Allergology International. 2006;55:121-129

REVIEW ARTICLE

Awarded Article, Annual Meeting of JSA

Fibroblasts as Local Immune Modulators in Ocular Allergic Disease

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ABSTRACT

Vernal keratoconjunctivitis (VKC), a severe form of ocular allergic disease, is characterized by the formation of giant papillae at the upper tarsal conjunctiva and corneal lesions that threaten vision. Recent evidence indicates that resident fibroblasts function as immune modulators in the pathogenesis of the chronic allergic inflammation associated with VKC. The T helper 2 (Th2) cell-derived cytokines interleukin (IL)-4 and IL-13 stimulate the migration and proliferation of conjunctival fibroblasts as well as protecting these cells from apoptotic cell death, effects that likely underlie the hyperplasia of fibroblasts that contributes to the formation of giant papillae. Conjunctival fibroblasts also synthesize extracellular matrix proteins and tissue inhibitors of metalloproteinases as well as down-regulate the expression of matrix metalloproteinases in response to these cytokines, effects that likely contribute to the excessive deposition of extracellular matrix that is characteristic of giant papillae. Stimulation of fibroblasts in the corneal stroma with the combination of a proinflammatory cytokine and either IL-4 or IL-13 results in up-regulation of the expression of the chemokine eotaxin and thymus- and activation-regulated chemokine as well as of vascular cell adhesion molecule-1, which together mediate the infiltration and activation of eosinophils and Th2 cells. Fibroblasts therefore appear to play a central role in the induction and amplification of ocular allergic inflammation and the consequent development of giant papillae and corneal disorders in individuals with VKC. Fibroblasts and fibroblast-derived factors thus represent new and potentially important therapeutic targets for treatment of the giant papillae and corneal disorders associated with VKC.

KEY WORDS

conjunctiva, cornea, cytokine, fibroblast, vernal keratoconjunctivitis

INTRODUCTION

Ocular allergic diseases are triggered by the invasion of antigens, such as grass or tree pollen, dead mites, and skin debris of various animals, into the conjunctiva of sensitized individuals. Both the tarsal and bulbar conjunctiva possess an extensive vasculature and are rich in immune cells such as mast cells, eosinophils, macrophages, and lymphocytes, characteristics that confer high susceptibility to allergic reactions (Fig. 1A). Allergic conjunctival diseases are classified into several subtypes–including allergic conjunctivitis, atopic keratoconjunctivitis, vernal keratoconjunctivitis (VKC),and giant papillary conjunctivitis–on the basis of the absence or presence of conjunctival proliferative changes, atopic dermatitis, and conjunctival foreign bodies. Individuals with allergic conjunctivitis experience ocular itching, tearing, or a watery discharge as a result of acute conjunctival inflammation, but they usually manifest only mild hyperemia or edema of the conjunctiva. The cornea is not involved in allergic conjunctivitis, with the result that vision is not disturbed.

In contrast to the acute nature of allergic conjunctivitis, VKC is a chronic and severe ocular allergic disease.[1] VKC is characterized by pronounced and persistent allergic inflammation of the conjunctiva that is accompanied by ocular itching, pain, mucous discharge, and visual disturbance. Conjunctival proliferative lesions, such as giant papillae of the upper tarsal conjunctiva (Fig. 1B), and swollen limbal lesions are characteristic of VKC; they are not associated with other types of ocular inflammatory disease with the exception of giant papillary conjunctivitis.

The cornea and conjunctiva are separated from each other by only a thin layer of tear fluid(Fig. 1A).

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Received 15 November 2005.

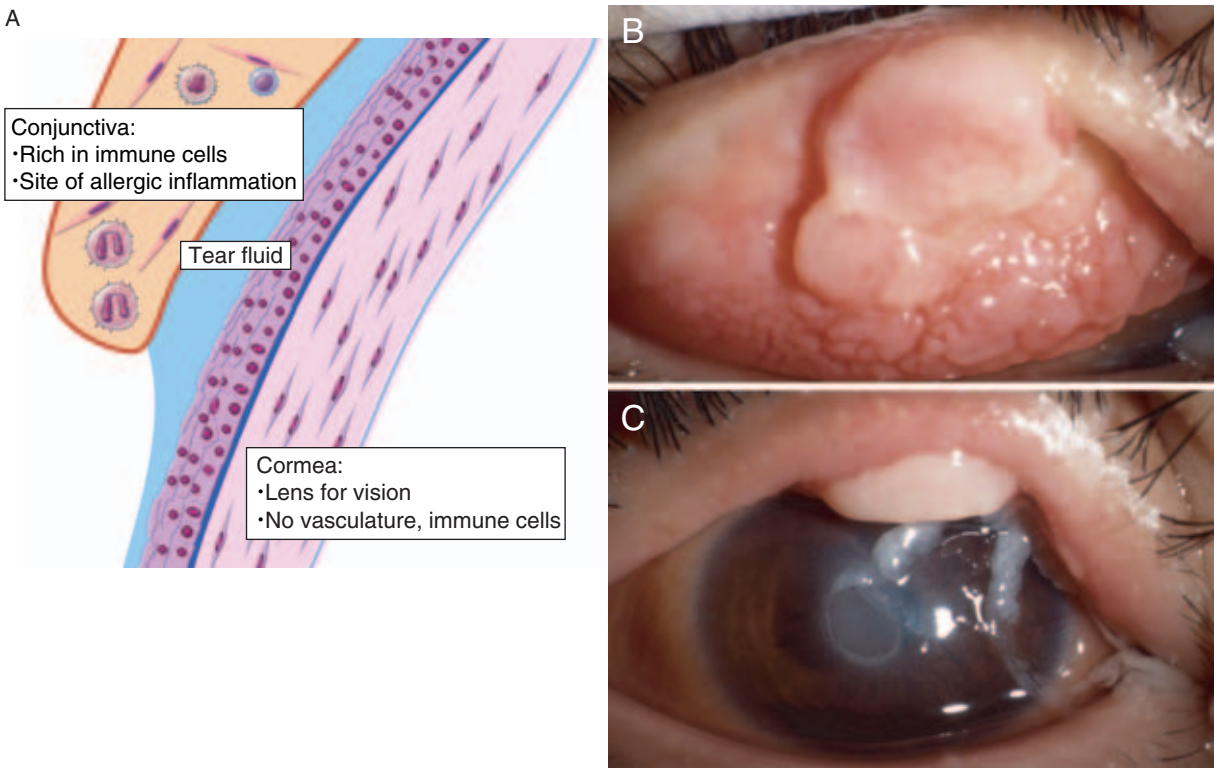

Fig. 1 Anatomy of the ocular surface and clinical characteristics of VKC. (**A**) The cornea and conjunctiva are separated by a thin layer of tear fluid. (**B**) Giant papilla of the tarsal conjunctiva in an individual with VKC. (**C**) Corneal shield ulcer in an individual with VKC.

The cornea has a unique structure because of its role in vision. It is a transparent and avascular tissue, lacks immune cells (with the exception of Langerhans' cells at the periphery of the corneal epithelium), and is composed of only three types of structural cells (epithelial cells, stromal fibroblasts, and corneal endothelial cells), with corneal epithelial cells forming a tight barrier between the tissue and the external environment (Fig. 1A) . These characteristics render the cornea resistant to primary allergic reactions. However, the cornea is influenced by allergic inflammation of the conjunctiva as a result of the release of bioactive substances such as histamine, enzymes, cytokines, and eosinophil-derived cytotoxic proteins into tear fluid as well as through direct contact and neuronal communication.[2-6] Indeed, more than 50% of individuals with VKC have corneal lesions (Fig. 1C) that result in disturbance of vision.[7] The symptoms of most patients with allergic conjunctivitis can be controlled by topical administration of antiallergy eyedrops such as those containing mast cell stabilizers or antihistamines, given the predominant role of the early-phase reaction induced by degranulation of mast cells in this disease. 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.[7]

Recent studies have revealed that interaction of immune cell-mediated inflammatory processes with a network of nonimmune cell types contributes to the development of VKC, with the effector activities of these latter cells being responsible for the giant papillae and corneal disorders associated with this condition.[8,9] Thus, although until recently, the nonimmune cell constituents of tissues, such as epithelial cells and fibroblasts, were regarded as mere targets of the inflammatory milieu and as secondary players in the development of allergic disease, new data implicate such cells as dominant players in the pathogenesis of VKC. This review focuses on our current knowledge of the biology of ocular resident fibroblasts and their role in the development of VKC.

CYTOKINES AND GROWTH FACTORS IN OCULAR ALLERGIC DISEASES

The concentrations of various inflammatory mediators, including growth factors and cytokines released from inflammatory cells, are increased in the conjunctiva and tear fluid of patients with VKC. The levels of transforming growth factor-β, platelet-derived growth factor, and fibroblast growth factor, for example, are increased in the giant papillae of such individuals.[10] Inflammatory cells associated with giant papillae include mast cells, eosinophils, and T helper (Th) cells.

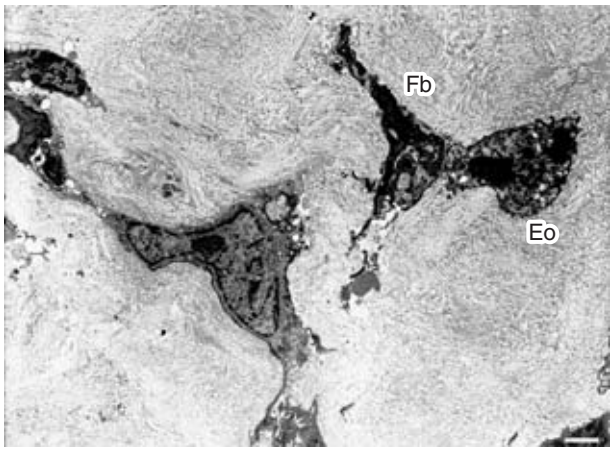

Fig. 2 Interaction between conjunctival resident fibroblasts and infiltrated eosinophils in giant papillae. Electron microscopy reveals the interaction of a fibroblast (Fb) with an eosinophil (Eo) and the accumulation of collagen fibers in a giant papilla of an individual with VKC. Scale bar, 2 μm.

Immunohistochemical analysis and *in vitro* cloning of T cells have revealed that infiltrated Th cells in the conjunctiva of VKC patients comprise mostly Th2 cells.[11] Whereas VKC results from Th2-dominant inflammation, most other types of conjunctivitis, including those associated with infection or autoimmune disease, result from Th1-dominant inflammation. It was thus thought possible that the activation of conjunctival fibroblasts during Th2-dominant inflammation contributes to the formation of the giant papillae that are largely specific to VKC. However, it has been unclear whether Th2 cell-derived cytokines such as interleukin (IL)-4 and IL-13 affect the functions of tissue-resident fibroblasts.

Until recently, Th2 cytokines were thought to act only on immune cells such as T cells and B cells. However, we and others have shown that not only immune cells but also the nonimmune cellular constituents of tissue, such as fibroblasts and epithelial cells, express receptors for these cytokines.[12,13] We have thus characterized the IL-4 receptor (IL-4R) complex expressed on the surface of human corneal and conjunctival fibroblasts,[13-15] showing that it consists of IL-4Rα, IL-2Rγc, IL-13Rα1, and IL-13Rα2 chains. Binding assays revealed that IL-4 binds with high affinity (dissociation constant, ～10 pM) to these receptors. Furthermore, our demonstration that IL-4 induced the activation of signal transducer and activator of transcription 6 (STAT6) in corneal fibroblasts and that neutralizing antibodies to IL-4 R inhibited IL-4-induced release of the chemokine eotaxin from these cells suggested that the IL-4R complex expressed on their surface is functional. Both IL-4 and IL-13, which also acts at the IL-4R complex, thus exert direct effects on ocular resident fibroblasts, suggesting that these cells might function as important effectors in the regulation of allergic inflammation by Th2 cytokines.

ROLE OF CONJUNCTIVAL FIBROBLASTS IN THE FORMATION OF GIANT PAPILLAE

Giant papillae, a characteristic lesion of VKC, consist of infiltrated inflammatory cells–such as mast cells, eosinophils, and Th2 cells–as well as conjunctival fibroblasts and extracellular matrix (ECM) molecules (Fig. 2). Immunohistochemical and biochemical studies have thus revealed increased deposition of collagen types I, III, and V as well as fibronectin, tenascin, and laminin in giant papillae.[16-18] An increased abundance of procollagen has also been detected in tear fluid and the conjunctiva of patients with active VKC.[19] As in other tissues, fibroblasts are responsible for ECM metabolism in the conjunctiva. Under normal conditions, resident fibroblasts maintain tissue integrity by both synthesizing and degrading ECM proteins, the latter of which is achieved by the release of matrix-degrading enzymes such as matrix metalloproteinases (MMPs). Degradation of ECM is further determined by the balance between the activities of MMPs and those of endogenous MMP inhibitors known as tissue inhibitors of metalloproteinases (TIMPs). Disturbance of the balance between the synthesis and degradation of ECM underlies various pathological conditions. If degradation exceeds synthesis, the loss of ECM proteins results in breakdown of the affected tissue. If synthesis exceeds degradation, excessive deposition of ECM proteins results in tissue fibrosis or hyperplasia.

The hyperplasia of fibroblasts associated with the development of giant papillae may result from a distorted homeostatic balance among fibroblast recruitment, proliferation, and death. We examined the effects of Th2 cytokines on these aspects of conjunctival fibroblast function. IL-4 (Fig. 3) was found to stimulate the migration of human conjunctival fibroblasts in a time- and concentration-dependent manner. Such effects of IL-4 were also observed with dermal fibroblasts but not with lung fibroblasts.[20,21] Among Th2 cytokines, only IL-4 and IL-13, not IL-5, IL-9, or IL-10, were shown to stimulate the proliferation of conjunctival fibroblasts.[15] Furthermore, only IL-4 and IL-13 (not IL-5, IL-9, or IL-10) protected conjunctival fibroblasts from nitric oxide (NO)-induced apoptosis, an effect that was mediated by activation of signaling by phosphatidylinositol 3-kinase and the protein kinase Akt.[14] These observations thus suggest that the chemotactic, mitogenic, and antiapoptotic effects of IL-4 and IL-13 on conjunctival fibroblasts might contribute to the hyperplasia of these cells that underlies the formation of giant papillae in individuals with VKC.

To clarify the contribution of Th2 cytokines to the excessive deposition of ECM in giant papillae, we and

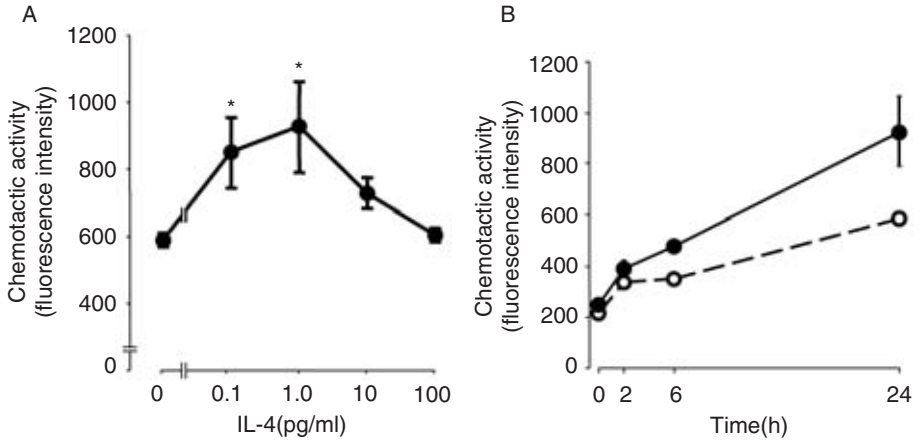

Fig. 3 IL-4-induced chemotaxis of conjunctival fibroblasts *in vitro*. Calcein-labeled human conjunctival fibroblasts were placed in the upper well of a Boyden blindwell chamber containing a filter with a pore diameter of 8 μm. The lower well contained IL-4 at the indicated concentrations (**A**) or at 0 (open circles) or 1.0 (closed circles) ng/ml (**B**). The chamber was incubated at 37°C for 24 h (**A**) or for the indicated times (**B**), after which the cells that had migrated into the lower well were detected by measurement of fluorescence with a microplate reader. Data are means ± SEM of values from three separate experiments. *$P < 0.05$ (Fisher's PLSD test) versus the corresponding value for cells incubated in the absence of IL-4.

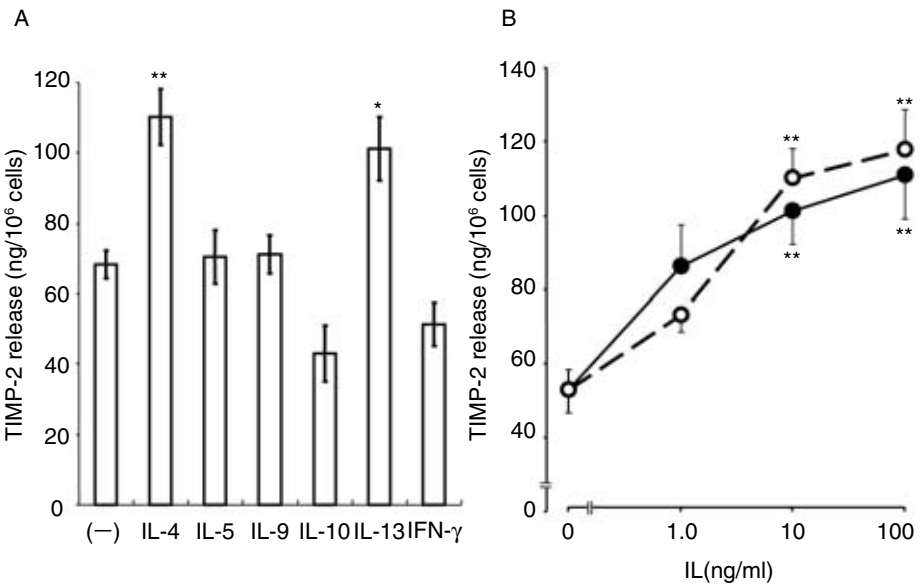

Fig. 4 IL-4- or IL-13-induced TIMP-2 release from human conjunctival fibroblasts. Cells were incubated for 24 h with the indicated Th2 cytokines at 10 ng/ml (**A**) or with the indicated concentrations of IL-4 (circles) or IL-13 (solid dots) (**B**). The concentration of TIMP-2 in culture supernatants was then determined by enzyme-linked immunosorbent assay. Data are expressed as nanograms of TIMP-2 released per 1×10^6 cells and are means ± SEM of values from four separate experiments. *$P < 0.05$, **$P < 0.01$ (Dunnett's test) versus the corresponding value for cells incubated in the absence of cytokine.

others investigated the effects of these cytokines on the metabolism of ECM by conjunctival fibroblasts. IL-4 and IL-13 were each found to stimulate the synthesis of collagen types I and III and fibronectin by

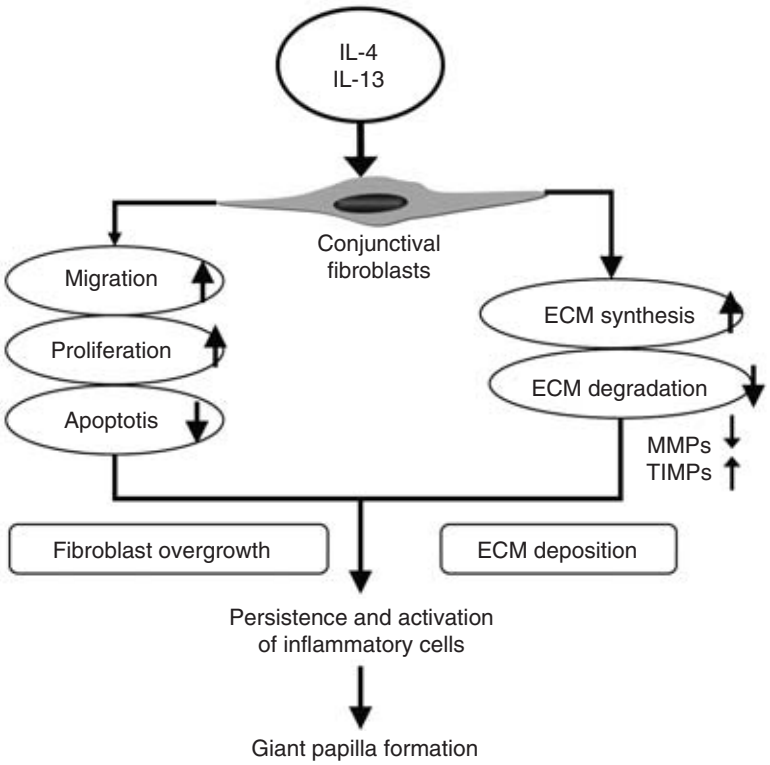

Fig. 5 Role of conjunctival fibroblasts in the pathogenesis of giant papillae. Stimulation of conjunctival fibroblasts by the Th2 cytokines IL-4 and IL-13 contributes to both the hyperplasia and excessive deposition of ECM that underlie the development of giant papillae associated with VKC.

these cells.[15] Furthermore, IL-4 inhibited the release by conjunctival fibroblasts of MMP-1,[22] which is largely responsible for the degradation of collagen type I. Conversely, IL-4 and IL-13 each promoted the release by conjunctival fibroblasts of TIMP-1,[22] which specifically inhibits the activities of interstitial collagenase, gelatinase, and stromelysin. In addition, IL-4 and IL-13, but not other Th2 cytokines or the Th1 cytokine interferon-γ (IFN-γ), induced TIMP-2 release from human conjunctival fibroblasts in a concentration-dependent manner (Fig. 4). Together, these observations indicate that IL-4 and IL-13 act as inhibitors of matrix degradation by conjunctival fibroblasts. Both the stimulatory effects of these cytokines on ECM synthesis in, and their inhibition of ECM degradation by, conjunctival fibroblasts may thus contribute to the excessive deposition of ECM in giant papillae.

The interaction of leukocyte integrins with the accumulated ECM proteins in giant papillae likely provides costimulatory signals to the infiltrating inflammatory cells. Adhesion of leukocytes to ECM components promotes their proliferation, prolongs their survival, or activates their expression of inflammatory cytokines, growth factors, and adhesion molecules, and the ECM also acts as a reservoir for inflammatory mediators or growth factors.[23-26] The increased deposition of ECM in giant papillae is thus thought to contribute to the persistence and activation of infiltrated inflammatory cells during conjunctival allergic inflammation (Fig. 5).

ROLE OF CORNEAL FIBROBLASTS IN CORNEAL DAMAGE ASSOCIATED WITH VKC

In contrast to the conjunctiva, primary allergic reactions do not occur in the cornea because of its lack of blood vessels and immune cells and because the barrier function of the corneal epithelium prevents antigens from invading the corneal stroma. Like the conjunctiva, however, the cornea is in contact with a thin layer of tear fluid, which, during allergic inflammation of the conjunctiva, is rich in bioactive molecules such as histamine, eosinophil-derived cytotoxic proteins, leukotrienes, proteinases, and cytokines. Exposure of the cornea to such molecules, especially to granule proteins released from infiltrated eosinophils, can damage corneal epithelial cells, destroy the normal structure of the cornea, and lead to the formation of corneal ulcers. Corneal damage is more important

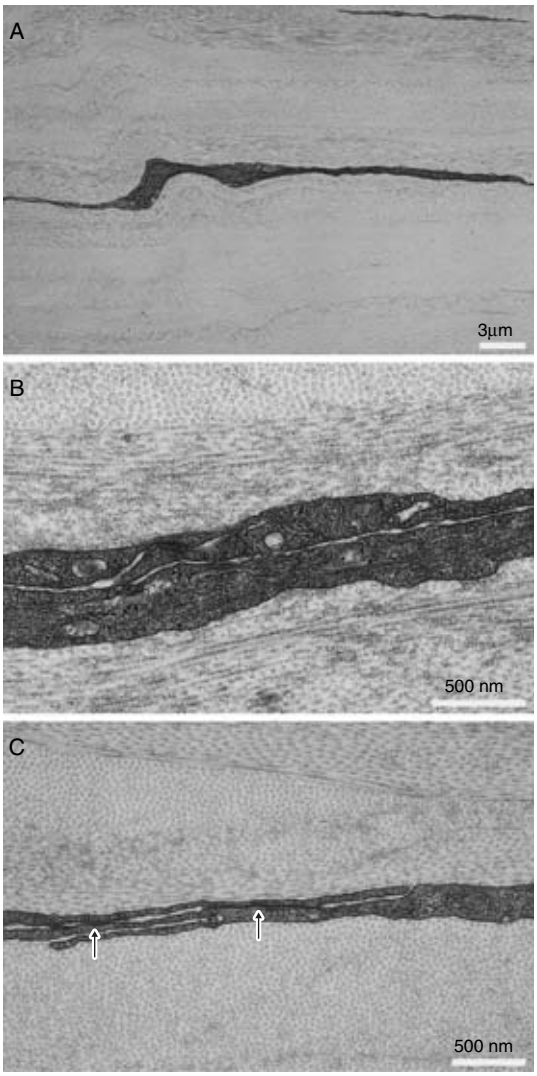

Fig. 6 Fibroblasts in the cornea. Electron microscopy reveals that fibroblasts in the human cornea are loosely arrayed between the lamellae formed by collagen fibers of the corneal stroma (**A**). The cytoplasmic processes of corneal fibroblasts are rich in intermediate filaments (**B**), and the long cytoplasmic processes of neighboring fibroblasts make contact with each other to form an extensive and continuous network structure parallel to the plane of the collagen lamellae (**C**). Typical gap junctions are apparent between these cytoplasmic processes (arrows).

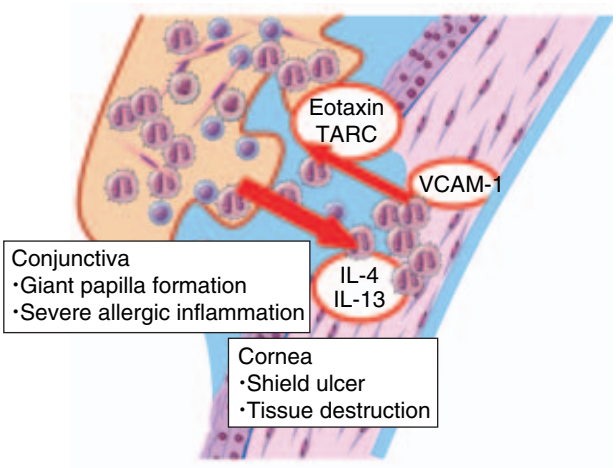

Fig. 7 Role of corneal fibroblasts in the pathogenesis of corneal lesions in VKC. Corneal fibroblasts are activated by IL-4 and IL-13 released into tear fluid as a result of conjunctival allergic inflammation. The activated fibroblasts regulate inflammatory cell recruitment through expression of chemokines and adhesion molecules. The infiltration of inflammatory cells then leads to corneal damage.

clinically than conjunctival changes because of the role of the cornea in vision. Recent studies have revealed that chemokines released from corneal cells contribute to eosinophil recruitment to the cornea and consequent corneal injury. The concentration of eotaxin (CCL11), a potent and specific chemokine for eosinophils, was thus found to be increased in the tear fluid of individuals with ocular allergic diseases, and both the number of eosinophils and the concentration of eotaxin in tear fluid correlated with the extent of corneal damage.[4]

We and others have investigated the cellular source of eotaxin present in tear fluid.[27,28] Human corneal epithelial cells in culture did not release detectable amounts of eotaxin into the culture medium in response to stimulation either with tumor necrosis factor-α (TNF-α), IL-4, or IL-13 alone or with combinations thereof. In contrast, stimulation of cultured human corneal fibroblasts (Fig. 6) with TNF-α in combination with either IL-4 or IL-13 resulted in a marked increase in eotaxin production that was both time- and concentration-dependent. These results suggested that cytokine-stimulated corneal fibroblasts, but not corneal epithelial cells, may represent a major source of eotaxin in tear fluid (Fig. 7).

At sites of inflammation, the interaction of leukocytes with tissue-resident cells promotes the infiltration, retention, and activation of the leukocytes. Adhesion molecules such as intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 expressed on the surface of these structural cells and integrin chains expressed on the surface of the leukocytes mediate these interactions. In vascularized tissue, the expression of adhesion molecules on the surface of endothelial cells facilitates the transmigration of leukocytes across the vessel wall and into the tissue proper. In the cornea, however, which lacks a vasculature, the retention and activation of leukocytes have been thought to be regulated by corneal epithelial cells or stromal fibroblasts. To clarify whether corneal cells might interact

with infiltrated eosinophils, we investigated the expression of ICAM-1 and VCAM-1 in human corneal epithelial cells and fibroblasts. ICAM-1, which is the ligand for leukocyte function-associated antigen (LFA)-1 expressed on the surface of both eosinophils and neutrophils, was found to be expressed on the surface of both corneal epithelial cells and fibroblasts in culture. Stimulation with the proinflammatory cytokine TNF-α increased the surface expression of ICAM-1 in both cell types, whereas the Th2 cytokines IL-4 and IL-13 had no such effect.[29] In contrast, VCAM-1, which is a ligand for very late antigen (VLA)-4 expressed on the surface of eosinophils (but not on that of neutrophils), was not expressed by corneal epithelial cells cultured in the absence or presence of cytokines. In corneal fibroblasts, however, stimulation with TNF-α, IL-4, or IL-13 alone induced a small increase in the basal level of VCAM-1 expression, and exposure to the combination of TNF-α and either IL-4 or IL-13 resulted in a marked synergistic increase in the surface expression of this adhesion molecule. These results suggest that the interaction of VCAM-1 on corneal fibroblasts with VLA-4 on eosinophils plays a key role in the pathogenesis of corneal lesions associated with VKC (Fig. 7).

CORNEAL FIBROBLASTS AS SENTINEL CELLS AND HETEROGENEITY OF FIBROBLASTS

IFN-γ inhibits the stimulatory effects of IL-4 on immunoglobulin E production *in vivo*[30] and on the development of Th2 cell clones *in vitro*.[31,32] The antagonism between the Th1 cell-derived cytokine IFN-γ and the Th2 cell-derived cytokine IL-4 apparent in hematopoietic cells suggested the possibility that IFN-γ might also inhibit the effects of Th2 cytokines on corneal fibroblasts. We therefore investigated whether IFN-γ affects Th2 cytokine-induced eotaxin synthesis in human corneal fibroblasts.[33] IFN-γ significantly inhibited the stimulatory effects of the proinflammatory cytokines TNF-α, IL-1α, and IL-1β on eotaxin release from corneal fibroblasts. Furthermore, it markedly inhibited the synergistic increase in eotaxin release induced by the combination of TNF-α and either IL-4 or IL-13. These results indicated that the Th1 cytokine IFN-γ opposes the effects of Th2 cytokines on corneal fibroblasts. Furthermore, this antagonism suggests that fibroblasts in the corneal stroma, which lacks immune cells under normal conditions, act as sentinel cells to sense the relative abundance of Th1 and Th2 cytokines and to respond accordingly by altering their expression of chemokines and adhesion molecules.

Fibroblasts are present throughout the body, but they do not comprise a homogeneous population of cells. They manifest distinct structural and functional features depending on their anatomic location within the body and their exposure to local stimuli.[34-36] In addition, the specific phenotypes of fibroblasts from different sites are maintained even after prolonged culture *in vitro*.[37-39] The expression of chemokines in response to exposure to lipopolysaccharide, for example, differs between nasal and lung fibroblasts, demonstrating heterogeneity of fibroblasts even within the respiratory tract.[40] Furthermore, unlike skin and lung fibroblasts, which are of mesenchymal origin, corneal fibroblasts are derived from the neural ectoderm. The specific contributions of fibroblasts in different organs to allergic inflammation, however, remain poorly understood.

Given that the cornea is avascular tissue that normally lacks hematopoietic and immune cells, corneal fibroblasts (Fig. 6) might be expected to have functions that differ from those of fibroblasts in other tissues. To characterize the differential contributions of fibroblasts in different tissues to the pathogenesis of allergic diseases such as VKC, atopic dermatitis, and asthma, we compared the effects of various cytokines on the production of thymus- and activation-regulated chemokine (TARC, CCL17), a potent and selective chemoattractant for Th2 cells, by human fibroblasts derived from the cornea, skin, and lungs.[41] In corneal and dermal fibroblasts, the combination of TNF-α and either of the Th2 cytokines IL-4 or IL-13 induced a marked increase in both the release of TARC and the intracellular abundance of TARC mRNA. In contrast, lung fibroblasts did not release detectable amounts of TARC in response to any of the cytokines examined. This differential response of corneal and dermal fibroblasts on the one hand and lung fibroblasts on the other suggests that these cells play different roles in the initiation of corresponding Th2-dominant allergic conditions (VKC, atopic dermatitis, and asthma, respectively). In addition to immune cells such as dendritic cells and T cells, tissue-resident epithelial cells in the nasal mucosa, lungs, and skin produce TARC in response to cytokine stimulation.[42-44] In contrast, we have shown that corneal epithelial cells do not produce TARC in response to cytokine stimulation.[45] These observations thus reinforce the notion that the resident cell types responsible for TARC production during allergic inflammation (only fibroblasts in the cornea, both epithelial cells and fibroblasts in the skin, and only epithelial cells in the lungs) differ among organs and tissues.

CONCLUSION

We have shown that fibroblasts not only confer mechanical strength to tissue through production of the supporting framework of the ECM but also constitute an important source of biological mediators that contribute to the initiation and amplification of allergic inflammation. The uncontrolled overproduction of such mediators by fibroblasts of the eye may prevent resolution of such inflammation and lead to tissue remodeling or destruction.[8,9,46] Fibroblasts thus function as

immune modulators during ocular allergic inflammation, modulating the activities of immune cells through expression of adhesion molecules, chemokines, and ECM proteins. Fibroblasts are not a homogeneous population of cells, however, with those resident in different tissues playing distinct roles in local allergic inflammation. Targeting of tissue-resident fibroblasts or fibroblast-derived factors may provide a basis for new treatments for the giant papillae and corneal disorders associated with VKC as well as for other allergic diseases.

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