Chemokine receptor gene expression in giant papillae of atopic keratoconjunctivitis

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Purpose: Major chemokine receptors in the giant papillae of atopic keratoconjunctivitis (AKC) have yet to be determined. We evaluated chemokine receptor genes and their ligand expressions in upper tarsal conjunctival giant papillae of AKC with atopic dermatitis and/or asthma.

Methods: CC, CXC, and CX3C chemokine receptor (R) gene expression levels in giant papillae of five clinically active AKC patients and in three age matched non-allergic control conjunctiva were measured with a multi-probe ribonuclease protection assay (RPA) system. The ligands of abundant chemokine receptors in the giant papillae were examined by immunohistochemistry or reverse transcription-polymerase chain reaction. Interleukin (IL)-4 and IL-13 gene expression levels were measured with RPA. Ligand expression in cultured human conjunctival fibroblasts was examined by reverse transcription-polymerase chain reaction.

Results: High CXCR4 and CCR4 gene expression levels were detected in the giant papillae of all (CXCR4) and four out of five (CCR4) patients. As a CCR4-ligand, thymus and activation regulated cytokine (TARC/CCL17) rather than macrophage derived chemokine (MDC/CCL22), was predominant immunohistochemically in the giant papillae. Giant papillae with high CCR4 gene expression levels showed high IL-4 and IL-13 expression. Cultured human conjunctival fibroblasts express stromal cell derived factor-1 (SDF-1/CXCL12) in vitro.

Conclusions: CXCR4 and CCR4 are the major chemokine receptor genes expressed in the giant papillae of AKC with atopic dermatitis and/or asthma. Our findings suggest a role for CXCR4 and CCR4 in the formation of giant papillae.

Atopic keratoconjunctivitis (AKC) is characterized by chronic allergic inflammation of the conjunctiva accompanied by ocular itching and conjunctival hyperemia. Histopathologically, cells that infiltrate the conjunctiva are eosinophils, T- and B-lymphocytes, mast cells, macrophages, basophils, plasma cells, and dendritic cells [1]. Vernal keratoconjunctivitis (VKC) and AKC patients have many elevated giant papillae, a gelatinous conjunctival elevation in the upper tarsal conjunctiva [2]. Clinically, the giant papillae and chemical mediators often produce corneal epithelial lesions subsequent to the conjunctival allergic reaction.

Chemokines are low molecular weight proteins critical to immune and inflammatory responses by their regulating leukocyte trafficking in a multistep process [3,4]. Chemokines are divided into families based on the relative positions of their cysteine residues [5]. The CXC (α) chemokine family has the first two cysteines separated by one amino acid residue. In the CC (β) chemokine group, the first two cysteine residues are adjacent to each other. Two other subfamilies, the C (XCL1, lymphotactin) and CX3C (CX3CL1, fractalkine) families, have only single members [5,6]. Chemokines form a complex functional network locally and systemically in a variety of inflammatory, infectious, and immune diseases [4,7]. In allergic diseases that T helper 2 cytokine are predominant, CCR3 and CCR4 are candidate chemokine receptors relevant to pathophysiology. In allergic conjunctivitis, CXCR3 is reported to be the predominant chemokine receptor in limbal form VKC [8]. Eotaxin and its receptor, CCR3, were detected in tear and mucus of AKC and VKC patients [9-11]. Major chemokine receptors in the giant papillae of AKC have yet to be determined by systemic comparison.

We evaluated CC, CXC, and CX3C chemokine receptor mRNA expression in the giant papillae of severe AKC patients. CXCR4 and CCR4 were the major chemokine receptors in the giant papillae, suggesting that they may play a role in the formation of tarsal conjunctival giant papillae.

METHODS

Patients: All the experiments followed the guidelines of the Declaration of Helsinki. The Institutional Review Board of the University of Tokyo School of Medicine approved the study. Giant papillae were surgically resected in cases where patients suffered from severe corneal damage and profound itching in spite of steroid or non-steroid treatment, and five patients with giant papillae of AKC were studied. After obtaining informed consent of the patients or persons with parental authority, giant papillae were resected for treatment, as described elsewhere [12], of five patients (Table 1). Normal conjunctivae were obtained during strabismus surgery from three non-allergic normal control patients (12, 16, and 32 years old) after informed consent was obtained. Papillae from each patient were homogenized separately in Isogen® (Nippon...
Gene, Tokyo, Japan) then quick frozen in OCT compound (Miles, Elkhart, IN) with liquid nitrogen. Normal conjunctiva used for total RNA extraction were processed as a group because of the small amount of total RNA available per sample. All samples were stored at -70 °C until further examination.

**RNA preparation and ribonuclease protection assay:*** After centrifugation to remove cellular debris, total RNA was isolated with Isogen® according to the manufacturer’s instructions, and the RNA pellet was resuspended in nuclease free water. Detection and quantification of chemokine receptor mRNAs were carried out with a multiprobe ribonuclease protection assay (RPA) system (BD PharMingen, San Diego, CA) as described previously [13]. Briefly, a mixture of [α-32P] UTP labeled antisense riboprobes was generated from the chemokine receptor template set hCR-5, hCR-6, and hCK-1 (BD PharMingen). Total RNA (5 µg) was used in each sample. Total RNA was hybridized overnight at 56 °C with 300 pg of the 32P-anti-sense riboprobe mixture. After ethanol purification of the nuclease protected RNA fragments, samples were separated on 5% polyacrylamide sequencing gels, and the gels dried and subjected to autoradiography. Protected bands were observed after exposure of the gels to x-ray film. Specific bands were identified by their distinctive migration patterns as compared to patterns of the undigested probes. Densitometric analysis (NIH Image, version 1.63) was used for quantitation. Bands were normalized to the liposome 32 (L32) housekeeping gene. Two sets of results on separate experiments were analyzed, and averaged density was shown as the results of densitometric analysis.

**Immunohistochemical study:** The antibody (Ab) used for immunostaining was mouse anti-human thymus and activation regulated cytokine (TARC/CCL17) Ab (54026.11; R&D Systems, Minneapolis, MN), goat anti-CCR4 polyclonal antibody (C-20; Santa Cruz biotechnology, Inc., Santa Cruz, CA), mouse anti-human macrophage derived chemokine (MDC/CCL22) Ab (57226.11; R&D Systems), anti-CXCR4 Ab (12G5; R&D Systems), and anti-stromal cell derived factor (SDF)-1/CXCL12 Ab (79018.111; R&D Systems). HCFs remaining in the wells were exposed with an ELISA kit according to the manufacturer’s protocol (R&D Systems). HCFs remaining in the wells were exposed to trypsin-ethylene diamine tetraacetate acid, and the average number present was determined with a hematocytometer. Protein concentration in the culture medium was normalized as nanograms of protein per 10⁶ cells. Plates were read with a Microplate Reader (Molecular Bioscience Group, Hercules, CA) at the optical densities of 450 nm for measurement and 550 nm for reference.

**Statistics:** One way analysis of variance (ANOVA) and Fisher’s protected least significant difference was used to compare the band densities in the RT-PCR. The α level was set at 0.05. The analysis was done with the Stat View statistical software package (Statview, version 5; Abacus Concepts, Berkeley, CA).

**RESULTS**

**CC, CXC, and CX3C chemokine receptor gene expression in upper tarsal conjunctiva:** Figure 1 shows the representative RPA autoradiographs (Figure 1A,B) and the amounts of chemokine receptor mRNA normalized to housekeeping gene.
L32. Averaged density of two sets of results was shown in Figure 1C,D. The CCR1, CCR2, and CCR4 genes are barely detectable in normal conjunctivae. The AKC, but not the normal, conjunctivae show appreciable CCR3 gene expression. Overexpression of CCR4 gene is present in four of five upper tarsal giant papillae as compared with the normal control conjunctivae. In contrast, patient 5 does not show a significant amount of CCR4 gene expression. In CXC and CX3C chemokine receptor, the CXCR1, CXCR2, CXCR4, and CX3CR genes are detected in the normal conjunctivae. There are no appreciable differences in CXCR1, CXCR2, or CX3CR1 between the normal and allergic conjunctivae. CXCR2 gene expression level in normal conjunctivae is higher than those in giant papillae of AKC patients, suggesting a comparatively high expression of neutrophil related chemokines/chemokine receptors in the normal conjunctivae [14-16]. The AKC, but not the normal, conjunctivae show appreciable CXCR5 gene expression. A significant high amount of CXCR4 gene is present in all five conjunctivae as compared with the normal control conjunctivae.

**Immunohistochemical study of CCR4 ligands, TARC/CCL17 and MDC/CCL22 in conjunctivae:** TARC/CCL17 and MDC/CCL22 expression were examined immunohistochemically in three AKC conjunctivae (patients 1, 2, and 4) and three normal ones. No positive staining was detected in the conjunctival epithelium of giant papillae. In the AKC conjunctivae, many TARC/CCL17 positive stainings are present in the subepithelial area, in the representative photograph of TARC/CCL17 positive stains in Figure 2A (Patient 1). In contrast, MDC/CCL22 positive staining was present, but not as much as compared with the number of TARC/CCL17 positive staining cells (Figure 2B). No positive staining was detected in the normal conjunctivae with anti-TARC/CCL17 positive staining cells and eosinophils are predominant (Figure 4A). Focal fibroblast-like cell infiltrating areas are also detected in giant papillae of patient 1. Similar findings are observed in patients 2 and 3 (data not shown). Marked fibroblast-like cells are infiltrating in giant papillae of patient 5 (Figure 4B). Some fibroblast-like cells are present under normal conjunctival epithelium (Figure 4C).

**SDF-1/CXCL12 gene expression in conjunctivae:** The CXC4 ligand’s SDF-1/CXCL12 gene expression level was measured by semi-quantitative RT-PCR, because commercially available antibody did not work immunohistochemically. The gene was present in both normal and patient conjunctivae. A representative photograph of 4 sets of data and the average density within the linear range of amplification obtained by NIH Image are shown in Figure 5. Significantly higher amounts of SDF-1/CXCL12 are present in all the conjunctivae from the patients as compared to the normal conjunctivae (p<0.01). TARC/CCL17 and SDF-1/CXCL12 expression in cultured human conjunctival fibroblasts: To examine the possibility that normal HCFs in addition to infiltrating cells expresses high CCR4 gene giant papillae (patients 1-4) as compared with the normal conjunctivae, whereas IL-4 gene expression level was low in giant papillae of patient 5 with no CCR4 gene expression. A high amount of IL-13 gene expression as compared with the normal conjunctivae was detected in high CCR4 gene expressing giant papillae (patients 1-4), but not in the giant papillae of patient 5.

**HE staining in giant papillae of AKC patients 1 and 5:** HE staining in patient 1 shows infiltrations of mononuclear cells and eosinophils are predominant (Figure 4A). Focal fibroblast-like cell infiltrating areas are also detected in giant papillae of patient 1. Similar findings are observed in patients 2 and 3 (data not shown). Marked fibroblast-like cells are infiltrating in giant papillae of patient 5 (Figure 4B). Some fibroblast-like cells are present under normal conjunctival epithelium (Figure 4C).

**IL-4 and IL-13 gene expression in giant papillae:** To examine the correlation between helper T2 (Th2) cytokine and CCR4 gene expression, IL-4 and IL-13 gene expression was measured with the RPA method. Figure 3 shows a representative autoradiograph and the averaged amounts of chemokine receptor mRNA of two sets of results normalized to housekeeping gene L32. IL-4 gene expression was upregulated in high CCR4 gene giant papillae (patients 1-4) as compared with the normal conjunctivae, whereas IL-4 gene expression level was low in giant papillae of patient 5 with no CCR4 gene expression.

### Table 1. Patient Background

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Corneal condition</th>
<th>Allergic disease</th>
<th>Position</th>
<th>Treatments</th>
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<tr>
<td>1</td>
<td>37</td>
<td>Ulcer</td>
<td>AKC, AD, AS</td>
<td>U</td>
<td>CR, CyA</td>
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<tr>
<td>2</td>
<td>13</td>
<td>Plaque</td>
<td>AKC, AD, AS</td>
<td>B</td>
<td>Betamethasone</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>Ulcer</td>
<td>AKC, AD, AS</td>
<td>B</td>
<td>Betamethasone</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>Ulcer</td>
<td>AKC, AD, AS</td>
<td>B</td>
<td>CR, Tacrolimus</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>Ulcer</td>
<td>AKC, AD, AS</td>
<td>B</td>
<td>CR, CyA, Fluoromethoron</td>
</tr>
</tbody>
</table>

Besides having atopic keratoconjunctivitis (AKC), most of these patients also had atopic dermatitis (AD) and asthma (AS). Patients were treated with different topical medications including betamethasone, sodium cromoglycate (CR), cyclosporine A (CyA), fluoromethorin, and tacrolimus. All patients were male. In the “Position” column, ‘U’ indicates unilateral and ‘B’ indicates bilateral.

### Table 2. Primer sequences for RT-PCR

<table>
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<tr>
<th>Gene</th>
<th>Sequence</th>
<th>Product size (bp)</th>
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<td>354</td>
</tr>
<tr>
<td></td>
<td>3’-AGTTTAGGTGCCACCCCCAAAAGGCC-5’</td>
<td>354</td>
</tr>
<tr>
<td>TARC/CCL17</td>
<td>5’-CGGACCCCAACAACAGAGAAT-3’</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>3’-CTCTCCCATCTGTGCTCTCT-5’</td>
<td>202</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-GTGCAATGGTGCTGATGACGA-3’</td>
<td>223</td>
</tr>
<tr>
<td></td>
<td>3’-TGTTAGTGGGGTCTCCCTCGTG-5’</td>
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</table>

Primer sequences for semi-quantitative RT-PCR of stromal cell derived factor-1 (SDF-1/CXCL12), TARC/CCL17, and glyceraldehyde phosphate dehydrogenase (GAPDH).
Figure 1. CC, CXC, and CX3C chemokine receptor gene expression in giant papillae in atopic keratoconjunctivitis. **A** shows an autoradiograph of CC chemokine receptors and **B** shows an autoradiograph of CXC/CX3C chemokine receptors. Specific bands for each chemokine were identified from undigested probe migration patterns. Lane M represents an undigested marker probe. Lane N is normal conjunctiva. Lanes 1-5 correspond to the five patients. **C, D:** Normalized analysis results are shown of the autoradiographs from **A** and **B.** The vertical axes have arbitrary units based on densitometry. The bands were normalized to the liposome 32 (L32) housekeeping gene. In comparison to chemokine receptor mRNA levels in normal conjunctiva, CCR4 mRNA is abundant in four out of five AKC patients (**C**). In CXC and CX3C chemokine receptor, CXCR4 mRNA levels are high in the giant papillae of all five patients (**D**). Two sets of results on separate experiments were analyzed, and averaged density of the individual bands was shown as the results of densitometric analysis.
the TARC/CCL17 and SDF-1/CXCL12, we tested whether cultured HCFs can express TARC/CCL17 and SDF-1/CXCL12. The TARC/CCL17 (38 cycles) and SDF-1/CXCL12 (30 cycles) genes were expressed in cultured HCFs (Figure 6A). Then cultured HCFs were stimulated with recombinant IFN-γ, IL-4, IL-13, TNF-α, or the vehicle (control group). As shown in Figure 6B, cultured HCFs produced SDF-1/CXCL12 in the serum free condition (control group). In comparison with the control samples, a significantly large amount of SDF-1/CXCL12 was produced in HCFs stimulated with recombinant IFN-γ, but not with IL-4, IL-13, or TNF-α. The high SDF-1/CXCL12 concentration produced with IFN-γ was decreased in the presence of the same amount of recombinant TNF-α. In ELISA, TARC/CCL17 expression was undetectable in HCFs stimulated with recombinant IFN-γ, IL-4, IL-13, TNF-α, or vehicle (data not shown).

**DISCUSSION**

We used a multiprobe RPA system to quantify a panel of CC, CXC, and CX3C chemokine receptor mRNA from individual samples (five samples five results) of the total RNA of giant papillae from five AKC patients. This RPA method allows the comparative analysis of different mRNA species in an mRNA sample. All five patients had corneal damage and giant papillae with atopic dermatitis, asthma, or both. Although the patient numbers were not satisfactory for comprehensive analysis, identification of common abundant chemokine receptor

Figure 2. Immunohistochemical expression of TARC/CCL17 and MDC/CCL22 in the giant papillae of an AKC patient. TARC/CCL17 and MDC/CCL22 were expressed in the giant papillae of all three available patients examined for immunohistochemistry (patients 1, 2, and 4). A: A representative photograph is shown (patient 2). TARC/CCL17 positive staining is present in the subepithelial part of the conjunctiva. B: MDC/CCL22 positive cells in the conjunctiva were detected, but were rare in each giant papilla of the 3 patients (Arrow). C: No positive staining is detected in the AKC conjunctivae with non-immunized control IgG. Positive staining is not detected in the normal conjunctivae (data not shown). Original magnification was 200x.

Figure 3. Th2 cytokine gene expression in giant papillae in atopic keratoconjunctivitis. To examine the correlation between helper T2 (Th2) cytokine and CCR4 gene expression, IL-4 and IL-13 gene expression was measured with the multi-probe ribonuclease protection assay RPA method. A: A representative autoradiograph from two sets of results. B: The averaged amounts of chemokine receptor mRNA (determined from densitometric analysis of the RPA analysis) of two sets of results normalized to housekeeping gene L32. The vertical axes have arbitrary units based on densitometry. In comparison to IL-4 mRNA levels in normal conjunctivae, comparatively high IL-4 mRNA levels are detected in four out of five AKC patients. The IL-4 mRNA expression level of patient 5 is similar to that of normal conjunctivae. In IL-13 mRNA expression, elevated amounts of IL-13 mRNA are present in the giant papillae of four out of five patients. In contrast, no apparent IL-13 mRNA is detected in giant papillae of patient 5. Two sets of results on separate experiments were analyzed, and the average density is shown as the results of densitometric analysis.
genes can become a clue to reveal the pathogenesis of giant papillae formation in allergic conjunctival diseases.

Generally, Th2 cytokines and some chemoattractants play a role in the pathogenesis of allergic inflammation. The effects of Th2 cytokines, such as IL-4, IL-5, IL-9, and IL-13, reflect the pathophysiological manifestations of allergy and asthma. Moreover, both Th2 cells and the effector cells (basophils, mast cells, and eosinophils) express chemoattractant receptors such as CCR3, CCR4, and CCR8 [17]. Therefore, interactions of eotaxin(s), eotaxin/CCL11, RANTES/CCL5, and MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, and MCP-4/CCL13 with CCR3 are responsible for the recruitment of basophils, eosinophils, and mast cells, whereas interactions of CCR4 with MDC/CCL22 or TARC/CCL17, and CCR8 with I-309/CCL1 have a role in the allergen induced recruitment of Th2 cells in the target tissues of allergic inflammation [18,19]. Our findings that an interaction of CCR4 with TARC/CCL17 rather than MDC/CCL22 among possible interactions of chemokine-chemokine receptors is predominant suggests a critical role of CCR4 with TARC/CCL17 in Th2 cytokine and effector cell recruitment in giant papillae of allergic conjunctivitis with atopic dermatitis and asthma.

Giant papillae of a patient (patient 5) did not show overexpression of CCR4 gene and Th2 cytokines, IL-4 and...
IL-13 genes. As shown in HE staining (Figure 4B), fibroblast-like cell infiltrations were predominant in giant papillae, whereas numerous eosinophilic and mononuclear cells were mainly infiltrating in the giant papillae of the other patients as shown in representative HE staining. Systemically, patient 5 also has asthma and severe atopic dermatitis including facial skin and clinical finding of giant papillae was similar to those of other patients, suggesting a systemic Th2 cytokine dominant condition. In this patient, resection of giant papillae was performed on the chronic stage after the proliferating stage. Although Th2 cytokine should affect formation of giant papillae, it may depend on the chronic or acute stage or the difference of giant papillae formation etiology.

SDF-1/CXCL12 is a member of a family of chemokines and initially was characterized as a growth stimulating factor for B cell precursors [20,21]. It is a potent chemotactic factor for T and pre-B lymphocytes [22,23], plasma cells [24], and dendritic cells (DCs) [25] and is a co-stimulatory factor for CD4+ T-cell activation [26] and affects T-cell rolling and tight adhesion to activated endothelial cells [27]. Moreover, SDF-1/CXCL12 and its specific receptor, CXCR4, are involved in tumorgenesis [28-30] and angiogenesis [31,32]. As a regulator of local inflammation in the rheumatoid arthritis synovium, CXCR4 and SD-1/CXCL12 accumulate CD4+ T cells within the inflamed synovium [33]. These findings suggest that SDF-1/CXCL12 may recruit a large number of mature and immature CXCR4 positive leukocytes to the giant papillae and stimulate neovascularization in the giant papillae of tarsal conjunctivae. Moreover, the enlargement of papillae in tarsal conjunctivae is due to the combination of conjunctival thickening, subepithelial fibrosis, mucous metaplasia, neovascularization, and scarring [1]. This tissue remodeling is involved in overproduction of the extracellular matrix promoted by T-helper 2 type cytokines and growth factors [34-36]. Although our data revealed that resident conjunctival fibroblasts express SDF-1/CXCL12, there is a possibility that the other infiltrating cells in giant papillae also express SDF-1/CXCL12. Massive induced CXCR4 positive inflammatory cell infiltration may enhance the formation of giant papillae by supplying an extracellular matrix, cytokines, and neovascularization.

CXCR4 is also expressed on eosinophils [37,38] and mast cells [39,40], critical components in allergic conjunctivitis [41]. CXCR4 and SDF-1/CXCL12 are considered to be essential factors of allergic airway disease in the mouse [42,43] and humans [44]. Human mast cells migrate across the vascular

Figure 6. SDF-1/CXCL12 and TARC/CCL17 gene and protein production in cultured human conjunctival fibroblasts (HCFs). A: Cultured conjunctival fibroblasts were homogenized in Isogen®. Water was used as a negative control. The TARC/CCL17 and SDF-1/CXCL12 genes are expressed in cultured conjunctival fibroblasts. Lane M shows a molecular weight marker (100 bp ladder). Lane C: Cultured conjunctival fibroblasts. Lane NC: Negative control. B: Cultured conjunctival fibroblasts were incubated in serum free medium for 24 h with IFN-γ (10 ng/ml), IL-4 (10 ng/ml), IL-13 (10 ng/ml), TNF-α (10 ng/ml), IFN-γ+TNF-α (10 ng/ml each), or the vehicle only. The SDF-1/CXCL12 and TARC/CCL17 concentrations in the culture supernatant were measured by ELISA. A significantly large amount of SDF-1/CXCL12 is produced in HCFs stimulated with recombinant IFN-γ, but not with IL-4, IL-13, or TNF-α in comparison with the control samples. The high SDF-1/CXCL12 concentration produced with IFN-γ is decreased in the presence of the same amount of recombinant TNF-α. The TARC/CCL17 concentration was undetectable with these cytokine stimulations (data not shown). Values presented are means; the error bars represent the standard deviations. The single asterisk indicates a statistical difference between IFN-γ and control (p=0.0057; Fisher’s protected least significant difference test); the double asterisk indicates a statistical difference between IFN-γ+TNF-α and IFN-γ (p=0.0115; Fisher’s protected least significant difference test). Similar results were obtained for a different set of culture dishes.
endothelium to tissues in response to SDF-1/CXCL12 [41]. Neutralization of CXCR4 and SDF-1/CXCL12 reduced lung eosinophilia and airway hyper-responsiveness in the mouse model of allergic airway disease [42,43]. Although we did not directly identify which cells express CXCR4 in this study, the blockade of CXCR4 and SDF-1/CXCL12 interaction may offer candidate treatment for tarsal conjunctival giant papillae.

Because corneal damage seen in allergic conjunctival disease is associated with the eosinophil infiltration of the conjunctiva and the release of various eosinophilic proteins [45], mast cells have critical roles in the pathogenesis of allergic conjunctivitis [41]. The findings that eotaxin and its receptor CCR3 expression have been detected in tear and mucous of AKC patients [8-11] are not contradictory to our data because our findings show comparatively predominant expression of CCR4 and CXCR4 genes in the giant papillae.

It is important to explain a limitation of this study. We showed comparative data of chemokine receptor gene expression levels, but not protein expression of chemokine receptor because commercially available antibody did not work well for immunohistochomical studies. To our knowledge, protein expressions of IL-4 and IL-13 have not been detected yet in the giant papillae of chronic allergy diseases, although Th2 cytokines are considered to be associated with formation of giant papillae. Further definitive protein level studies such as western blotting method may be required in the analysis of chemokines and cytokines.

In summary, CCR4 and CXCR4 are the major chemokine receptor genes of giant papillae in tarsal conjunctivae and TARC/CCL17, rather than MDC/CCL22, is a predominant CCR4 ligand. Cultured HCFs express SDF-1/CXCL12. Our findings point to a role for CXCR4 and CCR4 in the pathogenesis of allergic conjunctival giant papillae.

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REFERENCES


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