Experimental Eye Research 91 (2010) 748–754

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Role of TGF-β in tissue eosinophilia associated with vernal keratoconjunctivitis

Kaori Ohtomo [a], Nobuyuki Ebihara [a,*], Akira Matsuda [a], Tomoko Tokura [b], Toshinari Funaki [a], Akira Murakami [a]

[a] Department of Ophthalmology, Juntendo University School of Medicine, 3-1-3 Hongo, Bunkyo-ku, Tokyo 113-8431, Japan
[b] Allergy Research Center, Juntendo University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 14 July 2010
Accepted in revised form
29 August 2010
Available online 6 September 2010

Keywords:
vernal keratoconjunctivitis
TGF-β_1
eotaxin
tissue eosinophilia
integrin $\alpha v\beta_6$
tenascin-C
immunosuppressant

ABSTRACT

To determine the role of TGF-β_1 in the tissue eosinophilia associated with vernal keratoconjunctivitis (VKC), we investigated the immunohistochemical expression of TGF-β_1 and TGF-β_1-related proteins in giant papillae obtained from VKC patients. We also investigated the effect of TGF-β_1 on production of eotaxin by cultured conjunctival and corneal fibroblasts using ELISA. Finally, the effects of glucocorticoids, cyclosporine, and tacrolimus on eotaxin production by corneal fibroblasts were assessed. Our investigations revealed that eosinophils expressing TGF-β_1 and TGF-β_1-related proteins (such as phosphorylated Smad2, integrin $\alpha v\beta_6$, α-smooth muscle actin, type I procollagen, and tenascin-C) were expressed in the giant papillae. TGF-β_1 and IL-4/IL-13 caused a synergistic increase of eotaxin production in cultured conjunctival and corneal fibroblasts. This effect of TGF-β_1 and IL-4/IL-13 was inhibited by glucocorticoids, but neither by cyclosporine nor by tacrolimus. In conclusion, TGF-β_1 has an important role in the tissue eosinophilia associated with VKC.

1. Introduction

Severe allergic conjunctivitis, such as vernal keratoconjunctivitis (VKC) or atopic keratoconjunctivitis (AKC) is chronic inflammatory condition of conjunctivae characterized by prominent infiltration of eosinophils. For many years, it has been recognized that eosinophils have potential to damage the conjunctival and corneal epithelium by releasing basic proteins from their granules, such as major basic protein (MBP) or eosinophil cationic protein (ECP) (Trocmé et al., 1993, 1997). This observation, together with the overall correlation between the number of infiltrating eosinophils and the severity of allergic tissue damage, has led to the hypothesis that the eosinophil is the central effector cell in the ongoing process of conjunctival and corneal tissue injury. However, less attention has been given to possible mechanisms of the tissue eosinophilia associated with VKC. Eosinophils are a source of several molecules that have a role in the tissue eosinophilia. In particular, eosinophils are a rich source of TGF-β_1, which is a potent regulator of fibroblast/myofibroblast function and controls production of the extracellular matrix. Therefore, we investigated the immunohistochemical expression of TGF-β_1 and TGF-β_1-related proteins in giant papillae obtained from VKC patients. We also hypothesized that TGF-β_1 could be an important factor in the occurrence of the tissue eosinophilia associated with VKC. To explore this possibility, we investigated the role of TGF-β_1 in the production of eotaxin by cultured conjunctival or corneal fibroblasts. Eotaxin is a C–C chemokine with a strong direct chemoattractant effect on eosinophils. Finally, the effect of glucocorticoids, cyclosporine, and tacrolimus on the production of eotaxin by corneal fibroblasts was assessed. Cyclosporine and tacrolimus are inhibitors of the enzyme calcineurin. Recently, several reports revealed that ophthalmic solutions of cyclosporine and tacrolimus were effective for the treatment of VKC, AKC and superior limbic keratoconjunctivitis (SLK) (Ebihara et al., 2009; Ohashi et al., 2010; Sahin et al., 2008).

2. Materials and methods

2.1. Antibodies

Anti-human TGF-β_1 monoclonal antibody (mAb) was purchased from R&D systems (Minneapolis, MN, USA), anti-human major basic protein (MBP) polyclonal antibody (pAb) was obtained from Protein Tech (Chicago, IL, USA), and anti-human procollagen type I mAb was sourced from Chemicon (Billerica, MA, USA). In addition, anti-human tenascin-C mAb was purchased from Exbio (Praha, Czech Republic), anti α-smooth muscle actin (α-SMA) mAb was purchased from Dako Cytomation (Denmark), anti-phosphorylated Smad2

* Corresponding author. Tel.: +81 3 5802 1093; fax: +81 3 3817 0260.
E-mail address: ebihara@juntendo.ac.jp (N. Ebihara).

0014-4835/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2010.08.025

(Ser465/467) was obtained from Cell signaling Technology (Danvers, MA, USA), and anti-integrin $\alpha v\beta_6$ mAb was sourced from Chemicon. Anti-TGF-β_1 mAb used in this study reacts with a mature TGF-β_1.

2.2. Reagents

Human recombinant TGF-β_1, IL-4, and IL-13 were purchased from Peprotec (Rocky Hill, NJ, USA). Dexamethasone, fluorometholone, and cyclosporine were obtained from Sigma (St. Louis, USA). Tacrolimus was a kind gift from Astellas Co. (Tokyo, Japan). Dexamethasone and fluorometholone were dissolved in dimethyl sulfoxide (DMSO) and added to cultures at a final concentration of 100 nM. Cyclosporine and tacrolimus were dissolved in ethanol and Tween 80 (0.05%) and added to cultures at a final concentration of 1000 ng/ml. Tween 80 and ethanol or DMSO was added to the cultures at the same concentrations as the reagent controls for cyclosporine, tacrolimus, fluorometholone, and dexamethasone. Cell viability and the appearance of corneal fibroblasts did not differ among the various treatment groups at 24 h.

2.3. Specimens of giant papillae

All of these experiments followed the guidelines of the Declaration of Helsinki. The Ethical Review Board of Juntendo University School of Medicine approved this study. Giant papillae were removed surgically from patients who had severe corneal damage despite steroid or immunosuppressive treatment. A total of five patients with VKC and giant papillae were studied (4 males and one female with a mean age of 18 ± 7.2 years; range: 14–28 years). After obtaining the informed consent of the patients or their parents, giant papillae were resected as part of the treatment process. Normal conjunctival specimens were obtained during strabismus surgery from three patients without allergy (12, 16, and 32 years old) after informed consent was obtained. Papillae were rapidly frozen in OCT compound (Miles, Elkhart, IN) with liquid nitrogen and samples were stored at −80 °C until further examination.

2.4. Cell cultures

2.4.1. Conjunctival fibroblasts

Human conjunctival fibroblasts (HConF) were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA).

2.4.2. Corneal fibroblasts

Corneas were obtained from the Rocky Mountain Lions' Eye Bank at 3–5 days after the death of the donors (aged 62–70 years) and were maintained in storage medium (Optisol GS; Bausch & Lomb, Rochester, NY) at 4 °C until use. Corneas with focal or diffuse stromal opacity or those from patients with a history of corneal disease in the eye bank report were not used. The method of isolating keratocytes from the corneal stroma has been described previously (Ebihara et al., 2007). Isolated keratocytes were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% FCS. In this study, we used passage 3 keratocytes grown with 10% FCS. For phenotypic characterization, we investigated expression of the CD34, keratocan, and α-smooth muscle actin (α-SMA) gene products. Passage 3 keratocytes grown with 10% FCS strongly expressed α-SMA, but not CD34 or keratocan (Ebihara et al., 2007). CD34 and keratocan are markers of keratocytes, and α-SMA is a marker of corneal fibroblasts/myofibroblasts, so these results indicated that passage 3 keratocytes had differentiated corneal fibroblasts/myofibroblasts.

2.5. Immunohistochemistry

Frozen specimens were cut into 5 μm sections on a cryostat, air-dried, fixed in cold acetone for 10 min, and then washed with

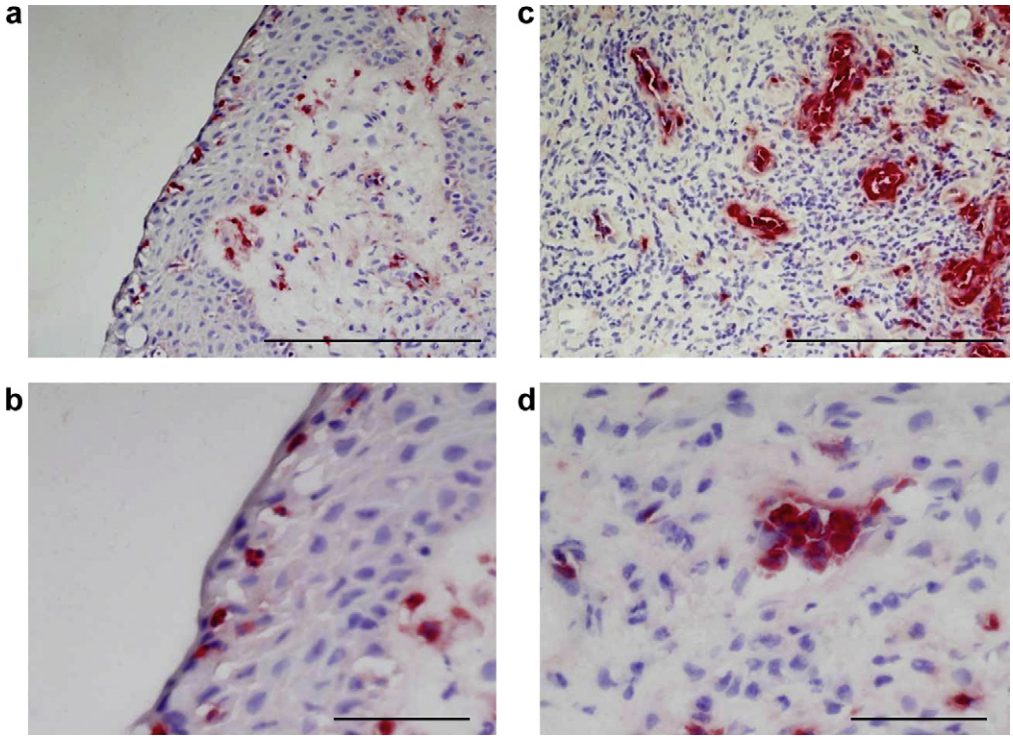

Fig. 1. Immunostaining for TGF-β_1 in giant papillae. Many inflammatory cells with cytoplasmic expression of TGF-β_1 were recognized in the epithelium, substantia propria (a,b), and vascular cavities (c,d) of the giant papillae. a,c: low magnification (Bar 200 μm), b,d: high magnification (Bar 50 μm).