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Cellular Infiltrate in Rheumatoid Arthritis Associated Paracentral Corneal Ulceration

Carolyn M. Kalsow, PhD, Steven S. S. T. Ching, MD, and Ronald D. Plotnik, MD

Department of Ophthalmology, University of Rochester School of Medicine and Dentistry
Rochester, New York, USA

Abstract

Purpose.—To investigate an immunopathogenesis of central and paracentral corneal ulceration associated with rheumatoid arthritis.

Methods.—Sparse infiltrating cells in the ulcer area were identified by immunohistochemistry applied to archived formalin fixed, paraffin embedded tissues that had been recovered from patients undergoing penetrating keratoplasty necessitated by rheumatoid associated central or paracentral corneal ulceration.

Results—Clinically the ulcers presented as noninfiltrated lesions with a modicum of other ocular inflammation. Sparse T lymphocytes were consistently identified in the subepithelial areas adjacent to the ulcer, with some neutrophils and macrophages in the stroma. B lymphocytes were not detected. MHC Class II antigens reactivity was noted on some infiltrating cells and on corneal endothelium of 2 specimens.

Conclusion—Immunohistochemistry of archival tissue facilitated detection and identification of sparse infiltrate into this infrequent corneal melting. Selective, consistent finding of T lymphocyte infiltration in the ulcer area supports an immunopathogenesis of this clinical entity.

Keywords

corneal ulcer; immunohistochemistry; infiltrate; rheumatoid arthritis; T lymphocytes

INTRODUCTION

Rheumatoid arthritis is a systemic autoimmune disease that may directly or indirectly target the cornea as dry eye syndrome, episcleritis, scleritis or peripheral ulcerative keratitis (PUK)¹. Central or paracentral corneal ulceration (CPCU) is an uncommon but serious ocular complication of rheumatoid arthritis^{2,3}. The central location and modicum of ocular surface inflammation of this sterile ulceration differentiate it from the more frequently documented PUK also associated with rheumatoid arthritis^{3,4}. These ulcers appear spontaneously, often with quiescent systemic arthritis. Clinically, the sterile lesions appear

Correspondence: Carolyn M. Kalsow Present addresses: Ocular Research Services, P. O. Box 202, Mendon, NY 14506, USA. ckalsow@att.net.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

non-infiltrated with no associated scleritis, iritis or conjunctivitis. Since the ulcers are difficult to manage and often progress to perforation, prompt recognition is critical to appropriate treatment.

The juxtaposition of corneal structural changes in a non-inflammatory milieu, complicates understanding the pathologic mechanisms of this entity. Since the associated rheumatoid arthritis pathogenesis involves both immune and collagen abnormalities, either or both could participate in the cornea. Immune pathogenesis in CPCU is supported by immunohistochemical demonstration of a few activated immune cells in the ulcer area^{2,4}, expression of inflammatory cytokines IL-6 and TNF- α ⁵, and a positive response to immunoregulatory treatment with cyclosporine⁴. Ultrastructural collagen alterations and biochemical changes have also been noted in the melting cornea³.

Further study of CPCU has been hampered by a paucity of quality samples for study in a given institution at a given time. Use of frozen tissue limits the number of cases that can be included in a given study and compromises the morphological detail of the specimens. Here we selected and validated antibodies that are reactive in formalin fixed/paraffin embedded tissue to extend identification of immune cells in the ulcer and adjacent area as well as to evaluate the state of host tissue.

MATERIALS AND METHODS

Case Selection and Specimens

Cases were selected from patients (SSTC and RDP) who had undergone penetrating keratoplasty as a result of rheumatoid arthritis-related central/paracentral corneal perforations. Standard formalin fixed, paraffin embedded tissue blocks of corneal tissue obtained at the time of penetrating keratoplasty were collected from the surgical pathology laboratories of Strong Memorial and St. Mary's Hospitals, Rochester, NY and University of Iowa Hospital, Iowa City, IA. Rochester Eye and Human Parts Bank, Rochester, NY provided negative control corneas and conjunctiva with no known or observable corneal pathology.

Cases

Case 1.—A 74 y.o. white male with a 30 year history of rheumatoid arthritis presented to the University of Rochester with bilateral central corneal ulcerations (Figure 1a). There was no clinically detectable infiltrate or active joint disease. The ulcerations were treated with cyanoacrylate tissue adhesive that subsequently failed necessitating penetrating keratoplasty. A second melt required another penetrating keratoplasty a year later.

Case2.—A 74 y.o. white female with a 20 year history of rheumatoid arthritis presented to the University of Rochester on 12/23/92 with a descemetocoele that ruptured while applying cyanoacrylate tissue adhesive. There was no clinically detectable infiltrate or active joint disease.

Case 3.—A 60 y.o. white female with a longstanding history of rheumatoid arthritis presented to the University of Iowa on 11/81 with paracentral corneal thinning and a small

area of pannus (Figure 1b). The epithelium was intact and there was no history of dry eye. On 2/13/82, she presented with an asymptomatic perforation in previously thinned cornea. There was no clinically detectable infiltrate or active joint disease.

Case 4.—A 60 y.o. black female with a 20 year history of rheumatoid arthritis presented to the University of Rochester on 3/20/92 with a two week history of blurred vision and epiphora. There was no clinically detectable infiltrate or active joint disease. A perforated paracentral corneal ulcer with a nasal pterygium bordering the ulcer bed was treated with tissue adhesive. This subsequently failed necessitating penetrating keratoplasty (Figure 1). The patient developed OD recurrent, paracentral ulceration and perforation on 7/26/93.

Immunohistochemistry

A peroxidase anti-peroxidase (PAP) method was used for immunoenzymatic visualization of monoclonal antibody (MAb) reactivity. Four to six μm sections were cleared and treated with 3% H_2O_2 for 5 min. to remove endogenous peroxidase and were blocked with 5% normal rabbit serum or SuperBlock Blocking Buffer (Pierce, Rockford, IL) for 20 min. at room temperature in a humid chamber. Rinsed sections were treated with MAb (DAKO Corp., Santa Barbara, CA) (Table) overnight at 4°C, and with rabbit anti-mouse immunoglobulins (10–2)(DAKO Corp., Santa Barbara, CA) and DAKO mouse PAP (5 $\times$ 10–2) for 20 min. at room temperature. Peroxidase was visualized with DAB Metal Enhanced Substrate Kit (Pierce) for 20 min. at room temperature. Sections were dehydrated for mounting in Refrax (Anatech, Ltd., Battle Creek, MI), viewed by brightfield microscopy and photographed with Royal Gold 100 film (Eastman Kodak Co., Rochester, NY). Reactivity of these MAbs on formalin fixed tissue was confirmed on formalin fixed human tonsil (Department of Surgical Pathology, University of Rochester, Rochester, NY.) and on formalin fixed human conjunctiva (Rochester Eye and Human Parts Bank, Rochester, NY). Immunohistochemical reactivity was compared with hematoxylin and eosin (H+E) stained sections of the corneal tissue.

RESULTS

Reactivity of the test monoclonal antibodies was validated as appropriate reactivity with human lymphoid and conjunctival tissue. Reactivity was not observed on normal human cornea.

Consistent with the clinical appearance, few inflammatory cells were observed in H+E sections of the excised corneas (Figure 2A,B 3) including failed grafts. In each of the specimens, cells reactive with the T lymphocyte specific antibodies were readily observed in areas of epidermolysis adjacent to the corneal ulceration (Figure 2C, 3). In these same areas scattered cells reactive for human neutrophil elastase were observed in the stroma but not in the epithelium (Figure 2D). Cells reactive with the B lymphocyte specific antibodies were not found in the corneas near the ulcers, although reactive cells were observed in the ruptured descemetocoele of Case 2 and the ulcer area adjacent to a pterygium and perforated ulcer of Case 4.

Some antibodies, although reactive, did not give similar patterns of staining for all specimens. Patterns of staining with the monocyte/histocyte specific antibody were not consistent. Very few cells reactive with the antibodies specific for macrophage CD68 were observed in some ulcer areas (Figure 2E), although there was also of strong reactivity in other regions of the cornea including diffuse staining in epithelial areas making distinction between specific infiltrating cells, resident cells and nonspecific staining difficult. Reactivity with antibodies specific for MHC Class II was also variable. In three of the cases (1,3,and 4) MHC Class II reactive cells were observed in the subepithelial or stromal area. MHC Class II reactivity in endothelium was observed in cases 1 and 2 (Figure 2F).

DISCUSSION

Immunohistochemistry of formalin fixed, paraffin embedded archival tissue was used to study a series of cases of rheumatoid associated central and paracentral corneal ulcers. In contrast to infiltrated peripheral ulcers (PUK) associated with rheumatoid arthritis or those associated with bacterial infection, corneal infiltrate in these cases was minimal. The method yielded consistent demonstration of subepithelial T lymphocytes in the areas of corneal ulceration, neutrophils in the stroma, with an absence of B lymphocyte reactivity. Although there was reactivity with the antibodies specific for MHC Class II, monocyte/histocyte and macrophages, there were no consistent patterns associated with CPCU.

The use of archival tissue was of value in providing multiple specimens from different institutions over an extended period of time. In this rare entity, permanence of tissue blocks and quality architecture of formalin fixed paraffin embedded tissue allowed visualization and localization of scarce participating cells that could be consistently identified and localized in affected corneas. Development of additional antibodies and antigen retrieval techniques can be used to further refine the identification and localization of infiltrating and resident cells.

The results reported here are similar to but not totally consistent with previous studies of central/paracentral rheumatoid associated corneal ulcers. Consistent reactivity of isolated T lymphocytes and neutrophils here is contrasted to the absence of T lymphocyte reactivity in one study² but more numerous T lymphocytes and neutrophils in another⁴. None of the studies reported any B lymphocyte reactivity. In contrast to a report of strong MHC Class II epithelial reactivity⁴, such reactivity was not observed here or in a previous study². This is the first report of MHC Class II reactivity in the endothelium of affected corneas. Since reactivity was not strong, it may not have been observable in frozen sections. Use of different antibodies and frozen sections of corneas as well as treatment prior to specimen collection could account for variances in results.

Just as the systemic immune response of rheumatoid arthritis contributes to joint pathologic changes, so this immune response can likewise promote corneal structural changes⁴. Even though the nature of these reactions are yet to be fully understood, collagen abnormalities in this corneal melting have been observed in electron microscopic demonstration of alterations of the corneal extracellular matrix⁴. Such changes in cases of CPCU have been described as similar to those of ruptured hand tendons in rheumatoid arthritis³. An imbalance of collagenase and its inhibitors has also been noted in sterile corneal ulceration⁵, and

collagenase was detected in the corneas of this study (data not shown). Changes in corneal biomechanical properties such as corneal hysteresis have been measured in corneas of patients with rheumatoid arthritis even in the absence of ocular involvement or active systemic disease⁶. Hence the systemic immune activity could affect corneal structural changes prior to clinical signs of corneal melting.

Results presented here provide further support for an immunopathogenesis of CPCU. Consistent presence of T lymphocytes and neutrophils along with MHC Class II expression on the endothelium indicates immune activation at these sites even with a modicum of inflammation and a central/paracentral location. Previous studies also provided evidence of an associated immune response at the ocular surface: immunohistochemical demonstration of activated immune cells^{2,4}, expression of inflammatory cytokines IL-6 and TNF- α ⁷, and a positive response to immunoregulatory treatment with cyclosporine⁴. The systemic immune response associated with rheumatoid arthritis has been suggested to contribute to CPCU following cataract surgery in patients with rheumatoid arthritis⁸. Furthermore, confocal microscopy showing beadlike formations on corneal nerves and activation of keratocytes⁹ and altered Langerhans cell density and morphology¹⁰ suggests a generalized state of activation of the cornea in cases of rheumatoid arthritis even in the absence of corneal involvement.

The paucity of inflammation in paracentral melting even with recurrent grafts suggests an immunodysregulation in the cornea. Just as premature immunosenescence has been observed in rheumatoid arthritis in general¹², this contraction of the T-cell compartment and resulting overcompensation by the remaining cells leading to immune responses including autoimmunity¹³ could also affect the cornea. Decreased graft survival noted here is consistent with decreased graft survival reported with rheumatoid associated corneal ulcers in general¹⁴. Hence the immunopathogenesis of both corneal and systemic manifestations of rheumatoid arthritis may involve aberrations of the immune response.

Central or paracentral corneal location of the ulcer along with minimal or absent accompanying inflammation of CPCU generally distinguishes it from other corneal conditions associate with systemic rheumatoid arthritis^{1,2,4}. In the CPCU cases reported here the rheumatoid arthritis was quiet and there was no clinical evidence of systemic or focal limbal vasculitis, overt dry eye, or neurotropic cornea. Since treatment of CPCU can be difficult and often lead to poor results, awareness and recognition of CPCU as a corneal melt associated with rheumatoid arthritis could initiate timely and appropriate treatment.

Evidence presented here supports an immunopathogenesis of CPCU even in the absence of overt ocular surface inflammation, thereby indicating a value of immunologic modulation in these cases^{4,13}. (Of note, one patient had been treated with topical cyclosporine 2% without improvement in her course.) A role of various immunomodulators both topical and systemic might be considered in treatment of this non infective central corneal ulceration in patients with rheumatoid arthritis.

In summary, immunohistochemistry of archival tissue facilitated detection and identification of sparse infiltrate into this infrequent corneal melting. Selective, consistent finding of T lymphocyte infiltration in the ulcer area and MHC Class II expression on the endothelium

supports an immune mediated pathogenesis of this central/paracentral corneal ulcers associated with rheumatoid arthritis and thus supports the value of immunomodulating treatment.

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REFERENCES

1. Artifoni M, Rothschild P-R, Brézin A et al. Ocular inflammatory diseases associated with rheumatoid arthritis. *Nat Rev Rheumatol*. 2014; 10108–116.
2. Michels ML, Cobo LM, Caldwell DS et al. Rheumatoid arthritis and sterile corneal ulceration. *Arth Rheum*. 1984;27606–614.
3. Adachi W, Nishida K, Quantock AJ et al. Ultrastructural alterations in the stroma adjacent to non-inflammatory corneal perforations associated with long standing rheumatoid arthritis. *Brit J Ophthalmol*. 1988;821445–1446.
4. Kervick GN, Pflugfelder SC, Haimovici R et al. Paracentral rheumatoid corneal ulceration. Clinical features and cyclosporine therapy. *Ophthalmol*. 1992;9980–88.
5. Riley GP, Harrall PG, Watson PG et al. Collagenase (MMP-1) and TIMP-1 in destructive corneal disease associated with rheumatoid arthritis. *Eye*. 1995;9703–718.
6. Prada J, Noelle B, Haatz H et al. Tumour necrosis factor alpha and interleukin 6 gene expression in keratocytes from patients with rheumatoid corneal ulcerations. *Brit J Ophthalmol*. 2003;87548–550.
7. Cohen KL. Sterile corneal perforation after cataract surgery in Sjögren's syndrome. *Brit J Ophthalmol*. 1982;66179–182.
8. Villani E, Galimberti D, Viola F et al. Corneal involvement in rheumatoid arthritis: an in vivo confocal study. *Invest Ophthalmol Vis Sci*. 2008;49560–564.
9. Marsovszky L, Resch M, Németh J et al. In vivo confocal microscopic evaluation of corneal Langerhans cell density, and distribution and evaluation of dry eye in rheumatoid arthritis. *Innate Immunity*. 2012;19348–354.
10. Can ME, Erten S, Can GD et al. Corneal biomechanical properties in rheumatoid arthritis. *Eye & Cont Lens*. 2015
11. Hohensinner PJ, Goronzy JJ, Weyand CM. Targets of immune regeneration in rheumatoid arthritis. *Mayo Clin Proc*. 2014;89463–475.
12. Weyand CM, Yang Z, Goronzy JJ. T-cell aging in rheumatoid arthritis. *Curr Opin Rheumatol*. 2014;2693–100.
13. Stylianides A, Jones MNA, Steward RMK et al. Rheumatoid arthritis-associated corneal ulceration: mortality and graft survival. *Ophthalmol*. 2013;120682–686.
14. Jayson MIV, Easty DL. Ulceration of the cornea in rheumatoid arthritis. *Ann Rheum Dis*. 1977;36428–432.

Figure 1.

Central/paracentral corneal ulcers associated with rheumatoid arthritis. Case 1: Central ulceration with no clinically detectable infiltrate at presentation but vascularization after glue application (A) and paracentral ulcer in graft (B). Case 3: Paracentral corneal thinning with central pedicle of stroma and inferior pannus (C) and asymptomatic perforation 3 months later in previously thinned cornea (D). Case 4: Recurrent ulceration in graft, low and higher magnification (E,F).

Figure 2.

Immunohistochemistry of central/paracentral corneal ulcers. H+E of excised corneal graft showing minimal infiltrate (original magnification 40X (A), 312.5X (B)).

Immunohistochemical reactivity with antibodies specific for T lymphocytes at edge of an ulcer (C), neutrophil Elastase reactivity of neutrophils in the stroma (D), myeloid/histiocytes in stroma (E), and MHC Class II reactivity at endothelium (F), (original magnification 312.5X).

Figure 3.

T lymphocyte infiltration detected in excised corneas from central/paracentral rheumatoid associated ulcers. T lymphocyte immunoreactivity in an area of epidermolysis adjacent to the ulceration (A,B) and in ulcer area of failed graft, (C,D) of case 1, in subepithelial area near ruptured descemetocoele of case 2 (E,F), in the subepithelial area of case 3 (G,H) and in subepithelial areas of epidermolysis of case 4 both in the perforated paracentral corneal ulcer (I,J), and ulcerated graft (K,L). (original magnification $\times 312.5X$)

Table

Primary Antibodies for immunohistochemistry.

Antibody	Clone	Specificity
B cell, CD145R	4KB5	CD45R
B cell, CD20	L26	CD20
HLA-DR	CR3/43	β chain DP, DQ, & DR
HLA-DR α	TAL 1B5	A chain HLA-DR
Macrophage, CD68	KP1	CD68
Myeloid/Histiocyte	MAC 387	L1 protein
Neutrophil elastase	M752	Human neutrophil elastase
T cell, CD45RO	UCHL1	CD45RO
T cell, CD43	DF-T1	CD43