The role of adhesion molecules ICAM-1 and VCAM-1 in herpes simplex keratitis

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Abstract
Purpose: To elucidate the role of adhesion molecules in the pathogenesis of herpes simplex keratitis.
Methods: Fifty female Balb/c mice (4–6 weeks old, 14–22 g weight) were divided into two groups randomly. Forty were infected by herpes simplex virus and the other 10 were used as normal controls. All mice were fed under the same conditions. Corneas of these mice were collected for immunohistochemical testing on day 14 and 21 after infection.
Results: ICAM-1 was mainly expressed in the basal cells of the corneal epithelia and vascular endothelia of the infected mice. A substantial amount of VCAM-1 was also expressed in the corneal vascular endothelial cells of infected mice, and was also found in inflammatory cells in the epithelial and stromal layers of the corneas.
Conclusion: Adhesion molecules ICAM-1 and VCAM-1 were involved in the progression of herpes simplex keratitis. They may accelerate the progress of inflammation by mediating the extravasation of inflammatory cells from vessels into the infected sites. (Eye Science 2011; 26: 61–64)
Keywords: herpes simplex keratitis; adhesion molecules; ICAM-1; VCAM-1

Introduction
Herpes simplex keratitis (HSK) is a commonly-encountered blinding disease caused by herpes simplex virus type 1 (HSV-1), leading to repeated recurrence of keratitis, gradual decrease in corneal transparency, and even blindness¹. Treatments for HSK are at present of limited efficacy. Therefore, in-depth study of the pathogenesis of this disease may be beneficial in improving available therapies.

HSK is mainly characterized by leucocytes infiltration and corneal angiogenesis. Until now, the fundamental pathogenesis underlying HSK has not been well understood. We have established an HSK-affected mice model of leucocytes infiltration and corneal angiogenesis by infecting Balb/c mice with herpes simplex virus, to investigate the roles of ICAM-1 (Intercellular adhesion molecule-1) and VCAM-1 (Vascular cellular adhesion molecule-1) in the pathogenesis of HSK, and to establish a basis for seeking novel treatments for this disease.

Materials and methods

Virus and cells
The virus used in this study was HSV-1 RE line provided by University of Pittsburgh in the United States. The viral titer was 2×10⁶ PFU/ml prior to initiating the experiment. Vero cells were cultured.

Establishment of the model
Fifty 4–6 weeks old female Balb/c mice were selected, weighing from 14 to 16 g, and were randomly divided into an infection group (n=40) and a control group (n=10). The mice were anesthetized by intraperitoneal injection of pentobarbital sodium (45 mg/kg body weight). A grid was incised on the subjects, corneas using the tip of a 1 ml syringe needle. This grid was exposed to 5 μl of 2×10⁵ PFU/ml viral solution in the injection group, and an equivalent quantity of 0.9% PBS in the control group. The animals in the two groups were fed in separate cages. The pathological changes in the corneas were observed daily post infection under slit-
lamp biomicroscopy.

**Pathological grading of HSV-1-infected corneas**

1+: mild opacity  
2+: moderate opacity, iris visible  
3+: severe opacity, iris texture unclear  
4+: severe opacity, iris texture unclear and corneal ulcer present  
5+: corneal perforation  

Grades 1+, 2+ were deemed to be mild, and 3+, 4+, 5+ were defined as severe.

**Immunohistochemical assay**

All antibodies were purchased from Serotech, Inc. (Toronto, Canada). Corneas of the mice were harvested at 14 (20 animals) and 21 (20 animals) days following infection, and fixed in 10% formalin solution. Immunohistochemical assay was performed according to manufacture’s instructions.

**Results**

**Pathological changes in mice corneas**

Ten mice in the infection group showed corneal epithelial repair 14 days following viral infection, though no lesions were noted. The other animals in the infection group presented with dendritic changes in the corneal epithelia, corneal endothelial folds and corneal opacity with the iris remaining visible. These were graded as 2+ (mild corneal pathology). In the control group, all mice showed smooth corneas without lesions. Twenty-one days post-infection, 8 animals in the infection group had smooth corneas without lesions, while another 12 mice had severe corneal disease, presenting with swollen epithelial and stromal cells, and dense corneal opacity obscuring the iris. Among these animals, 4 had corneal ulcers, which were graded as 3+ to 4+ (severe). The mice in the control group had smooth corneas without visible lesions.

**HE staining results in mice corneas**

Staining indicated intact corneal structures in all control animals. The mice with corneal disease graded as “mild” displayed vacuolation of the corneal epithelium, karyopyknosis, and a relatively number of inflammatory cells. Additionally, the stromal layer of these corneas was intact, though both corneal edema and inflammatory cell infiltration were documented. In the mice with clinically severe disease, more significant vacuolation was observed in epithelial cells compared with those in mild group, and substantial inflammatory cell infiltration was noted. Evident edema, cell lysis, and disordered cell arrangement were observed in the stromal layer of these corneas. Neovascularization was present at the corneal limbus and extended towards the central cornea, as shown in Figure 1.

![Figure 1 HE staining results in corneas; a substantial amount of infiltrating inflammatory cells, cellular edema, lysis, and disorganization of stromal fibers. Angiogenesis begins at the corneal limbus and extends towards the central cornea.](image)

**Expression levels of adhesion molecules**

Expression of ICAM-1

ICAM-1 was expressed in inflammatory cells in the mice corneas at both 14 (mild group) and 21 days (severe group) after viral infection. In the epithelium, the inflammatory cells expressing ICAM-1 were mainly distributed in the basal layer, and seldom in the surface layer. A large number of inflammatory cells in the stromal layer expressed ICAM-1, and a high level of ICAM expression was present in vascular endothelial cells located at the corneal limbus. The number of inflammatory cells expressing ICAM-1 in the severe group was significantly higher than among animals in the mild group. In the control group, a small number of ICAM-1+ cells were distributed only in the vessels surrounding the corneal limbus, but ICAM-1 expression was not observed in the corneal epithelial and stromal layers, as indicated in Figure 2.

The expression of VCAM-1

Both in the mild and severe groups, corneal vascular endothelial cells expressed a substantial amount
of VCAM-1. In addition, corneal epithelial and stromal inflammatory cells also expressed VCAM-1. However, no VCAM-1 was expressed in corneal cells in the normal controls.

**Discussion**

The process of leucocytes extravasation and infiltration serves as a critical step during the pathological reactions occurring in inflammatory disorders and immunological disease. Under normal circumstances, Langerhan’s cells (LC) are distributed in vessels near the corneal limbus. Once a corneal inflammatory reaction occurs, LCs may extravasate from vessels and migrate into the cornea. Neovascularization may be observed in corneal tissues after a relatively long course of disease. Polymorphonuclear cells (PMN) extravasate from vessels and migrate into inflammatory sites, which may be accomplished with the assistance of adhesion molecules. Previous studies have confirmed that diverse adhesion molecules expressed in endothelial cells play an important role in leucocytes transendothelial migration and infiltration into other tissues. The synergy between leucocytic infiltration and neovascularization may eventually induce blindness.

Leucocyte extravasation and infiltration into target tissues is a key step during inflammatory processes. Leucocytes pass out of the zone of rapidly-flowing blood, roll along the surface of vascular endothelial cells, and loosely adhere to vascular walls. The whole adhesion process is mediated by the adhesion molecules in the selectin family and their surface ligands. Leucocytes are able to stably adhere to vascular endothelial cells through the participation of adhesion molecules, such as ICAM-1 and VCAM-1, etc. Adhesion molecules play a significant role in
leucocyte extravasation from tight junction between vascular endothelial cells into gaps between vessels, and their subsequent migration into sites of infection.

Additionally, adhesion molecules are involved in inflammatory reactions by promoting neovascularization in infected sites. The inflammatory media in lesion sites are able to enhance angiogenesis directly or indirectly. Angiogenesis then promotes more severe inflammatory and pathological reactions. Thus, inflammatory cell infiltration and neovascularization are intimately associated. Yasyda M et al proved that PMN can induce angiogenesis in vitro, with the underlying mechanisms including PMN-endothelial cell interaction via E-selectin and ICAM-1. In previous studies focusing upon VEGF-induced corneal angiogenesis, results indicate that the number of adherent leucocytes is markedly increased. However, the number of adherent leucocytes decreased, while free rolling leucocytes increased, in those subjects treated by ICAM-1 antibody. Besides, the number of new vessels decreased in regions of angiogenesis.

In this investigation, ICAM-1 was expressed in the basal layers of corneal epithelial cells in infected mice, and also in PMN distributed in the vascular layers. ICAM-1 was expressed in all vascular endothelial cells. A substantial amount of VCAM-1 was expressed in corneal endothelia, and also in inflammatory cells in corneal epithelial and stromal layers. The above results demonstrate that ICAM-1 and VCAM-1 are associated with infiltrating inflammatory cells. ICAM-1 was even more widely distributed compared with VCAM-1, being particularly highly expressed in vascular epithelial cells and monocytes. Philip has reported that a substantial amount of ICAM-1 is expressed in surface cells, corneal cells and corneal endothelial cells among 4 cases of disc-like HSK. Rajasagi has also detected ICAM-1 expression in HSK patients.

Our study confirms the existence of adhesion molecules in HSK mice. Both ICAM-1 and VCAM-1 participated in the migration of multiple leucocytes towards affected sites. Adhesion molecules play a vital role in inflammatory cell infiltration and neovascularization in inflammatory sites. Inhibiting the bond between ICAM-1 and its receptors or decreasing ICAM-1 expression may lead to a cascade of events, including blocking or partially blocking the combination between leucocytes and vascular endothelia, leucocyte extravasation from vessels, leucocyte migration into corneal tissue, and eventually corneal opacity. This investigation provides a novel target for treatment of HSK.

References