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Local Ocular Immunotherapy for Experimental Allergic Conjunctivitis

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Purpose: We studied the efficacy of eyedrops as local ocular immunotherapy against experimental allergic conjunctivitis.

Methods: Guinea pigs were sensitized with an intraperitoneal injection of ovalbumin (OA). Three weeks after the sensitization, a low concentration (10 μg/mL) of OA eyedrops was administered once a day for 3 weeks. Six weeks after the sensitization, an allergic inflammation was provoked with 20 mg/mL of OA eyedrops.

Results: In conjunctival clinical scores 30 minutes after the allergen challenge, there was no significant difference between the controls and the treated group. The total number of inflammatory cells in the conjunctiva 8 hours after the allergen challenge was significantly decreased in the treated group (60.8 ± 23.2 cells/field) compared with the control group (199.1 ± 83.4 cells/field). Eosinophils in the conjunctiva 24 hours after the allergen challenge were also significantly decreased in the treated group (22.1 ± 15.5 cells/field) compared with the control group (50.3 ± 15.0 cells/field).

Conclusions: In this study, local ocular immunotherapy mainly suppressed the late phase reaction of allergic inflammation. These results coincide with previous studies of immunotherapy in which a subcutaneous injection was used. Local ocular immunotherapy is effective against experimental allergic conjunctivitis. **Jpn J Ophthalmol 2000;44:634–638** © 2000 Japanese Ophthalmological Society

Key Words: Experimental allergic conjunctivitis, eyedrops, guinea pig, immunotherapy, ovalbumin.

Introduction

Antigen-specific immunotherapy by a subcutaneous injection against type I allergic disorders has been used since Noon et al[1] first described this method about 90 years ago. While many treatments against allergic disease are palliative, immunotherapy is a radical one. Today, the efficacy of immunotherapy is recognized,[2,3] but subcutaneous injections of allergens have a risk of anaphylactic shock, so it is required that the patient attend a hospital frequently. Thus, immunotherapy by subcutaneous injection is difficult for patients, especially for those with allergic conjunctivitis.

Recently, local immunotherapy for pollen- and mite-induced allergic rhinitis by the nasal route with a powder form of allergen was reported and met with good results.[4,5] In ophthalmological literature, there are few reports of local immunotherapy using eyedrops for allergic conjunctivitis,[6,7] and no experimental results have been reported.

We performed local ocular immunotherapy using eyedrops containing a low concentration of allergen in an experimental allergic conjunctivitis model, and report on its efficacy.

Materials and Methods

Experimental Allergic Conjunctivitis

Eight-week-old male Hartley guinea pigs (Japan SLC, Hamamatsu) were divided into treated and

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control groups and used in this study. All experiments complied with the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research. One mg/mL of ovalbumin (OA, Grade V; Sigma, St. Louis, MO, USA) as an antigen, 20 mg/mL of $A1(OH)_3$ and 10^{10} cells/mL of inactivated *Bordetella pertussis* (Denka Seiken, Tokyo) as an adjuvant, were mixed in a solution of inactivated *Bordetella pertussis.* Then, 1 mL of this solution was injected intraperitoneally. One week later, the same sensitization was performed. Twenty days after the second sensitization, 20 mg/mL of OA eyedrops were administered in the right eye, provoking an allergic reaction.

Local Immunotherapy with Eyedrops

Twenty-one days after the second sensitization, 10 μg/mL of OA eyedrops, the maximum concentration that did not provoke an allergic reaction macroscopically in a preparatory experiment, were administered in the right eye once a day for 3 weeks. For the controls, saline eyedrops were administered for the same period.

Allergen Challenge and Evaluation of Inflammation

Six weeks after the second sensitization, 20 mg/mL of OA eyedrops were administered in the right eye, provoking an allergic reaction.

Thirty minutes after the allergen challenge, conjunctival clinical symptoms were evaluated according to the following scores: 1, mild conjunctival injection; 2, severe conjunctival injection; 3, conjunctival injection and mild to moderate chemosis; 4, severe chemosis. These symptoms were considered to indicate an early phase reaction.

Eight and 24 hours after the allergen challenge, the animals were sacrificed with sodium pentobarbital. The eyes were enucleated with the eyelids. After fixating with 10% formalin, hematoxylin-eosin stained histological sections were made with a sagittal slice. According to histological studies reported by Shoji et al[8,9], in a section 8 hours after the allergen challenge, the number of total inflammatory cells (lymphocytes, neutrophils, and eosinophils) in the conjunctiva was counted using a light microscope. In a section 24 hours after the allergen challenge, the number of eosinophils in the conjunctiva was counted. The number of cells was counted in a full field of a light microscope at 400× magnification, in any randomly chosen three fields, and averaged. Each cell count number was considered to indicate a late phase reaction.

Results

Early Phase Reaction

Clinical scores 30 minutes after the allergen challenge ranged between 3 and 4 with a median score of 4 in the control group (n = 12), and between 2 and 4 with a median score of 3 in the treated group (n = 15). The score in the control group was slightly higher than in the treated group, but the difference between the two groups was not significant ($P = .1492$, Mann–Whitney *U*-test) (Figure 1).

Late Phase Reaction

In the section 8 hours after the allergen challenge, an infiltration of various inflammatory cells (lymphocytes, neutrophils, and eosinophils) was seen in the conjunctival stromal layer. The change was predominant in the control group (Figure 2). The number of total inflammatory cells was 199.1 ± 83.4 cells/field in the control group (n = 6), and 60.8 ± 23.2 cells/field in the treated group (n = 9, mean ± SD). Statistically, there was a significant difference between the two groups ($P = .0004$, Student *t*-test) (Figure 3).

In the section 24 hours after the allergen challenge, an infiltration of eosinophils was seen mainly in the conjunctival stromal layer and partially in the epithelium. The change was also predominant in the control group (Figure 4). The number of eosinophils was 50.3 ± 15.0 cells/field in the control group (n = 6), and 22.1 ± 15.5 cells/field in the treated group (n = 6, mean ± SD). Statistically, there was a significant dif-

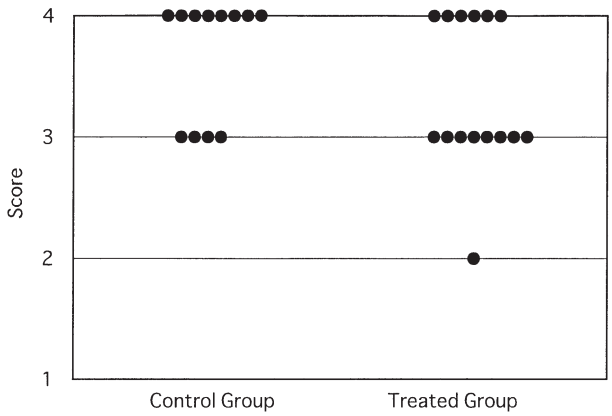

Figure 1. Conjunctival clinical scores 30 minutes after allergen challenge. 1: mild conjunctival injection, 2: severe conjunctival injection; 3: conjunctival injection and mild to moderate chemosis; 4: severe chemosis. •: One guinea pig. There was no significant difference between control and treated groups ($P = .1492$, Mann–Whitney *U*-test).

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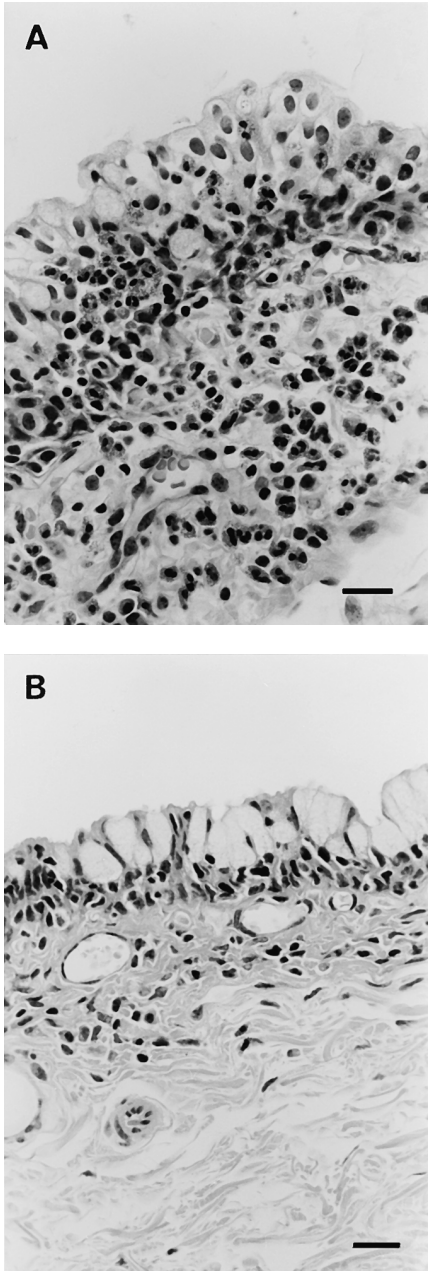

Figure 2. Representative light micrographs of conjunctiva 8 hours after allergen challenge. Infiltration of various inflammatory cells (lymphocytes, neutrophils, and eosinophils) was observed in conjunctival stromal layer. Goblet cells were decreased and epithelium was thinned. Change was predominant in control group (**A**) compared to treated group (**B**) (hematoxylin-eosin staining). Bar = 20 μm.

ference between the two groups ($P = .0092$, Student *t*-test) (Figure 5).

These histological changes agree with the results shown by Shoji et al.[8,9]

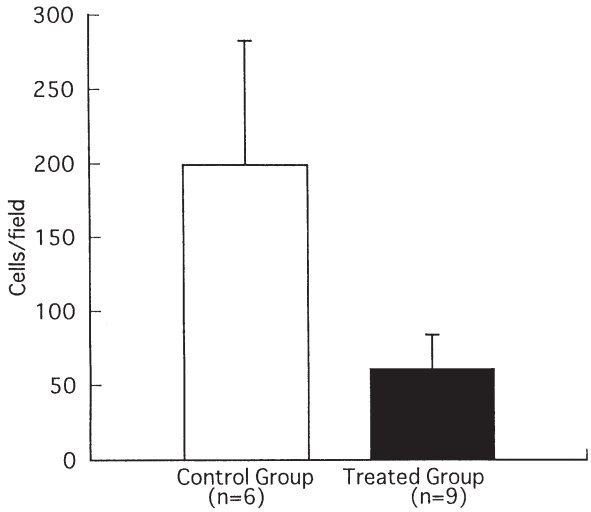

Figure 3. Number of total inflammatory cells in section 8 hours after allergen challenge. Number of total inflammatory cells was significantly decreased in treated group (n = 9) compared to control group (n = 6) ($P = .0004$, Student *t*-test). Error bars = 1 SD.

Discussion

Del Prete et al[6] reported the success of local ocular immunotherapy in patients with seasonal allergic conjunctivitis. The patients were allergic to different kinds of allergens (Dermatophagoides, pollen, and fungus). They received eyedrops of each allergen diluted at one tenth the concentration required to obtain a 3-mm-diameter weal in the prick test. In the treated group, there was a significant improvement in subjective symptoms, objective clinical scores and cytology. In this study, various kinds of allergen were used and all were effective. This indicates that local ocular immunotherapy may be effective for any kind of allergen.

In bronchial asthma, Warner et al[10] reported that after immunotherapy there was no change in the immediate response. However, a late reaction was not observed in half the patients. In ragweed allergic patients, Pienkowski et al[11] reported that the cutaneous late phase reaction was suppressed in patients receiving immunotherapy. Thus, immunotherapy suppresses mainly the late phase reaction of allergic inflammation. In our study, there was no significant difference between the control and the treated group 30 minutes after the allergen challenge. This may represent the early phase reaction. Eight and 24 hours after the allergen challenge, the number of inflammatory cells significantly decreased in the treated group. This may represent the late phase re-

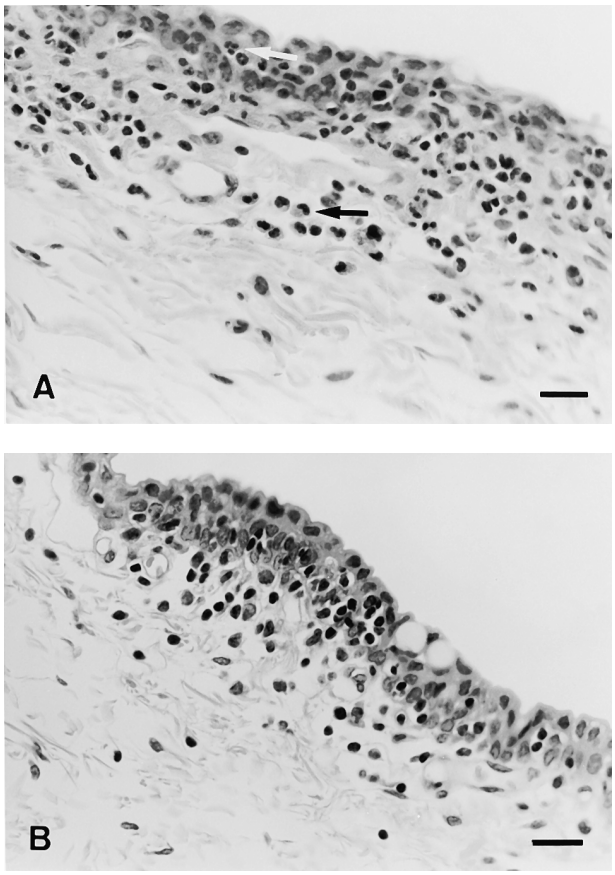

Figure 4. Representative light micrographs of conjunctiva 24 hours after allergen challenge. Infiltration of eosinophils was observed mainly in conjunctival stromal layer (black arrow) and partially in epithelium (white arrow). Change was predominant in control group (**A**) compared to treated group (**B**) (hematoxylin-eosin staining). Bar = 20 μm.

action. These results coincide with the previously mentioned reports.

In allergic inflammation, the late phase reaction with eosinophil infiltration is related to the severity of the disease, so suppression of the late phase reaction is an important factor in treating allergic inflammation. Therefore, immunotherapy is the ideal treatment for allergic inflammation. Immunotherapy by subcutaneous injection has a risk of anaphylactic shock, but local administration of low dose allergen eyedrops is thought to have few systemic complications, and patients can instill eyedrops by themselves. In this study, local immunotherapy using eyedrops was effective for experimental allergic conjunctivitis. We consider this method a safe and effective regimen for allergic conjunctivitis.

The mechanisms of immunotherapy and why immunotherapy mainly suppresses the late phase reaction are still unclear. Recently, the change of the cytokine profile in helper T cells has been noted as an important mechanism of immunotherapy.[12–14] In these studies, the cytokine profile changed from the Th2 pattern to the Th1 pattern after immunotherapy. It is still unclear why immunotherapy changes the cytokine profile in helper T cells, yet this may be one of the most important factors in elucidating the detailed mechanisms of immunotherapy.

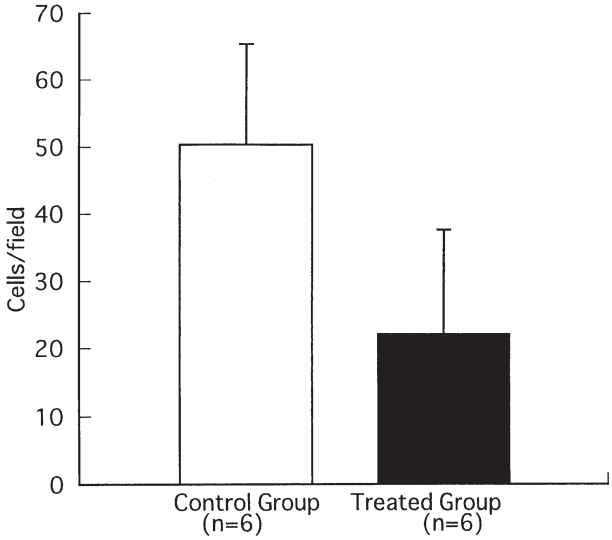

Figure 5. Number of eosinophils in section 24 hours after allergen challenge. Number of eosinophils was significantly decreased in treated group (n = 6) compared with control group (n = 6) (P = .0092, Student t-test). Error bars = 1 SD.

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