neovascularization, disease severity and corneal opacification in a rabbit model of HSV stromal keratitis [13]. Off-label topical and subconjunctival use of bevacizumab in humans for a variety of corneal neovascular conditions has been reported with clinical regression of corneal vessels [14–16]; however, there is no US FDA approval for this indication.

Current treatment for HSV stromal keratitis consists primarily of anti-inflammatory medications, usually corticosteroid eye drops, used in conjunction with antiviral medications to protect against the recurrence of epithelial disease. Despite these treatments, many patients with HSV stromal keratitis progress to develop visually significant corneal opacification and require PKP for visual rehabilitation. Patients undergoing PKP for sequelae of HSV keratitis are at higher risk for adverse corneal allograft outcomes when compared with individuals undergoing grafting for non-inflammatory corneal conditions. The postoperative course can be complicated by high rates of HSV recurrence, graft rejection and graft failure [17]. These adverse outcomes have been shown to be significantly reduced, but not eliminated, with postoperative use of systemic antiviral medications [18]. It is important to more clearly understand the pathogenesis of HSV stromal keratitis in an effort to more effectively treat this condition and improve patient outcomes.

Corneal histopathology in HSV stromal keratitis

Corneal allografts are unlike other solid-organ transplants in that allograft tissue is placed in a bed of the host's residual diseased tissue. In the case of HSV-infected corneas, this may predispose the allograft to adverse outcomes, such as rejection or failure. Information gathered from histopathologic evaluation of corneal tissue removed at the time of PKP can have clinical relevance for patient outcomes. Recent retrospective studies showed correlations between corneal histopathologic findings and subsequent clinical outcomes of PKP performed for sequelae of HSV keratitis [19–21]. These studies showed correlations between the histopathologic presence of corneal stromal inflammation and subsequent allograft rejection [19,20], as well as the histopathologic presence of neovascularization and subsequent allograft failure [20,21].

There is evidence that substantial inflammation exists in corneas with a history of HSV keratitis, even in the absence of clinical signs of activity [19,20,22]. In the cohort of patients studied, histopathologic evidence of inflammation was found in 74% of patients, despite the fact that 81% of patients had no clinical evidence of HSV keratitis for at least 6 months prior to PKP. The presence of subclinical levels of inflammation found in clinically quiescent corneal tissue probably reflects the host immune response to HSV viral antigens even after successful

clearance of intact virus. Corneal tissue removed at the time of PKP with histopathologic evidence of inflammation was also found to have increased the expression of HLA-DR antigens and ICAM-1, molecules known to enhance antigen recognition [19,20]. IL-8 and MCP-1, two principle cytokines known to attract and stimulate various leukocyte subsets, were also found in inflamed corneas [19,20]. The analysis of the tissue removed from these patients at the time of PKP reveals that subclinical inflammation seen histopathologically predicts rejection.

Clinically evident corneal neovascularization is a known risk factor for poor allograft outcomes. The histopathologic presence of neovascularization in corneal tissue removed at the time of PKP correlates with subsequent allograft failure [20,21]. The presence of blood vessels in the corneal specimen indicates that the neovascularization extends to the edge of the host corneal bed and is therefore present at the graft margin where the new donor tissue is attached. The presence of blood vessels at the graft–host junction may be essential to the delivery of cellular and serum components initiating and propagating inflammation and host immune responses, setting the stage for allograft rejection and failure.

A recent retrospective study reviewing all corneal tissue submitted to the pathology laboratory at the University of California at San Francisco (CA, USA) from 1972 to 2001 reported on the pathological findings in corneas with a clinical diagnosis of HSV keratitis [22]. Of the 4207 corneal buttons submitted for histologic examination during this time period, 76 (1.8%) were from individuals undergoing primary PKP for HSV keratitis. Corneas removed with a clinical diagnosis of HSV keratitis had histopathologic evidence of inflammatory cells in 87% and neovascularization in 59% of specimens [22]. The authors did not comment on any possible effects of these histopathologic findings on subsequent allograft outcomes. Another retrospective study evaluating histopathologic findings of corneal tissue removed at the time of transplantation surgery in France also describes the presence of inflammatory infiltrates in tissue removed for the sequelae of HSV keratitis [23].

Several studies have evaluated corneal tissue removed at the time of PKP for the presence of HSV DNA [24–27]. These studies indicate that viral DNA is present in detectable quantities in clinically quiescent corneas. Although one study with short follow-up found no correlation between the presence of viral DNA and subsequent allograft outcomes [24], others found that the presence of HSV DNA correlated negatively with graft survival [25,26], and that significantly higher numbers of copies of HSV DNA were detectable in corneas from patients with a clinical history of HSV keratitis [27].

Studies have also identified the HSV genome in a small number of donor corneas without any clinical history of HSV [26,27]. Theoretically, the presence of HSV DNA within donor cornea tissue could lead to infection transmission to corneal transplant recipients at the time of PKP. Animal studies [28] as well as a human case report [29] have documented suspected transmission from donor to host. However, given the ubiquity of HSV infection, it is difficult to ascertain with certainty the source of postkeratoplasty HSV infection [30].

Expert commentary

Histopathologic evaluation of tissue both improves our understanding of the underlying disease process in HSV keratitis and is clinically useful. Histopathologic evidence of inflammatory cells and the presence of neovascularization can be assessed in tissue processed and assessed in the usual fashion for corneal surgical pathology specimens. Corneal tissue can also be evaluated for the presence of HSV DNA. These findings are of practical significance to the clinician in care of patients after PKP because they provide information that can be used to customize the postoperative treatment plan for patients based on their expected level of risk for poor allograft outcomes. Furthermore, this establishes an important role for the pathologist in assisting the clinician in their choice of postoperative patient management.

In recent years, lamellar corneal transplant surgeries have increased in prevalence for the treatment of localized corneal dysfunction. In some cases, HSV-related scarring can be treated surgically with deep anterior lamellar keratoplasty [31,32]. However, HSV disease, with its full-thickness corneal manifestations, often remains an indication for full-thickness PKP. Thus, a larger percentage of the full-thickness transplants that are performed will be for the indication of HSV-related corneal scarring. Maximizing survival of these high-risk transplants will continue to be a priority for corneal surgeons.

Five-year view

The next step beyond postoperative histopathologic evaluation of tissue will be to translate these findings into preoperative corneal evaluation that provides similar information. The *in vivo* clinical confocal biomicroscope is able to non-invasively image inflammation and neovascularization in corneal tissue prior to corneal transplantation surgery (Figure 1). A recent study used the clinical confocal biomicroscope to assess corneal endothelial changes caused by HSV keratitis [33]. Utilization of this technology could become routine in the preoperative evaluation of patients. As the presence of subclinical inflammation and neovascularization are known to affect subsequent allograft outcomes, preoperative discovery of these findings could dictate a course of treatment prior to surgery to optimize outcomes in high-risk PKP.

Key issues

- Corneal transplantation performed for complications of HSV keratitis is associated with a high risk for allograft rejection and failure.
- Molecular biology studies have elucidated the role of the innate immune response in the pathogenesis of HSV keratitis.
- Interference with innate immune mechanisms has been shown to reduce the severity of HSV keratitis and its complications.
- Innate immune responses are important new targets for patients at risk for HSV keratitis complications and to reduce post-transplantation risks.
- Histopathologic evaluation of corneal tissue removed at the time of corneal transplantation can help predict patients who are at risk for allograft rejection or failure.
- Histopathologic presence of corneal neovascularization in corneal tissue removed at penetrating keratoplasty is associated with increased risk of corneal allograft failure.
- Histopathologic presence of subclinical levels of corneal inflammation is associated with increased risk of allograft rejection.
- Newer imaging techniques, such as *in vivo* clinical confocal biomicroscopy, might be useful in identifying factors that place patients at high risk for poor allograft outcomes prior to corneal transplantation.

References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

1. Wickham S, Carr DJJ. Molecular mimicry versus bystander activation herpetic stromal keratitis. *Autoimmunity* 2004;37:393–397. [PubMed: 15621563]
2. Araki-Sasaki K, Tanaka T, Ebisuno Y, et al. Dynamic expression of chemokines and the infiltration of inflammatory cells in the HSV-infected cornea and its associated tissues. *Ocul. Immunol. Inflamm* 2006;14(5):257–266. [PubMed: 17056459]
3. Inoue Y. Immunologic aspects of herpetic stromal keratitis. *Semin. Ophthalmol* 2008;23(4):221–227. • Concise review of immunologic aspects of HSV corneal infections.
4. Komatsu K, Miyazaki D, Morohoshi K, et al. Pathogenesis of herpetic stromal keratitis in CCR5-and/or CXCR3-deficient mice. *Curr. Eye Res* 2008;33(9):736–749. [PubMed: 18798077]
5. Li H, Zhang J, Kumar A, Zheng M, Atherton SS, Yu FS. Herpes simplex virus 1 infection induces the expression of proinflammatory cytokines, interferons and TLR7 in human corneal epithelial cells. *Immunology* 2006;117(2):167–176. [PubMed: 16423052]
6. Hayashi K, Hooper LC, Chin MS, Nagineni CN, Detrick B, Hooks JJ. Herpes simplex virus 1 (HSV-1) DNA and immune complex (HSV-1-human IgG) elicit vigorous interleukin 6 release from infected corneal cells via Toll-like receptors. *J. Gen. Virol* 2006;87(8):2161–2169. [PubMed: 16847112] • Human stromal keratocytes in culture demonstrate upregulated innate immune response in response to neutralized HSV immune complexes.
7. Divito SJ, Hendricks RL. Activated inflammatory infiltrate in HSV-1-infected corneas without herpes stromal keratitis. *Invest. Ophthalmol. Vis. Sci* 2008;49:1488–1495. [PubMed: 18385067] • Murine model demonstrates the importance of nonspecific host immune responses in the development of HSV stromal keratitis.
8. Biswas PS, Banarjee K, Kim B, Kinchington PR, Rouse BT. Role of inflammatory cytokine-induced cyclooxygenase 2 in the ocular immunopathologic disease herpetic stromal keratitis. *J. Virol* 2005;79(16):10589–10600. [PubMed: 16051851] • Murine model indicates that COX2 is an important nonspecific mediator of inflammation and corneal scarring produced by uninfected stromal cells and inflammatory cells in the cornea early after HSV infection.
9. Lee S, Zheng M, Kim B, Rouse BT. Role of matrix metalloproteinase-9 in angiogenesis caused by ocular infection with herpes simplex virus. *J. Clin. Invest* 2002;110(8):1105–1111. [PubMed: 12393846]
10. Biswas PS, Banarjee K, Kinchington PR, Rouse BT. Involvement of IL-6 in the paracrine production of VEGF in ocular HSV-1 infection. *Exp. Eye Res* 2006;82:46–54. [PubMed: 16009363] • IL-6 can stimulate the production of VEGF and is associated with the development of neovascularization in HSV stromal keratitis.
11. Biswas PS, Banerjee K, Zheng M, Rouse BT. Counteracting corneal immunoinflammatory lesion with interleukin-1 receptor antagonist protein. *J. Leukoc. Biol* 2004;76(4):868–875. [PubMed: 15258192]
12. Kim B, Tang Q, Biswas PS, et al. Inhibition of ocular angiogenesis by siRNA targeting vascular endothelial growth factor pathway genes: therapeutic strategy for herpetic stromal keratitis. *Am. J. Pathol* 2004;165(6):2177–2185. [PubMed: 15579459] • The inhibition of VEGF can reduce the severity of HSV keratitis in a murine model.
13. Saravia M, Zapata G, Ferraiolo P, Racca L, Berra A. Anti-VEGF monoclonal antibody-induced regression of corneal neovascularization and inflammation in a rabbit model of herpetic stromal keratitis. *Graefes. Arch. Clin. Exp. Ophthalmol* 2009;247(10):1409–1416. [PubMed: 19655160]
14. Gerten G. Bevacizumab (avastin) and argon laser to treat neovascularization in corneal transplant surgery. *Cornea* 2008;27(10):1195–1199. [PubMed: 19034142] • Combined argon laser photocoagulation and bevacizumab injection may reduce neovascularization prior to penetrating keratoplasty.
15. Kim SW, Ha BJ, Kim EK, Tchah H, Kim TI. The effect of topical bevacizumab on corneal neovascularization. *Ophthalmology* 2008;115(6):e33–e38. [PubMed: 18439681]
16. Doctor PP, Bhat PV, Foster CS. Subconjunctival bevacizumab for corneal neovascularization. *Cornea* 2008;27(9):992–995. [PubMed: 18812760]
17. Garcia DD, Farjo Q, Musch DC, Sugar A. Effect of prophylactic oral acyclovir after penetrating keratoplasty for herpes simplex keratitis. *Cornea* 2007;26:930–934. [PubMed: 17721290]

18. Ghosh S, Jhanji V, Lamoureux E, Taylor HR, Vajpayee RB. Acyclovir therapy in prevention of recurrent herpetic keratitis following penetrating keratoplasty. *Am. J. Ophthalmol* 2008;145:198–202. [PubMed: 18222189]
19. Shtein RM, Garcia DD, Musch DC, Elnor VM. Herpes simplex virus keratitis: histopathologic inflammation and corneal allograft rejection. *Ophthalmology* 2009;116:1301–1305. [PubMed: 19576497]
20. Shtein RM, Garcia DD, Musch DC, Elnor VM. HSV Keratitis: histopathologic predictors of corneal allograft outcomes. *Trans. Am. Ophthalmol. Soc* 2008;106:161–170. [PubMed: 19277231]
21. Garcia DD, Shtein RM, Musch DC, Elnor VM. Herpes simplex virus keratitis: histopathologic neovascularization and corneal allograft failure. *Cornea* 2009;28:963–965. [PubMed: 19724220]
22. Branco BC, Gaudio PA, Margolis TP. Epidemiology and molecular analysis of herpes simplex keratitis requiring primary penetrating keratoplasty. *Br. J. Ophthalmol* 2004;88(10):1285–1288. [PubMed: 15377552]
23. Léger F, Vital C, Négrier ML, Bloch B. Histologic findings in a series of 1,540 corneal allografts. *Ann. Pathol* 2001;21(1):6–14. [PubMed: 11223555]
24. Robert PY, Adenis JP, Denis F, Alain S, Ranger-Rogez S. Herpes simplex virus DNA in corneal transplants: prospective study of 38 recipients. *J. Med. Virol* 2003;71(1):69–74. [PubMed: 12858411]
25. Aydemir O, Türkçüoğlu P, Bulut Y, Kalkan A. The relationship of graft survival and herpes simplex virus latency in recipient corneal buttons. *Clin. Ophthalmol* 2007;1(2):127–131. [PubMed: 19668501]
26. Remeijer L, Duan R, van Dun JM, Wefers Bettink MA, Osterhaus AD, Verjans GM. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. *J. Infect. Dis* 2009;200(1):11–19. [PubMed: 19476433] • The presence of HSV DNA in corneas removed at the time of penetrating keratoplasty correlates with disease severity and poor allograft outcomes.
27. Shimomura Y, Deai T, Fukuda M, Higaki S, Hooper LC, Hayashi K. Corneal buttons obtained from patients with HSK harbor high copy numbers of the HSV genome. *Cornea* 2007;26(2):190–193. [PubMed: 17251811]
28. Zheng X. Reactivation and donor–host transmission of herpes simplex virus after corneal transplantation. *Cornea* 2002;21(7 Suppl.):S90–S93. [PubMed: 12484706]
29. Remeijer L, Maertzdorf J, Doornenbal P, et al. Herpes simplex virus 1 transmission through corneal transplantation. *Lancet* 2001;357:442. [PubMed: 11273067]
30. Borderie VM, Meritet J-F, Chaumeil C, et al. Culture-proven herpetic keratitis after penetrating keratoplasty in patients with no previous history of Herpes disease. *Cornea* 2004;23:118–124. [PubMed: 15075879]
31. Sarnicola V, Toro P. Deep anterior lamellar keratoplasty in herpes simplex corneal opacities. *Cornea* 2010;29:60–64. [PubMed: 19907304]
32. Leccisotti A. Air-assisted manual deep anterior lamellar keratoplasty for treatment of herpetic corneal scars. *Cornea* 2009;28:728–731. [PubMed: 19574918]
33. Hillenaar T, Weenen C, Wubbels RJ, Remeijer L. Endothelial involvement in herpes simplex virus keratitis: an *in vivo* confocal microscopy study. *Ophthalmology* 2009;116:2077–2086. [PubMed: 19744733] • Describes the use of *in vivo* clinical confocal microscopy to detect corneal endothelial changes in patients with HSV keratitis.

Figure 1. Corneal scarring, neovascularization and inflammation detected by clinical confocal biomicroscopy and histopathology

(A) Clinical slit-lamp photograph of cornea exhibiting dense opacity and neovascularization, but without apparent inflammation, prior to penetrating keratoplasty. (B) Preoperative clinical confocal biomicroscopic image of neovascularization (arrows to vessel lumen). (C) Preoperative clinical confocal biomicroscopic image showing inflammatory cells (arrowheads) in scarred stroma. (D) Histopathologic photomicrograph of tissue removed at penetrating keratoplasty showing evidence of superficial neovascularization (arrows to vessel lumen). (E) Histopathologic photomicrograph of tissue removed at penetrating keratoplasty showing infiltrate of inflammatory cells (arrowheads) in scarred stroma.