

Treatment of Corneal Lesions in Individuals with Vernal Keratoconjunctivitis

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ABSTRACT

Vernal keratoconjunctivitis, a severe form of allergic conjunctival disease, is characterized by the development of various types of corneal lesions in conjunction with proliferative changes in the conjunctiva. Expression of bioactive substances, such as chemokines and adhesion molecules, by corneal fibroblasts likely contribute to the formation of corneal lesions by promoting local infiltration, activation, and survival of immune cells. Proliferation and deposition of extracellular matrix by conjunctival fibroblasts also may provide conditions which support the activation and survival of immune cells. Topical administration of corticosteroids is the principal mode of treatment for conjunctival inflammation in individuals with vernal keratoconjunctivitis. In some individuals, however, the surgical removal of conjunctival giant papillae or of corneal plaques is indicated.

KEY WORDS

adhesion molecule, chemokine, cornea, corticosteroid, vernal keratoconjunctivitis

INTRODUCTION

CORNEAL LESIONS IN ALLERGIC CONJUNCTIVAL DISEASES

Allergic conjunctival diseases are induced by invasion of antigens, such as those associated with tree pollen or dead mites, into the conjunctival sac of sensitized individuals. These diseases are classified into several subtypes—including allergic conjunctivitis (AC), atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), and giant papillary conjunctivitis (GPC)—on the basis of the presence or absence of conjunctival proliferative changes, atopic dermatitis, and conjunctival foreign bodies such as contact lenses, surgical sutures, or prostheses (Fig. 1).

In individuals with milder forms of allergic conjunctival diseases, such as AC, the main symptoms include ocular itching, tearing, and watery eye discharges. Vision in such individuals is not disturbed because of a lack of corneal involvement. In contrast, VKC is characterized by visual disturbances in addition to prominent ocular itching, discharge, pain, and secondary ptosis. These symptoms are caused by severe allergic inflammation of the conjunctiva and the

resulting development of corneal lesions. According to a nationwide study of allergic conjunctival diseases conducted by the Japanese Ophthalmologist Society, more than 50% of individuals with VKC have corneal lesions (Fig. 2a).¹ These lesions include superficial punctate keratopathy, corneal erosions, persistent corneal epithelial defects, corneal ulcers, and corneal plaques (Fig. 2b–e). VKC is also characterized by the presence of proliferative changes in the conjunctiva, which manifest clinically as giant papillae on the upper tarsal conjunctiva and swelling of the corneal-conjunctival limbic region.

The symptoms of most patients with AC can be controlled by topical administration of antiallergy eye-drops such as those containing mast cell stabilizers or antihistamines. However, corneal lesions and conjunctival proliferative changes in individuals with VKC are often resistant to such therapy and remain a challenge in the treatment of ocular allergy.

ALLERGOLOGICAL CHARACTERISTICS OF THE OCULAR SURFACE

The ocular surface possesses a unique anatomic structure and this structure may contribute to the

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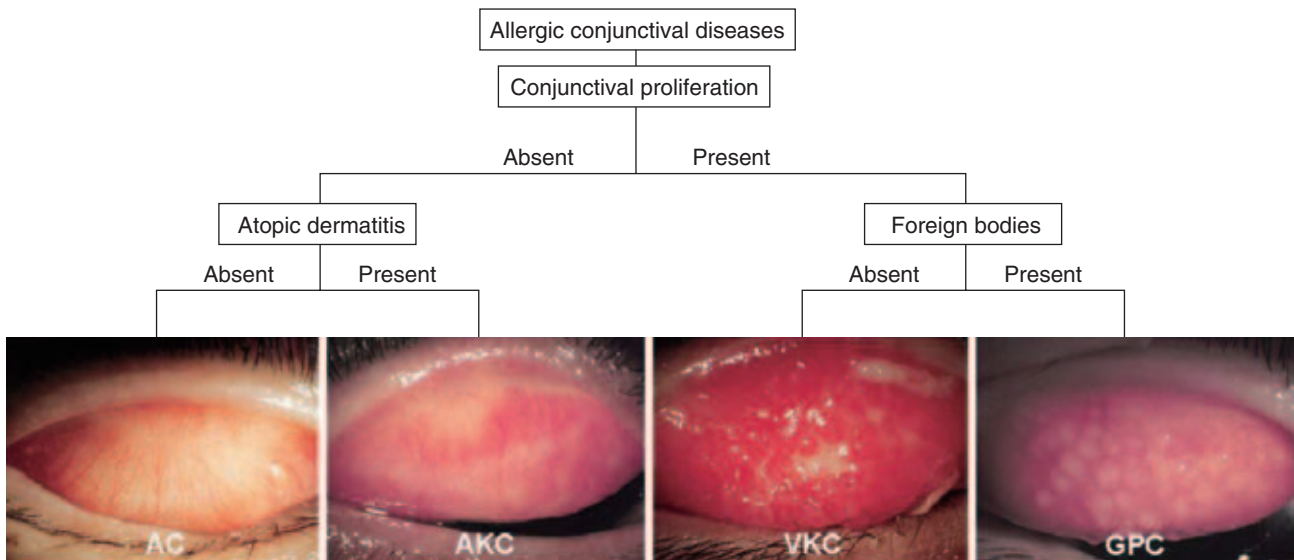


Fig. 1 Classification of allergic conjunctival diseases. Allergic conjunctival diseases are classified as allergic conjunctivitis (AC), atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), and giant papillary conjunctivitis (GPC) on the basis of the presence or absence of conjunctival proliferative changes, atopic dermatitis, and conjunctival foreign bodies.

specificity of ocular allergy. Allergic inflammation is readily evoked in the tarsal and bulbar conjunctiva given that (1) the conjunctiva has an extensive vasculature, (2) immune cells, such as mast cells, lymphocytes, and eosinophils, are abundant in the normal as well as the diseased conjunctiva,²⁻⁴ and (3) the conjunctiva is a mucous tissue that is moistened by tear fluid, which extracts antigens from dry particles of tree pollen or dead mites. In contrast, primary allergic reactions do not occur in the cornea because (1) the cornea is an avascular tissue, (2) no immune cells are present in the cornea, with the exception of Langerhans' cells at the periphery of the corneal epithelium,⁵ and (3) the barrier function of corneal epithelial cells prevents antigens from invading the corneal stroma. Like the conjunctiva, the cornea is in contact with a thin layer of tear fluid (Fig. 3). The cornea is thus exposed to bioactive molecules—such as histamine,⁶ eosinophil-derived cytotoxic proteins,⁷ leukotrienes,⁸ and proteinases⁹ that are released into tear fluid as a result of allergic inflammation in the conjunctiva. Exposure to such agents, especially to eosinophil-derived cytotoxic proteins,¹⁰ can result in damage to corneal epithelial cells. Corneal damage is clinically more important than conjunctival changes because of the prominent role of the cornea in vision.

ROLE OF RESIDENT CELLS OF THE CORNEA IN THE PATHOGENESIS OF ALLERGIC CONJUNCTIVAL DISEASES

Expression of Chemokines by Resident Cells of the Cornea

Resident cells of the cornea, such as epithelial cells and fibroblasts, are not considered to contribute di-

rectly to the pathophysiology of ocular allergic reactions. Rather, these cells are thought to be bystanders or victims of such reactions, which are induced and maintained by the activation of immune cells such as mast cells, lymphocytes, and eosinophils. However, recent studies have shown that these corneal resident cells play an important role in the pathogenesis of allergic conjunctival diseases.

Allergic conjunctival diseases are characterized pathologically by the local infiltration of eosinophils, a process that is triggered by the presence of the chemokine eotaxin in the affected tissue.¹¹ The concentration of eotaxin in tear fluid is increased in individuals with VKC, especially in those with corneal lesions.¹² Eosinophils accumulate at the tips of giant papillae, which are located adjacent to the tear fluid and the cornea. These giant papillae are characterized by erosion of the overlying conjunctival epithelium (Figs. 4a, b). The numbers of eosinophils in the conjunctiva as well as in tear fluid and ocular discharge are increased in individuals with VKC¹³ and most of these cells are degranulated (Figs. 4c, d). The concentrations of the proinflammatory cytokine tumor necrosis factor- α (TNF- α),¹⁴ T helper cell 2 (Th2) cytokines interleukin (IL)-4,¹⁵ and IL-13 in tear fluid are also increased in VKC patients.

We examined the effect of these cytokines on the expression of eotaxin by corneal epithelial cells, corneal fibroblasts, and conjunctival fibroblasts in culture. Corneal fibroblasts expressed a small amount of eotaxin in response to stimulation with either TNF- α , IL-4, or IL-13. However, the production of eotaxin by these cells was increased synergistically by the combination of TNF- α and either IL-4 or IL-13.¹⁶ In con-

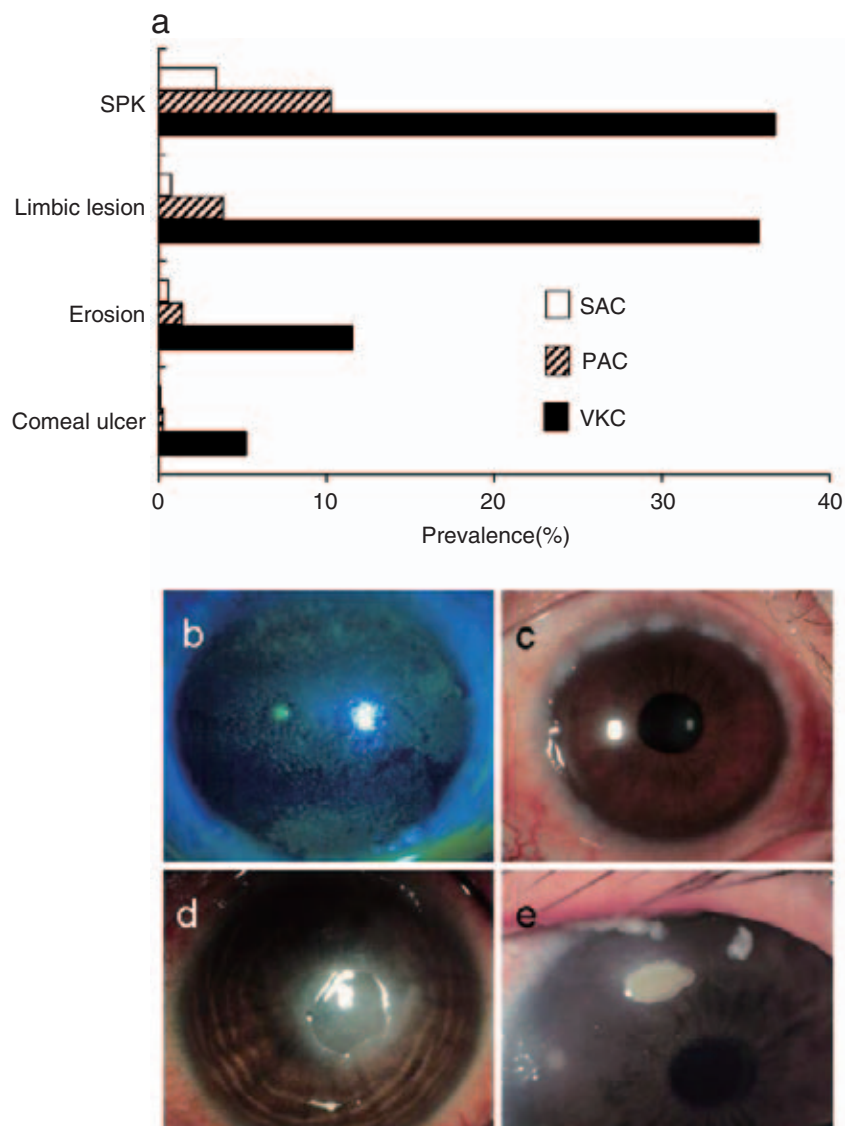


Fig. 2 Corneal lesions associated with allergic conjunctival diseases. **(a)** Prevalence of various types of corneal lesion in individuals with seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), or vernal keratoconjunctivitis (VKC) in Japan. SPK, superficial punctate keratopathy. Modified with permission from reference 1. **(b)** Superficial punctate keratopathy revealed by fluorescein biostaining. **(c)** Proliferative lesion in the corneal-conjunctival limbic region. **(d)** Persistent corneal epithelial defect. **(e)** Corneal plaque.

trast, corneal epithelial cells did not express eotaxin in response to stimulation with $\text{TNF-}\alpha$, IL-4, or IL-13 or with combinations thereof. Conjunctival fibroblasts produced eotaxin on exposure to these same cytokines, but the amount of chemokine synthesized by these cells was much smaller than that produced by corneal fibroblasts.

VKC, similar to allergic diseases in other organs, is also characterized by the local infiltration of Th2 cells,¹⁷ which is controlled mainly by the production of thymus- and activation-regulated chemokine

(TARC)¹⁸ and macrophage-derived chemokine (MDC).¹⁹ The regulation of TARC expression in corneal cells is similar to that of eotaxin expression. Cultured corneal fibroblasts thus do not express a detectable amount of TARC protein or mRNA in response to stimulation with various individual cytokines, including $\text{TNF-}\alpha$, IL-4, and IL-13; exposure of these cells to the combination of $\text{TNF-}\alpha$ and either IL-4 or IL-13, however, results in the production of large amounts of TARC.²⁰ Corneal epithelial cells do not express this chemokine in response to stimulation

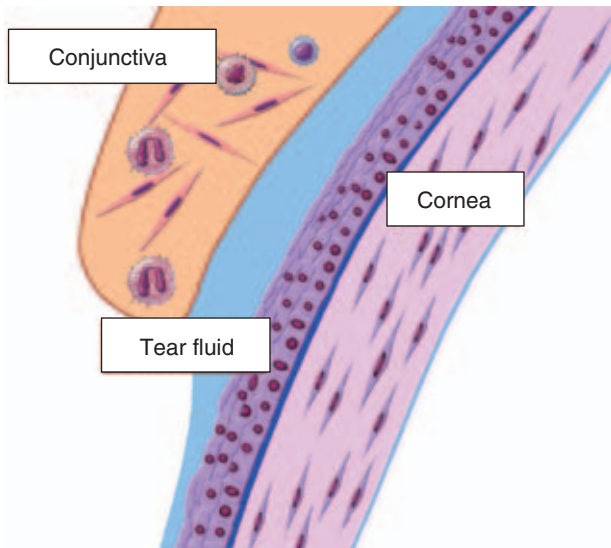


Fig. 3 Structure of the outer eye. The cornea and conjunctiva are separated by a thin layer of tear fluid.

with various cytokines alone or in combination, whereas cytokine-stimulated conjunctival fibroblasts produce TARC in amounts much smaller than those synthesized by corneal fibroblasts. MDC is not expressed by cultured corneal epithelial cells, corneal fibroblasts,²¹ or conjunctival fibroblasts. These various observations indicate that corneal fibroblasts, but not corneal epithelial cells, play an important role in the local infiltration of eosinophils and Th2 cells by expressing the chemokines eotaxin and TARC.

Expression of Adhesion Molecules by Resident Cells of the Cornea

Expression of adhesion molecules by resident cells also contributes to the local infiltration and activation of immune cells.²² The local accumulation of neutrophils is mediated predominantly by the interaction of leukocyte function-associated antigen-1 (LFA-1) with intercellular adhesion molecule-1 (ICAM-1), which are expressed on the surface of neutrophils and resident cells, respectively. Similarly, the infiltration of eosinophils and lymphocytes is mediated by the attachment of very late antigen-4 (VLA-4) on the leukocytes to vascular cell adhesion molecule-1 (VCAM-1) on resident cells.

We examined the expression of ICAM-1 and VCAM-1 and its regulation in cultured corneal cells. Corneal epithelial cells expressed a constitutive level of ICAM-1 on the cell surface and the abundance of this adhesion molecule was increased by stimulation of the cells with TNF- α .²³ These cells did not express VCAM-1, however, in the absence or presence of various cytokines. Similar to corneal epithelial cells, corneal fibroblasts expressed ICAM-1 at the cell surface under basal conditions and the abundance of this ad-

hesion molecule was increased by cell stimulation with TNF- α . Although these cells did not express a constitutive level of VCAM-1, they produced small amounts of this adhesion molecule in response to stimulation with TNF- α , IL-4, or IL-13 and exhibited a synergistic response to stimulation with TNF- α and either IL-4 or IL-13. Given that specific infiltration of eosinophils and lymphocytes, rather than that of neutrophils, is observed in the ocular region of individuals with VKC, these results indicate that corneal fibroblasts, rather than corneal epithelial cells, play an important role in ocular allergic reactions by expressing VCAM-1 in response to cytokine stimulation.

Mechanism of Exacerbation of Ocular Allergic Reactions

On the basis of our observations with cultured corneal cells, we propose the following model for the exacerbation of ocular allergic reactions: (1) Primary allergic reactions are induced in the conjunctiva, where immune cells are abundant, (2) The concentrations of bioactive substances, such as eosinophil-derived cytotoxic proteins, proinflammatory cytokines, and Th2 cytokines, increase in the tear fluid, (3) The corneal epithelium is damaged as a result of exposure to these agents, especially the eosinophil-derived cytotoxic proteins, found in tear fluid, (4) Corneal fibroblasts, which are located in the corneal stroma, also come into contact with the bioactive molecules as a result of the impaired barrier function of the injured corneal epithelium, (5) Corneal fibroblasts activated by exposure to cytokines produce chemokines and adhesion molecules, (6) Local infiltration of immune cells is triggered by the chemokines and adhesion molecules expressed by corneal fibroblasts, (7) The immune cells and corneal fibroblasts likely engage in mutual stimulation and thereby promote inflammation and tissue destruction.

ROLE OF CONJUNCTIVAL FIBROBLASTS IN THE PATHOGENESIS OF OCULAR ALLERGY

Individuals with corneal lesions associated with VKC manifest prominent proliferative changes of the conjunctiva, such as the development of giant papillae of the upper tarsus and limbic lesions. Histological analysis reveals that these changes are characterized by the accumulation of immune cells, such as eosinophils, mast cells, and lymphocytes, as well as by an increase in the number of conjunctival fibroblasts.¹⁴ Deposition of extracellular matrix, including fibronectin and collagen types I and III, is also increased in giant papillae.¹⁴ Given that the concentrations of various Th2 cytokines in tear fluid are increased in individuals with VKC,^{15,24} we examined the effects of such cytokines on cell proliferation and deposition of extracellular matrix by cultured conjunctival fibroblasts.²⁵ Conjunctival fibroblasts ex-

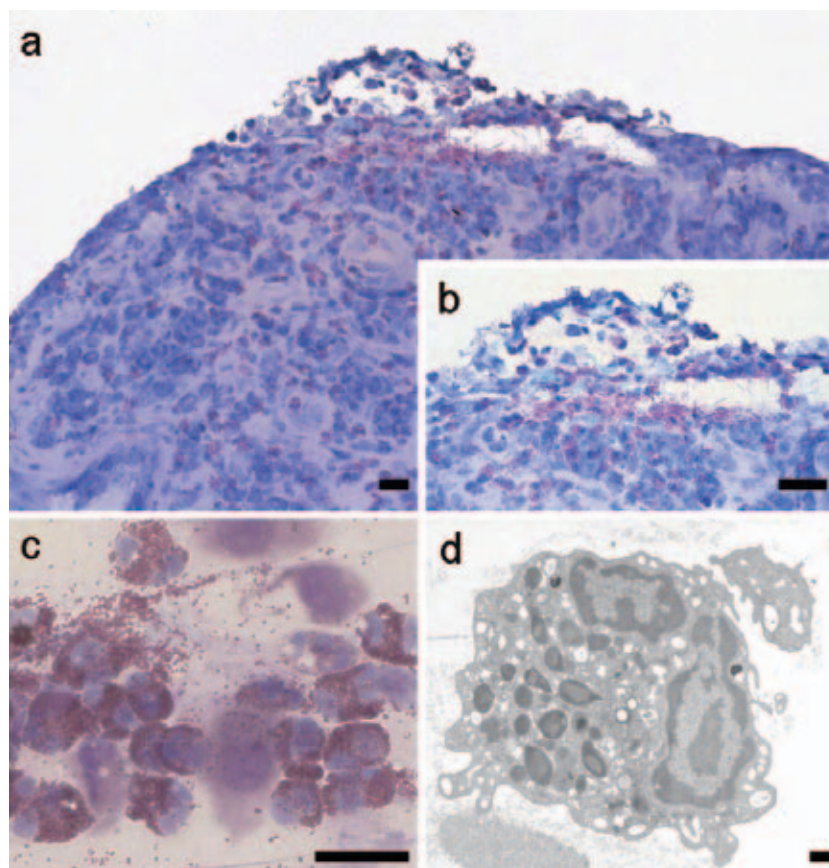


Fig. 4 Eosinophil infiltration in VKC. (a, b) Cross-section of a giant papilla from an individual with VKC. The accumulation of eosinophils under a conjunctival epithelial erosion is revealed by Hansel staining. Panel b is a higher-magnification view of the upper part of the image in panel a. (c) Ocular discharge of an individual with VKC. Hansel staining reveals the presence of numerous degranulated eosinophils. (d) Electron microscopical image of eosinophils in the ocular discharge of an individual with VKC. Scale bars: 20 μm (a–c) or 1 μm (d).

press receptors for IL-4 and IL-13²⁵ and each of these cytokines promoted both the proliferation of these cells and deposition of extracellular matrix. Coculture with conjunctival fibroblasts enhances the survival and activation of immune cells such as eosinophils.²⁶ The presence of extracellular matrix also promotes the survival of immune cells. Giant papillae therefore likely provide an environment that is conducive to the activation of eosinophils and other immune cells.

TREATMENT OF VKC

Pharmacological Therapies

Pharmacological therapies, including those based on mast cell stabilizers, antihistamines, and corticosteroids, constitute an important mode of treatment of corneal lesions in individuals with VKC. Local administration of mast cell stabilizers or antihistamines alone is usually not sufficient to suppress active allergic inflammation in such patients, although these

drugs are the first choice for the treatment of AC. However, these agents are routinely used because their administration allows the dose of corticosteroid to be reduced. The efficacies of oral antiallergy drugs in individuals with VKC are not well characterized.

Corticosteroids play a central role in the treatment of VKC patients, especially those with corneal lesions. These drugs are usually administered as eye-drops, and, among which dexamethasone and betamethasone are the most effective therapeutically but also frequently associated with pronounced side effects. Fluorometholone eyedrops are less efficacious but have fewer side effects; they are thus not usually sufficient to control conjunctival inflammation or heal corneal lesions in individuals with VKC. Dexamethasone or betamethasone is therefore often needed to treat VKC patients with corneal lesions. The effects of these drugs should be monitored, however, by clinical imaging of the cornea and conjunc-

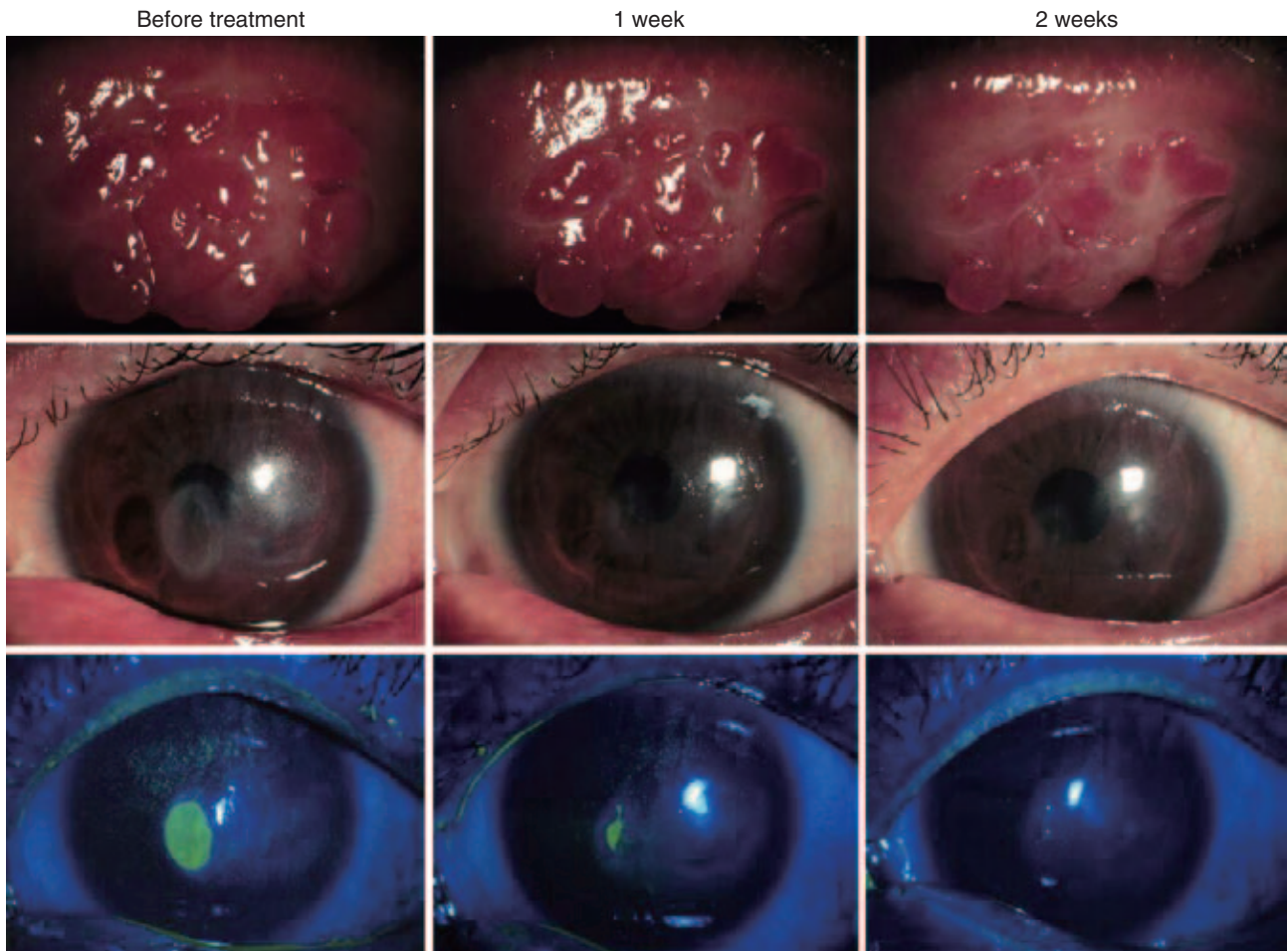


Fig. 5 Effect of corticosteroid eyedrops on the conjunctival proliferative changes and persistent corneal epithelial defect in an individual with VKC. Top row, photographs of the upper tarsal conjunctiva; middle row, photographs of the cornea; bottom row, fluorescein staining of the cornea, showing the corneal epithelial defect.

tiva. Effective treatment with corticosteroids is manifest by the relatively rapid (within few days) resolution of conjunctival changes such as edema, hyperemia, and exudation. The giant papillae also gradually decrease in size, but several months of treatment are required for their complete disappearance (Fig. 5). The area and density of superficial punctate keratopathy and the area of corneal epithelial erosion are important measures for judging the effectiveness of corticosteroid therapy. Corneal plaques, once formed, are not usually affected by corticosteroid treatment. In the most severe cases of VKC, short-term administration of oral corticosteroids (for example, prednisolone at a daily dose of 0.6 mg per kilogram of body mass) is usually effective (Fig. 6). Subconjunctival or subdermal injection of corticosteroids at the upper tarsus should also be considered in the most severe cases.

In general, corticosteroids have local as well as systemic side effects. In the ocular region, treatment

with corticosteroids can result in steroid cataract, steroid glaucoma, or microbiological infection of the cornea. The induction of steroid cataract differs little among individuals and is closely related to the total dose of corticosteroid administered. Steroid cataract can thus be induced in virtually anyone by treatment with corticosteroids in a dose dependent manner. In contrast, the incidence of steroid glaucoma differs markedly among individuals. Even small doses of corticosteroid can induce steroid glaucoma in so-called "steroid responders". The development of steroid glaucoma is related to the one-time dose, rather than the total dose, of corticosteroid administered. Steroid glaucoma is induced at a much greater frequency by local administration of corticosteroid than by systemic administration. Local administration of corticosteroids is also a risk factor for corneal infection.

Staphylococcus aureus and herpes simplex virus are the major causes of corneal infection in individuals with atopic dermatitis, which is often associated with

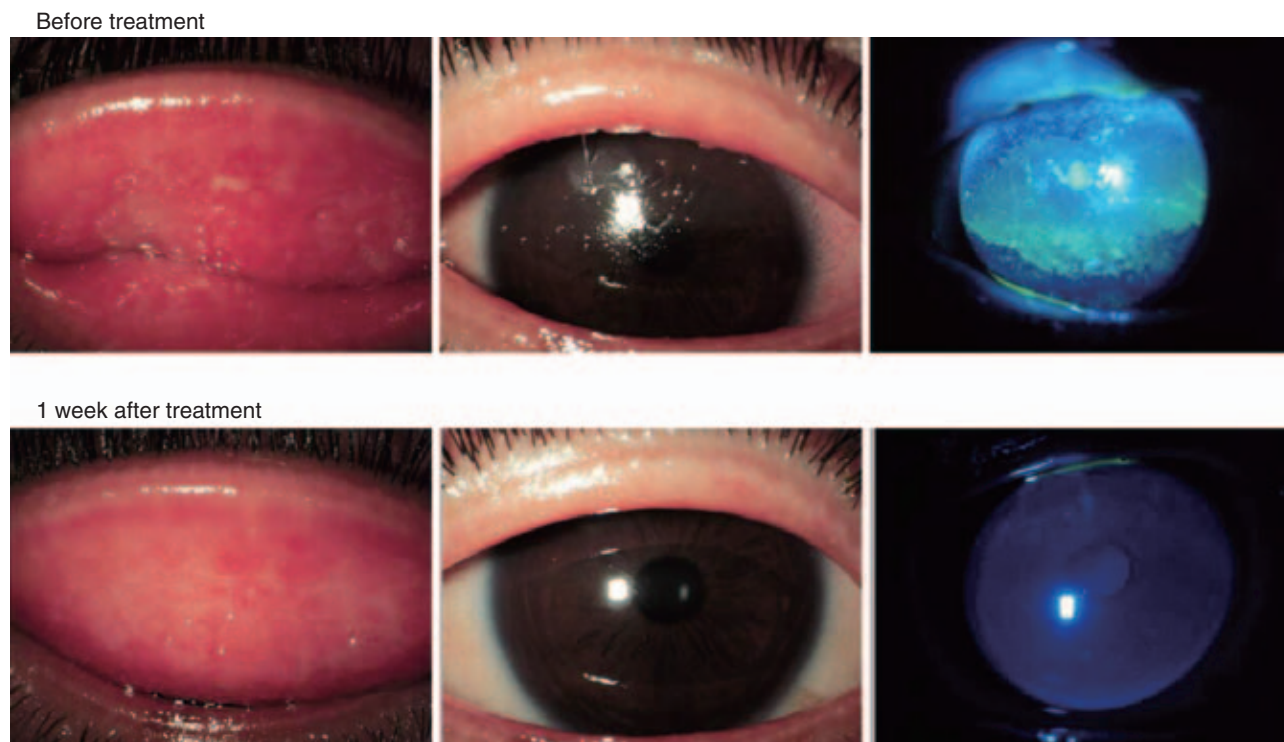


Fig. 6 Effect of oral corticosteroid (prednisolone, 0.6 mg/kg per day) treatment on the corneal lesions of an individual with VKC. Left column, photographs of the upper tarsal conjunctiva; middle column, photographs of the cornea; right column, fluorescein staining of the cornea, showing the corneal epithelial defect.

VKC.

Surgical Therapies for Corneal Lesions in VKC

Surgical removal of giant papillae is indicated in individuals with VKC and corneal erosion, corneal ulcer, or superficial punctate keratopathy covering the entire cornea, even in those treated with frequent administration of corticosteroid eyedrops. All giant papillae on the upper tarsal conjunctiva should be removed mechanically using local anesthesia. Corneal epithelial lesions usually diminish within several days after surgery (Fig. 7). Although conjunctival papillae gradually recur in most individuals several months after the operation, surgery is a rapid and reliable treatment for severe corneal lesions associated with VKC. Elimination of giant papillae relieves conjunctival inflammation, at least in part, through the removal of conjunctival fibroblasts and extracellular matrix, the consequent loss of the supportive environment for inflammatory cells, as well as through the removal of the inflammatory cells themselves.

Corneal plaques form in individuals with VKC subsequent to corneal ulceration or the persistent corneal epithelial defects. Once formed, corneal plaques persist for many months or years and their surface remains unexposed by the corneal epithelium even if conjunctival inflammation is well controlled. This is possibly explained by the fact that corneal epithelial

cells cannot attach to or migrate on the debris of corneal epithelial cells and eosinophils that constitutes such plaques. The presence of corneal plaques thus inhibits normal corneal epithelial healing and they are usually removed surgically in order to promote healing. Before treatment, however, conjunctival inflammation must be adequately controlled. We administered eyedrops containing fibronectin isolated from the patient's own blood to facilitate healing of the corneal epithelium after plaque removal (Fig. 8).

CONCLUSION

Treatment of ocular allergic reactions, including that of corneal lesions in individuals with VKC, relies predominantly on the application of eyedrops containing antiallergy drugs developed for systemic use. To date, only corticosteroids, mast cell stabilizers, and antihistamines are sold as eyedrops. The introduction of other types of antiallergy drugs, such as leukotriene antagonists and inhibitors of immunoglobulin E production, in eyedrop form will likely improve the treatment options for individuals with allergic conjunctival diseases.

Allergic conjunctival diseases exhibit pathophysiological characteristics that are distinct from allergic diseases of other organs. These characteristics are closely associated with the function of resident cells in the cornea and conjunctiva as well as with the anat-

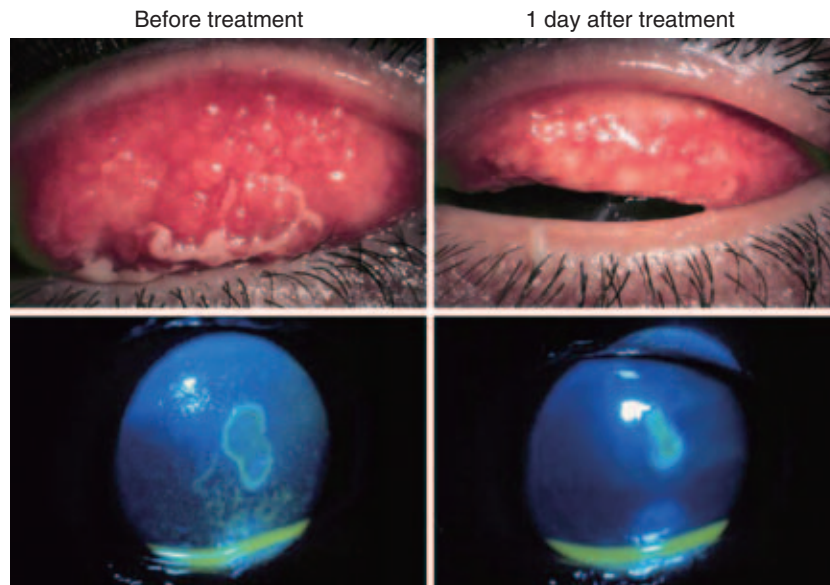


Fig. 7 Effect of resection of giant papillae on corneal lesions in an individual with VKC. Upper row, photographs of the upper tarsal conjunctiva; lower row, fluorescein staining of the outer eye, showing the corneal epithelial defect.

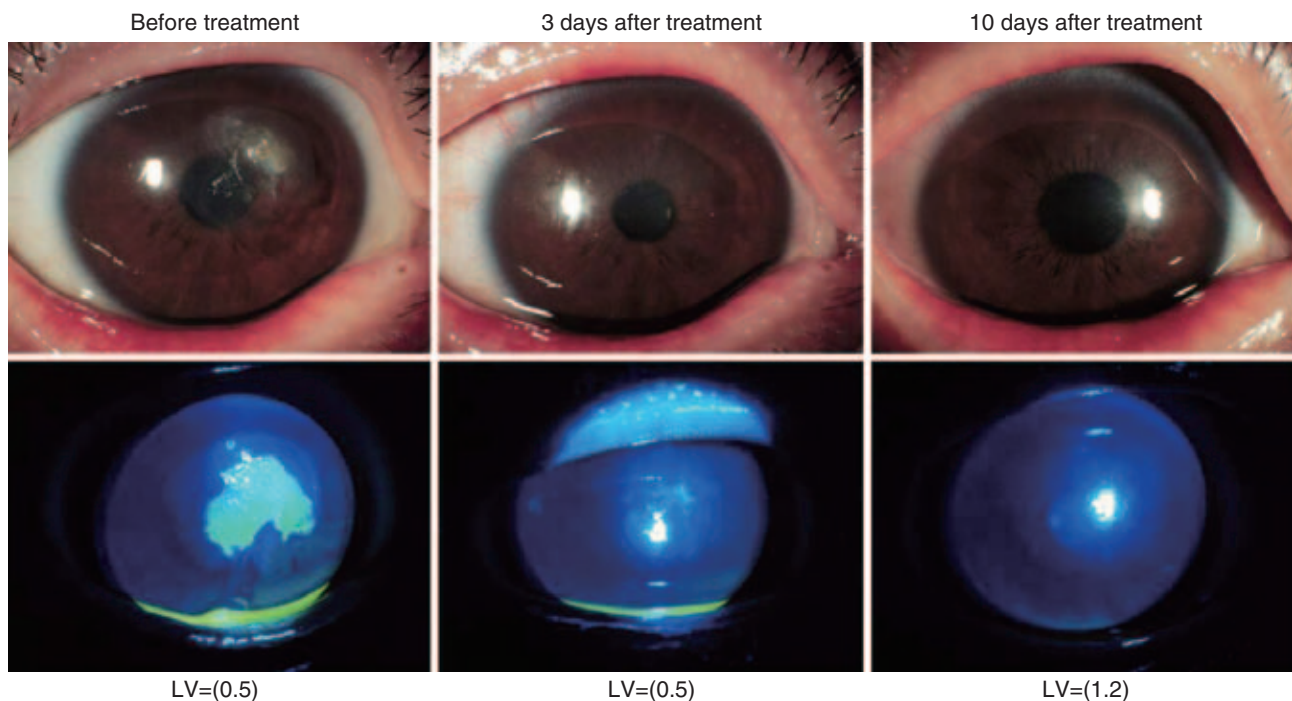


Fig. 8 Effect of corneal plaque removal and fibronectin eyedrops on corneal epithelial erosion in an individual with VKC. Upper row, photographs of the cornea; lower row, fluorescein staining of the cornea, showing the corneal epithelial defect. LV, left visual acuity.

omy of the cornea and conjunctiva and the physiological properties of tear fluid. Future treatment of these ophthalmic disorders should be directed at the regulation of resident cells in the cornea and the conjunctiva.

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