

HSV KERATITIS: HISTOPATHOLOGIC PREDICTORS OF CORNEAL ALLOGRAFT COMPLICATIONS

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ABSTRACT

Purpose: To identify histopathologic features predictive for adverse allograft outcomes following penetrating keratoplasty for herpes simplex virus (HSV) keratitis.

Methods: Retrospective, interventional case series of 62 consecutive patients with HSV keratitis who underwent penetrating keratoplasty at the Kellogg Eye Center from 1990 through 2000. A detailed chart review and review of the histopathology of the excised corneal button were performed to identify associations between clinical data (disease activity, vascularity, adverse allograft outcomes) and histopathologic data (inflammation, neovascularization, biomarkers). The main outcome measure was to find histopathologic features that may predict HSV recurrence, allograft rejection, or failure.

Results: Although 81% of patients had clinically quiescent disease, histopathology revealed that 74% had active corneal inflammation, a finding that was associated with the presence of clinical neovascularization ($P = .01$). Histopathologic inflammation was a risk factor for allograft rejection ($P = .02$) but not failure ($P = .98$) or HSV recurrence ($P = .45$). Histopathologic neovascularization did not predict rejection ($P = .19$) but did predict failure ($P = .002$) and HSV recurrence ($P = .05$). Biomarkers, including HLA-DR, ICAM-1, and IL-8(CXC) and MCP-1 (CC) chemokines, were all significantly increased in fresh corneal specimens demonstrating moderate to severe inflammation. IL-10 treatment ex vivo significantly inhibited HLA-DR, IL-8 ($P = .006$), and MCP-1 ($P = .01$) but did not reduce ICAM-1 expression.

Conclusion: Histopathologic inflammation, neovascularization, and the presence of specific biomarkers are risk factors for corneal allograft morbidity.

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INTRODUCTION

Patients undergoing penetrating keratoplasty (PKP) for sequelae of herpes simplex virus (HSV) keratitis are at higher risk for adverse corneal allograft outcomes when compared to individuals undergoing grafting for conditions such as keratoconus and Fuchs corneal dystrophy.^{1,2} The postoperative course can be complicated by high rates of HSV recurrence, graft rejection, and graft failure.³⁻⁶ In an effort to identify which histopathologic features present in corneas affected by HSV are predictive for subsequent HSV recurrence, graft rejection, and graft failure, we investigated excised corneal tissue from a series of patients who underwent PKP for visual rehabilitation.

We hypothesize that patients have subclinical inflammation in their corneas in spite of the clinical impression of quiescent HSV disease and that this inflammation places their allografts at risk for subsequent complications. By examining host corneal tissue removed at the time of surgery, we sought to identify inflammation and neovascularization as important histopathologic features that may identify patients at high risk for HSV recurrence, graft rejection, and graft failure. We graded inflammation by the histopathologic presence of inflammatory cells as well as the immunohistopathologic presence of markers of inflammation, specifically, HLA-DR, intercellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), and interleukin 8 (IL-8). Such patients with increased inflammation would be likely to benefit from more careful postoperative monitoring of their allografts.

METHODS

PATIENTS

All PKPs performed for sequelae of HSV keratitis at the University of Michigan from August 1990 to December 2000 were included in this study. A total of 79 allografts were performed on 73 patients. Data were not available for 3 patients. Six patients were grafted twice during this time period and only their first grafts were eligible for inclusion, resulting in a total of 70 allografts. Eight other patients had primary grafts done prior to 1990 and had subsequent grafts done during our study period. These subsequent keratoplasties were excluded, leaving 62 primary grafts in this study. All surgeries were performed by corneal subspecialists. Charts were reviewed for the following information: disease-free time before surgery, quadrants of preoperative host vascularity, HSV recurrence, allograft rejection, allograft failure, and histopathologic presence of inflammation and neovascularization in the excised corneal tissue. This study received Institutional Review Board approval at the University of Michigan Medical Center.

Active HSV keratitis was diagnosed by the presence of dendritic or geographic epithelial keratitis, and/or ulceration. HSV keratouveitis was identified by the presence of keratic precipitates on both the donor and host endothelium. Graft rejection was defined by an anterior chamber reaction with keratic precipitates on the donor endothelium only, by an endothelial or epithelial rejection line, or by graft edema with associated keratic precipitates on the donor endothelium. Graft failure was defined as irreversible loss of graft clarity. Clinical quiescence of HSV infection was defined as no change on clinical examination for at least 6 months.

Postoperative oral acyclovir prophylaxis was prescribed in 51 of the 62 patients (82%). The initial dose used was variable, as was

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the tapering regimen; however, patients were taking at least 800 mg/day for an average of 6 months, and at least 400 mg/day for an average of 17 months. Postoperative topical prednisolone acetate 0.1% eye drops (averaging 4 times daily and subsequently tapered) were used in all patients. Episodes of HSV recurrence were treated with oral acyclovir or trifluridine eye drops (Viroptic, Glaxo Wellcome). Episodes of rejection were treated with prednisolone acetate 1% eye drops, tapered over several weeks.

PATHOLOGY

Each specimen removed from all 62 patients was examined grossly for regions of maximal vascularization, opacity, and variations in thickness. The specimen was then bisected along a secant 0.5 mm from and parallel to the diameter demonstrating, in order, maximal vascularization, opacity, and/or variable thickness. After routine processing, 6- μ m paraffin step sections were obtained at 100- μ m intervals for 1 mm of the specimen, straddling the diameter of maximal gross pathology. For some specimens the remainder of the fresh tissue was preserved at -70°C for further study. The paraffin sections were stained with hematoxylin and eosin. The sections from each specimen were evaluated and graded in the week subsequent to its removal by an ophthalmic pathologist (V.M.E.), who was masked as to all clinical details except for the diagnosis.

Each of the 62 specimens was rendered a pathologic diagnosis and graded for the presence of inflammation into 1 of 4 groups (none visible, mild, moderate, severe) and for the presence of neovascularization (none visible, present). Corneas designated as having no inflammation had no discernible leukocytes (Figure 1, top left). The presence of scattered or focal collections of leukocytes resulted in a designation of mild inflammation (Figure 1, top right). Moderate inflammation was characterized by more intense, patchy leukocytic infiltrates (Figure 1, bottom left). Severe inflammation comprised full-thickness, diffuse, deep and/or necrotizing inflammation (Figure 1, bottom right).

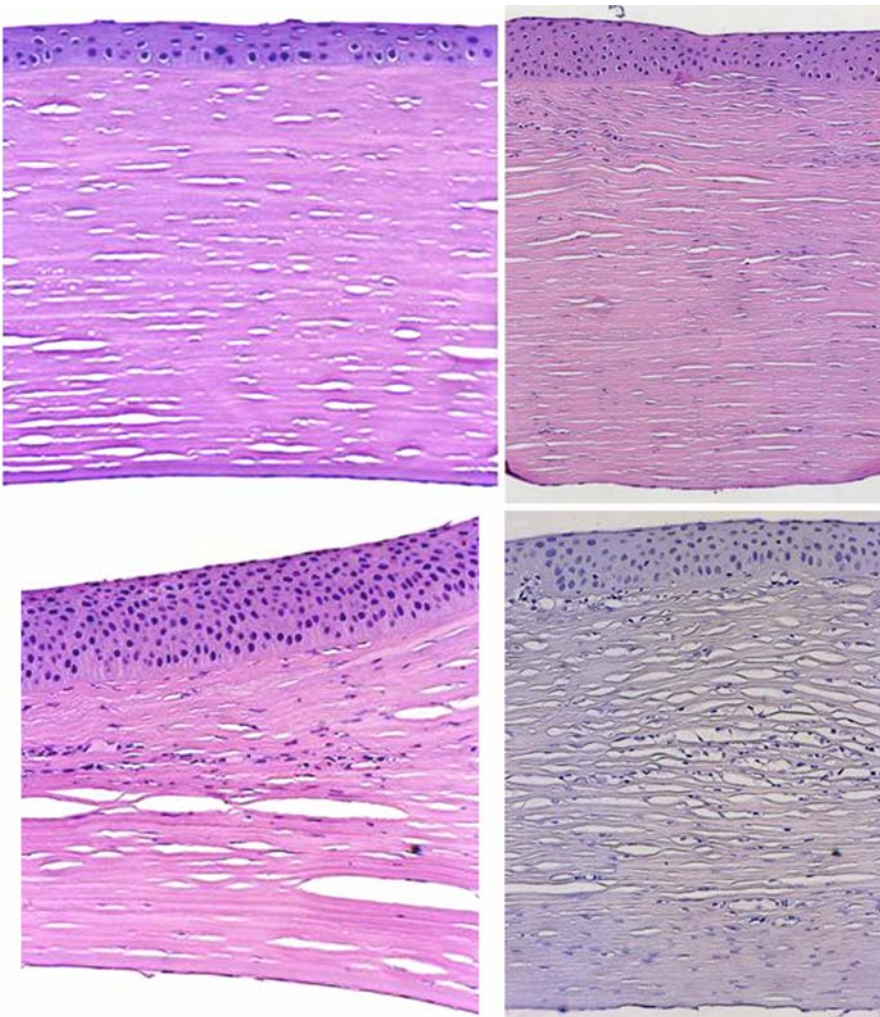


FIGURE 1

Degrees of inflammation in HSV stromal keratitis. Inflammation of tissue removed from patients at the time of penetrating keratoplasty was graded as none visible (top left), mild (top right), moderate (bottom left), and severe (bottom right) (hematoxylin-eosin, $\times 250$).

For 8 specimens, 4 without apparent inflammation and 4 with moderate to severe inflammation, fresh tissue was divided and portions of each were submerged in media alone or in media containing IL-10 (100 ng/mL) at 37°C for 24 hours. A portion of each specimen, treated and untreated, was processed for immunohistochemical detection and grading of HLA-DR and ICAM-1 as previously described.⁷ In brief, immunohistochemically stained tissue sections of corneal specimens were graded as 0 (no visible

staining), 1+ (intense staining in < 25% of cells), 2+ (intense staining in < 50% of cells), 3+ (intense staining in < 75% of cells), or 4+ (intense staining in > 75% of cells). Portions of these fresh corneal specimens were also processed for IL-8 and MCP-1 enzyme-linked immunosorbent assay (ELISA) as previously described.⁸

STATISTICAL ANALYSIS

The clinical and histopathologic data were analyzed using the chi-square test, Fisher exact test, analysis of variance, Kaplan-Meier survival curves, the log-rank test, and Cox regression. ELISA results were analyzed by *t* test with equal (IL-8) or unequal (MCP-1) variance and paired *t* test (IL-10 inhibition). Unless otherwise indicated, data are given as mean \pm SD. SAS 9.0 statistical software (SAS Institute, Cary, North Carolina) was used for the data analyses and comparisons.

RESULTS

The average patient age at surgery was 55 ± 22 years (range, 5-85 years), and the average disease duration was 19 ± 12 years (range, 0.25-72 years). Fifty-three percent of patients were female. The average duration of clinical quiescence before surgery was 50 ± 78 months (range, 3-360 months). Average follow-up was 43 ± 32 months (range, 3-142 months). Indications for surgery were corneal scarring in 60 patients (97%), descemetocele in 1 (1.5%), and perforation in 1 (1.5%). Nine patients had an HSV recurrence in their allograft during the study follow-up; 1 patient manifested with keratouveitis, 1 with geographic epithelial keratitis, and the remaining 7 with dendritic epithelial keratitis.

In spite of the fact that 50 of the patients (81%) had clinically quiet HSV disease for more than 6 months prior to PKP, only 16 patients (26%) had no histopathologically visible inflammation; 12 (19%) had mild, 15 (24%) had moderate, and 19 (31%) had severe inflammation (Figure 1). There was no association between the degree of histopathologic inflammation and the duration of clinically quiet disease prior to surgery ($P = .32$, ANOVA).

Of the 16 patients without any histopathologic inflammation in their corneas, only 1 (6.3%) experienced an allograft rejection. On the other hand, 20 (43.5%) of the 46 patients with histopathologic inflammation (mild, moderate, or severe) experienced a rejection. Figure 2 shows a Kaplan-Meier time to allograft rejection analysis in these 2 groups ($P = .02$, log-rank). Patients whose corneal button was graded in any of the 3 categories of histopathologic inflammation showed elevated hazard ratios (HRs) for allograft rejection relative to those without any inflammation, but the HR was statistically significant only in those with mild inflammatory signs: HR = 10.1 (95% confidence interval [CI], 1.2-84.6) for mild inflammation, HR = 6.6 (95% CI, 0.8-55.1) for moderate inflammation, and HR = 7.3 (95% CI, 0.9-59.1) for severe inflammation. We did not find any significant relationship between the presence of histopathologic inflammation and either graft failure ($P = .98$, log-rank test) or HSV recurrence ($P = .45$, log-rank test) in the allograft.

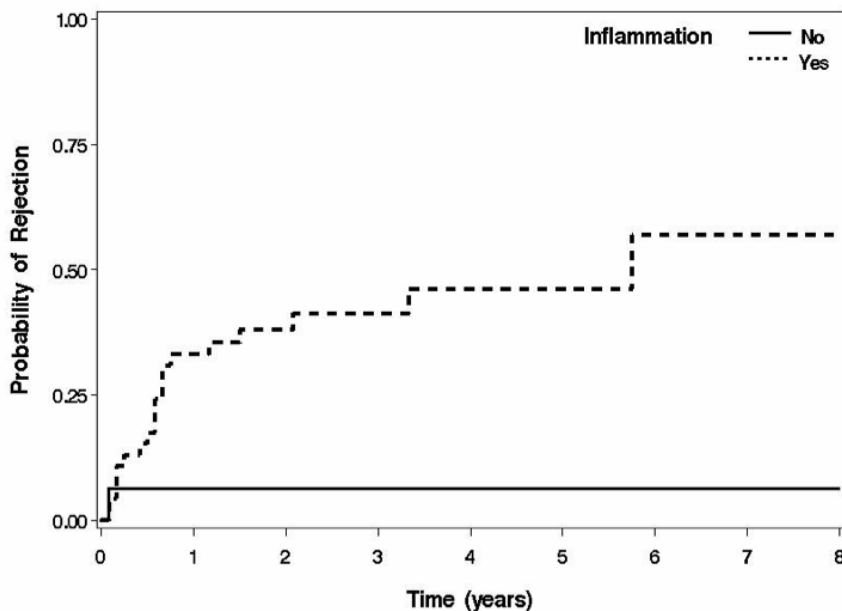


FIGURE 2

Kaplan-Meier survival curves of corneal allograft rejection in patients with HSV stromal keratitis with and without inflammation on histopathologic evaluation of their excised corneal tissue.

Corneal neovascularization was clinically present preoperatively in 39 patients (63%) but was seen histopathologically in only 19 (31%) of the specimens excised during PKP. In contrast, there was a statistically significant association between preoperative neovascularization and the histopathologic presence of inflammation ($P = .01$, extended Fisher exact test).

The histopathologic presence of neovascularization in the removed host corneal tissue did not predict allograft rejection but did predict graft failure ($P = .19$ and $P = .002$, respectively, log-rank test) (Figure 3). Of the 19 patients whose corneal specimens exhibited neovascularization, 4 (21%) subsequently had graft failure. In contrast, none of the 43 patients whose corneal tissue did not

have neovascularization developed graft failure. Kaplan-Meier analysis shows that histopathologic evidence of neovascularization was also associated with an increased rate of HSV recurrence ($P = .05$, log-rank test) in the allograft.

Immunohistochemical analysis was performed on 4 specimens with moderate or severe inflammation and 4 with none visible. These tissues were stained for the presence of HLA-DR and ICAM-1, both of which were increased in all 4 specimens with inflammation. In the presence of exogenous IL-10, HLA-DR staining in these same tissues was noticeably reduced (Figure 4); however, IL-10 had no effect on the amount of ICAM-1 immunopositivity (not shown).

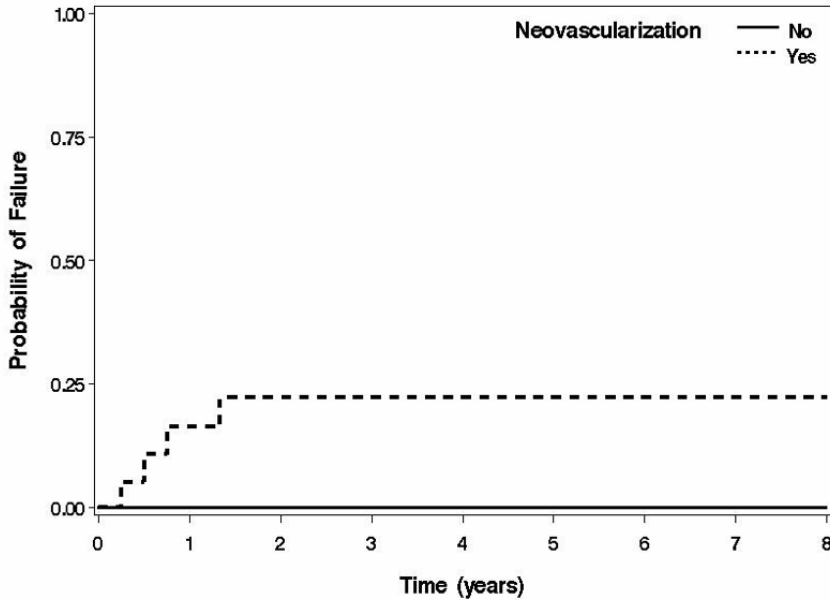


FIGURE 3

Kaplan-Meier survival curves of corneal allograft failure in patients with HSV stromal keratitis with and without neovascularization on histopathologic evaluation of their excised corneal tissue.

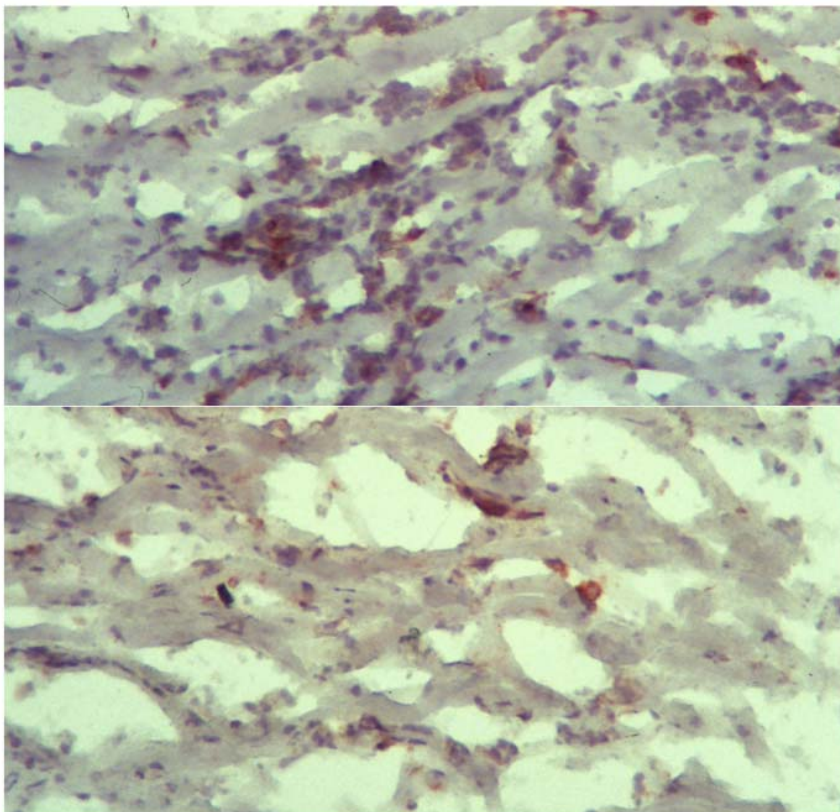


FIGURE 4

Immunohistochemical staining of HLA-DR antigens in corneal tissue with HSV stromal keratitis with moderate to severe inflammation untreated (top) or treated (bottom) with IL-10 ex vivo (hematoxylin counterstain, $\times 160$).

The same 8 corneal specimens were also evaluated for the presence of IL-8 and MCP-1 by ELISA. IL-8 (38 ± 16 ng/mg tissue)

and MCP-1 (4.9 ± 2.2 ng/mg tissue) were substantially increased in corneal specimens with moderate to severe inflammation as compared to IL-8 (4.6 ± 3.8 ng/mg tissue) and MCP-1 (1.7 ± 0.46 ng/mg tissue) in specimens with no visible inflammation (Figure 5). Inflamed specimens treated with media containing IL-10 (100 ng/mL) demonstrated significant inhibition of IL-8 ($82 \pm 14\%$) and MCP-1 ($54 \pm 16\%$) compared to tissue treated with media alone (Figure 6).

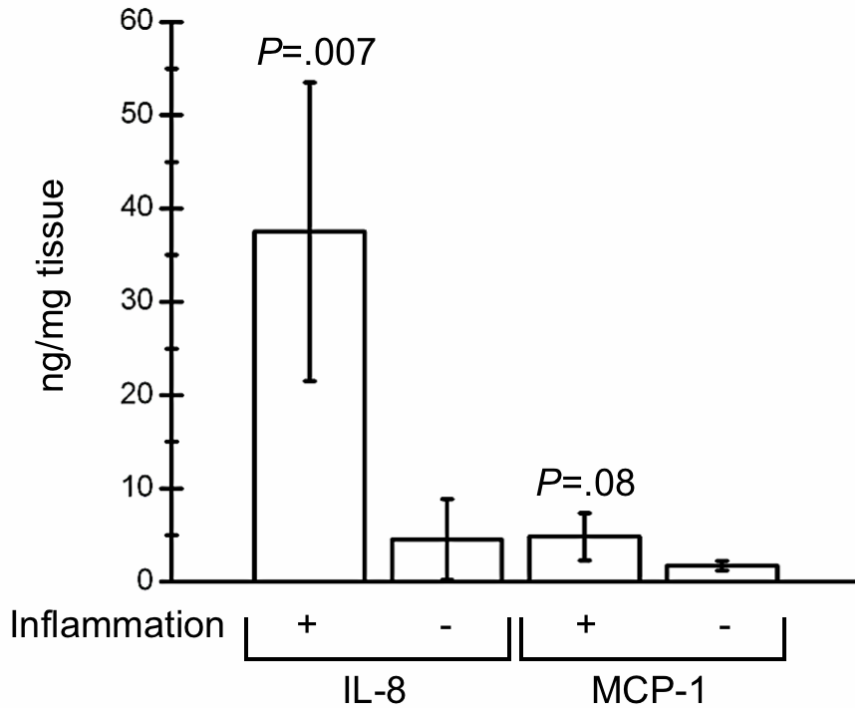


FIGURE 5
IL-8 and MCP-1 in corneal tissue with HSV stromal keratitis. IL-8 (left) and MCP-1 (right) ELISA of corneal tissue with moderate to severe (+) and without (-) visible inflammation.

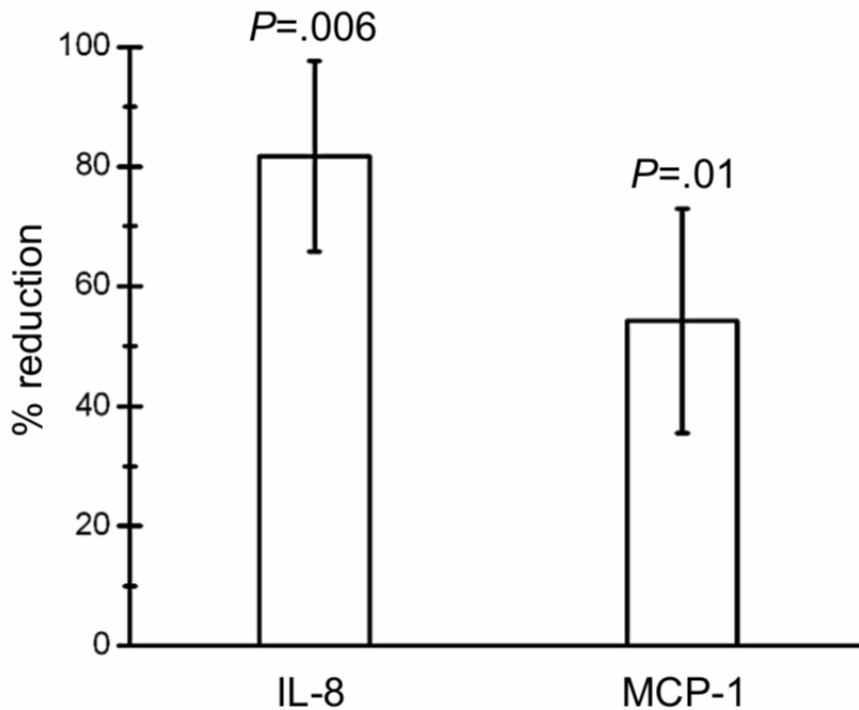


FIGURE 6
IL-8 and MCP-1 in corneal tissue with HSV stromal keratitis treated with IL-10. Percent inhibition of IL-8 (left) and MCP-1 (right), as measured by ELISA, of corneal tissue with moderate to severe inflammation after treatment with IL-10 ex vivo.

DISCUSSION

To our knowledge, there are no other published studies examining the relationship between histopathology of excised host corneal tissue and subsequent allograft outcomes. A study published in 2004 by Branco and associates⁹ looked at the records of all corneal tissue submitted from 1972 to 2001 to the pathology laboratory at the University of California at San Francisco. There were 4,207 grafts performed, 76 (1.8%) of which were for HSV keratitis. The investigators reported on the pathologic findings in corneas with a clinical diagnosis of HSV keratitis, including inflammatory cells in 87% and neovascularization in 59%. They did not comment on what effect the presence of these and other histopathologic findings had on subsequent graft rejection, failure, or HSV recurrence.

The significance of the histopathologic, immunohistochemical, and ELISA results in this study is emphasized by the fact that there were no statistically significant clinical variables predictive for HSV recurrence, graft rejection, and failure rates in this cohort.¹⁰ Our analysis of the tissue removed from these patients at the time of PKP reveals that subclinical inflammation predicts rejection, whereas histopathologic neovascularization predicts HSV recurrence and allograft failure. These findings are of practical significance to the clinician in care of patients after PKP.

Despite the fact that 81% of patients demonstrated clinically quiescent disease for at least 6 months, 74% had inflammation on histopathologic evaluation of their corneal tissue. In fact, over half of the specimens had moderate or severe histopathologic inflammation. This supports our hypothesis that substantial inflammation exists even when clinical signs are absent. Inflammation within the clinically quiescent corneal tissue probably reflects the putative mechanisms of host immune responses to residual viral antigens or virally altered cell proteins as propagators of inflammation even after successful clearance of intact virus.^{11,12} Our immunohistochemical and chemokine data of the inflamed corneal tissues improve our understanding of the corneal inflammatory response to HSV infection. We previously showed HLA-DR and ICAM-1 expression to be increased in HSV stromal keratitis¹³ and demonstrated reduced expression of HLA-DR, but not ICAM-1 due to IL-10 treatment,⁷ findings that were confirmed in this study. We now also show that there are substantial levels of leukocytic chemokines in corneas with clinically quiescent HSV stromal keratitis. These chemokines are known to attract and stimulate various leukocyte subsets, and their presence is likely to participate in the perpetuation of the stromal disease.

Studies in murine models have shown that IL-10 has the ability to lessen the severity of HSV keratitis without impairing viral clearance or reducing host resistance to the virus.¹⁴ Initial observations in a murine model of HSV keratitis suggested that MCP-1 did not play an important role.¹⁵ This may be due to the fact that a neutrophil response predominates in the murine model and appears to be driven by neutrophil chemokines, principally MIP-2.¹⁵ A subsequent study, however, showed that even in the murine model, in which mononuclear phagocytes compose only a minority of the corneal cell infiltrate, there is some protective effect of MCP-1 against the development of HSV keratitis.¹⁶

In humans, HSV stromal keratitis is characterized by a mixed infiltrate composed of chronic inflammatory cells, including lymphocytes, neutrophils, and mononuclear phagocytes.¹⁷ Corresponding to the histopathologic findings in human disease, we found that both IL-8, a neutrophil and lymphocyte chemokine, and MCP-1, a mononuclear phagocyte chemokine, were elevated in our samples. In addition, both were substantially suppressed by ex vivo IL-10 treatment of the excised corneal buttons that demonstrated moderate to severe inflammation histopathologically. Our observations of IL-10 effects on IL-8, MCP-1, and HLA-DR raise the possibility that IL-10 is a potential therapeutic agent to reduce the severity of keratitis in humans while permitting viral clearing as it does in the murine model.

Pathogenetically, the presence of inflammation, which we found to correlate with subsequent graft rejection, may be due to the fact that such inflammation in HSV keratitis is associated with increased corneal expression of HLA-DR antigens and ICAM-1.^{7,13,18} In this study, expression of these markers was found preferentially at sites of active keratitis and correlated with the degree of inflammation. Expression of these molecules is known to enhance antigen recognition and subsequent allograft rejection, which we found to correlate with the presence of inflammation.¹³ Although these grafts were prone to rejection, careful follow-up and intensive therapy of rejection episodes were able to preserve functioning grafts in many cases.^{10,19}

Corneal neovascularization was present preoperatively in 63% of patients on clinical examination. However, it was found histopathologically in only 31% of the specimens excised during PKP. This discrepancy may have resulted from clinical examinations, which documented peripheral preoperative corneal neovascularization in tissue not removed during PKP. It is unlikely that histopathologic sectioning did not include an axis involved with neovascularization, as the specimens were examined under a dissecting microscope and sectioned along the axis of greatest neovascularization as described above.

There was a statistically significant correspondence between the presence of preoperative clinical corneal neovascularization and the histopathologic presence of inflammation in the same corneal specimens after removal at PKP ($P = .01$). This suggests that an important preoperative clinical factor indicative of actual inflammation in the diseased cornea is the presence of corneal neovascularization, and not necessarily the interval of apparent clinical quiescence before surgery. This association between preoperative clinical corneal neovascularization and the histopathologic presence of inflammation in the excised tissue has important pathogenetic and clinical implications. Neovascularization may be essential to the delivery of cellular and serum components of inflammation and host immune responses, setting the stage for graft rejection and failure.^{20,21} Further, this study showed that the presence of any histopathologic inflammation in the excised host corneal tissue was associated with a statistically significant increase in graft rejection rate ($P = .02$). The histopathologic presence of neovascularization in the corneal tissue is an important predictor of graft failure ($P = .002$). Its presence within the central corneal tissue that is removed at the time of PKP probably indicates that these patients had severe stromal disease, placing them at higher risk for subsequent failure. Identifying these features in patients and their

excised tissues may be helpful in identifying the patients who are at highest risk for adverse allograft outcomes. These patients would have the most to gain from careful postoperative monitoring.

Although this study was retrospective, the histopathologic features of neovascularization and leukocyte infiltration and the immunohistochemical findings regarding HLA-DR, ICAM-1, IL-8, and MCP-1 in the removed host corneal tissue were well defined, as were the clinical end points of graft rejection, failure, and HSV recurrence. This lends confidence that the conclusions drawn from the study are likely to be true and clinically relevant. Moreover, as the histopathologic findings were determined in tissue processed in the usual fashion for corneal surgical pathology specimens, the observations made are relevant to routine clinical practice. This establishes a role for the pathologist in assisting clinicians in their choice of postoperative patient management.

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Conformity With Author Information: This study received Institutional Review Board approval at the University of Michigan Medical Center.

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PEER DISCUSSION

DR. STEVEN E. WILSON: Shtein and coworkers are to be congratulated for an exceptional study analyzing histopathologic abnormalities in corneal tissue removed at penetrating keratoplasty for herpes simplex virus keratitis that potentially correlate with clinical events such as allograft rejection and corneal graft failure. The investigators found several important findings that have clinical implications for optimal care for patients undergoing corneal transplantation for herpes simplex keratitis in this study of sixty-two eyes. First, although eighty-one percent of patients had clinically quiescent disease—defined by the investigators as no change on clinical examination for at least six months—seventy-four percent had corneal inflammation detected in the excised tissue, analyzed with hematoxylin and eosin staining of corneal sections. It seems likely that more sensitive methods for detecting inflammation—such as immunofluorescent staining for inflammatory cell markers—would have detected at least low-grade inflammation in an even higher proportion of corneas. As expected, active corneal inflammation was associated with the presence of clinical neovascularization. Interestingly, histopathologic inflammation was a risk factor for allograft rejection, but not graft failure in this series. This is likely related to effective treatment of allograft rejection by clinicians caring for the patients. Histopathologic neovascularization was a predictor for graft failure and recurrence of herpes simplex keratitis. Although histopathologic neovascularization was not a predictor of allograft rejection in this study ($p = 0.19$), this correlation might have been found if more transplanted eyes had been included in the study.

As with any retrospective analysis, there are limitations in the present study. These include variability in acyclovir prophylaxis and corticosteroid treatment after penetrating keratoplasty. It would have also been useful for the authors to include a non-herpes simplex keratitis control group in both the histopathologic studies and the studies of the expression of markers such as HLA-DR and ICAM-1. For example, this would have allowed the authors to determine whether the surprising level of inflammation detected in corneas that were quiescent was related directly to herpes simplex or a characteristic of corneas undergoing penetrating keratoplasty for many disorders.

The results of this study suggest the intriguing possibility that long term suppression of inflammation following corneal transplantation for sequelae of herpes simplex keratitis infection could decrease the incidence of allograft rejection. Long term administration of corticosteroids in these patients is not only associated with complications such as glaucoma and cataract, but also may promote reactivation of herpes keratitis and worsen the course of infection when there is a recurrence.¹ An alternative approach that could be considered is long-term administration of topical cyclosporine. As a member of the team that designed and implemented the phase III clinical trial for topical cyclosporine A for chronic dry eye, I recall my major concern with that study was the potential for the topical drug to reactivate herpes simplex keratitis, even in patients with no known history of herpes simplex keratitis. However, in hundreds of thousands of patients who have taken 0.05% cyclosporine A since the FDA approval in December of 2002, this concern has not been realized. In fact, I personally have yet to observe a single case of herpes simplex keratitis, primary or recurrence, in a patient taking topical cyclosporine A. This is indeed surprising. By chance alone, I would have expected to see herpes keratitis in a patient concurrently administering topical cyclosporine A. Why has this been such a rare association? I have found myself wondering whether cyclosporine A has some anti-viral effect on herpes simplex virus that blocks corneal infection. Surprisingly, I could find no studies published on potential anti-viral effects of cyclosporine on herpes simplex virus in vitro, even though anti-viral effects of cyclosporine A on hepatitis C virus have been reported.^{2,3} One clinical study has noted that topical cyclosporine A can suppress stromal inflammation associated with herpes simplex keratitis.⁴ Further investigation would seem to be warranted to determine whether topical cyclosporine A treatment could be used effectively to decrease the risk of allograft rejection, and possibly long-term transplant viability, in patients with herpes simplex keratitis.

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DR. RALPH C. EAGLE, JR: No conflicts. From my personal experience, I totally agree that corneal buttons excised from patients with herpetic keratitis have inflammation; in fact, I was surprised there were so many corneas that were not inflamed in this series. I do have a question about the subcategory of inflammation in these cases. You typically find lymphocytes and plasma cells, and not infrequently, epithelioid histiocytes. Textbooks typically emphasize a giant cell reaction to Descemet's membrane, but the latter is relatively uncommon in my experience. I wonder how many of the patients had granulomatous inflammation and whether any of them had a giant cell reaction to Descemet's membrane. Thank you.

DR. MARK A. TERRY: I have no financial interest in this topic. First of all, this is an excellent paper. I just wanted to bring up the point that there is a subset of patients with herpes simplex keratitis with viable corneal endothelium, regardless of the level of corneal inflammation. In fact, it is a large proportion of the cases that I have seen. Those patients with viable endothelium in the face of anterior stromal scars from HSV are eligible for a technique of deep anterior lamellar keratoplasty (DALK), with which we can preserve the endothelium of the recipient and replace everything else in the cornea. We have found that this option of DALK surgery has allowed us minimize the problem of graft rejection. Although it certainly is possible to have a stromal graft rejection, in my experience with DALK suggests that it does not occur in herpes simplex keratitis. In these patients we can restore vision quickly and more easily control the herpes simplex recurrences because we can reduce the corticosteroid dose within a matter of months and keep them off corticosteroids. I think DALK is a very good option in this situation.

DR. WOODFORD S. VAN METER: I have no financial conflicts to disclose. I commend the authors for helping to identify some features that explain why herpes simplex grafts do poorly. We know that these eyes fall into the poor prognostic category of corneal transplants. I would like to inquire if they can provide more information on the postoperative medical regimen regarding whether the patients received drops every four hours, every two hours, or every one hour. If given every one hour, then did they take them during the night? Certainly there is a wide variation in the quality of corticosteroid drops. We know that brand name Pred Forte®, prednisolone acetate, is a different preparation than some of the generics that are frequently pushed by drug companies. We also know that compliance with medications varies and thanks to Allan Flach we know that not all patients occlude their tear ducts or close their eyes after they have instilled their drops. Since some of these inflammatory features are very difficult for pathologists or surgeons to recognize, I believe this places special importance on the postoperative medication regimen. We may need to rethink our postoperative treatment of herpes simplex corneal grafts. Although many patients and certainly some ophthalmologist believe that the goal of the procedure is to achieve a clear graft without topical medications, we may need to establish some protocol that utilizes topical or systemic medications to preserve a clear graft and not expect patients to discontinue topical corticosteroids.

DR. VERINDER S. NIRANKARI: I have no financial conflict. I really enjoyed your talk. What struck me was that 81% of your patients were clinically quiescent, but by histopathologic examination, 74% had active inflammation. I believe that the only reliable way to determine if patients with previous herpes simplex keratitis or any other kind of chronic inflammatory disease have corneal neovascularization is to perform corneal fluorescein angiography. This subject was part of my AOS thesis. Many times you will clinically see scar tissue with ghost vessels, and incorrectly conclude that inflammation is not present, but when you perform corneal angiography many of these blood vessels will fill with the dye and become clinically apparent. We know that abnormal blood vessels leak inflammatory products into the cornea and that active inflammation exists even though clinically you cannot see it. Unless you can demonstrate that there are limited corneal blood vessels, I believe that you will most likely face active inflammation after the transplant and obviously this will influence the postoperative care of these patients. I believe corneal fluorescein angiography is a very useful technique in these eyes

DR. CHRISTOPHER J. RAPUANO: No financial interest. Very nice paper. I have two quick questions. First, did the histopathologically demonstrated inflammation correlate with the time since the last episode of clinical inflammation? If so, then maybe waiting six months for surgery just is not long enough? Perhaps we need to wait for 9, 12 or 24 months. Second, to piggy back on Woody Van Meter's question, was there a difference in the corticosteroid dose at the time of the rejection episode in the patients with inflammation versus the patients without inflammation? Thank you.

DR. REZA DANA: No conflict. Nice paper. I have two questions and a comment. In terms of your data, did you look at the potential correlation of this cell infiltration and the type of chemokines that you exhibited were elevated? As you know, the CXC chemokine isolate that you looked at is primarily functional for neutrophils, whereas MCP1 works on macrophages and to some extent on CCR2 - expressing T cells. Could you share some insight into that? Secondly, we have shown that the presence of lymphatics is also very important in terms of predicting the alloimmune response, because that is the venue that allows antigen-presenting cells to carry antigenic information from the graft to the lymphoid compartment. I wondered if you have looked at it histopathologically, for example, staining for LYVE-1+ lymphatics. Lastly, as a quick comment, we have been very impressed with the use of confocal microscopy for detecting inflammation, when it is not appreciable with standard slitlamp biomicroscopy. I wonder if you have used that technique in other studies. Thank you.

DR. RONI M. SHTEIN: Thank you for your comments and questions. I agree with Dr. Wilson's opinion that immunofluorescent studies would identify cell subsets yielding more details about the type of inflammation present in these corneas. However, they were not performed in our retrospective study. Although this is certainly information that would be useful in elucidating the pathophysiology of disease, our study, performed in a retrospective manner, did not allow for analysis of fresh tissue. Nevertheless, we did find clinically relevant information since we reviewed the histopathologic findings in corneal tissue, as they are reported to the

clinician by the pathologist, based on routine surgical pathology evaluation. Certainly, additional useful information could be obtained from further investigations of HSV corneal tissue in a prospective manner.

The clinical regimen of acyclovir prophylaxis and prednisolone acetate used in these patients for postoperative treatment and graft rejection episodes was variable. There is a related paper on this cohort that was published last year evaluating the significance of various clinical parameters, including medication regimens, on allograft outcomes. A multivariate analysis of various clinical characteristics, including medication regimen, did not reveal any individual clinical variables that correlated with poor allograft outcomes. Certainly, a control group would be helpful in evaluating our findings further, and I also thought that Dr. Wilson's comments about the use of cyclosporine A in these patients was interesting and worth further investigation.

I also agree that deep anterior lamellar keratoplasty could be potentially very useful in these patients. We know that they are at high risk for allograft rejection and failure, and any technique that minimizes these risks will be useful. The comments about clinically undetected neovascularization of the cornea are relevant as subclinical neovascularization may well be of critical importance in herpes simplex and other chronic inflammatory diseases. Most likely, conditions that are difficult to treat have contributing factors that we are unable to detect with our current clinical examination. The use of the confocal microscope and anterior segment fluorescein angiography would provide more detailed information regarding inflammation and neovascularization that might be of clinical relevance in guiding the specific treatment for these patients. Ultimately, a prospective study to investigate more details of the histopathology of these corneas would certainly be useful. Thank you.