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Flow Cytometry Tear Analysis in Patients with Chronic Allergic Conjunctivitis

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Abstract: Tears from patients with chronic allergic conjunctivitis were analyzed with flow cytometry to determine the function of the T lymphocyte-related immunological reactions in the disease pathogenesis. Twenty-eight patients and 22 normal volunteers were studied; tears were obtained with capillary tubes. T helper/T suppressor ratios and the percentages of HLA DR+, CD23+, and CD3+ cells were significantly higher in patients than in controls. This study provides support for the hypothesis that chronic allergic conjunctivitis results from T lymphocyte-related immunological reactions. **Jpn J Ophthalmol 1997;41:67–70** © 1997 Japanese Ophthalmological Society

Key Words: Activated B lymphocyte, allergy, chronic allergic conjunctivitis, flow cytometry, human tears, T helper lymphocyte, T lymphocyte.

Introduction

Allergic conjunctivitis is a common condition that results from a type I reaction to an airborne allergen.[1] The most common allergen is ragweed, but a variety of antigens can be responsible: other plant pollen, dust, animal dander, and mold spores.[2] In the acute stage, observations include conjunctival hyperemia, edema, chemosis, and mucous discharge.[3] A mild papillary response, especially on the upper tarsal conjunctiva, and mild conjunctival hyperemia constitute the findings in chronic cases. In the chronic stage, patients' chief complaints are itching, hyperemia, and nonspecific eye discomfort.[1] The symptoms are often seasonal, related to the amount of the allergen present in the air; the pollen of trees in early spring and grasses in the late spring and summer are responsible for much distress.[1]

The pathogenesis of allergic conjunctivitis is not fully understood. Acute exacerbations reflect an immediate hypersensitivity reaction to an environmental antigen, to which the patient has previously been exposed. These reactions are IgE mediated, involving eosinophils and mast cells; the mast cells degranulate and liberate histamine.[4] Many of these patients manifest other forms of immediate hypersensitivity such as asthma, food and drug allergies, and urticaria-angioedema. Conjunctival scrapings often show eosinophils[5] and the patient's serum contains specific IgEs to common airborne allergens. In the milder and chronic forms, however, conjunctival scrapings usually lack eosinophils.[4] In some forms of chronic allergic conjunctivitis, patients do not develop immediate skin reactions to allergens nor do they have specific IgE antibody in the plasma, although allergen specific IgG antibodies in the conjunctiva are increased.[6] It appears, therefore, that some immunological mechanism other than the type I hypersensitivity reaction is involved in chronic allergic conjunctivitis.

We used flow cytometric (FCM) analysis to study specific inflammatory cell types from the tears of patients with chronic allergic conjunctivitis and compared the results with normal volunteers whose ocular examinations were normal, except for refractive errors.

Materials and Methods

Subjects in this study were 18 men and 10 women between the ages of 17 and 41 years (mean 27.8 ±

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2.3 SD) who had had conjunctivitis for at least 2 years, with a history of acute episodes of sudden conjunctival hyperemia, itching, burning, and tearing that usually occurred in the spring or early summer. Ophthalmic examination found conjunctival hyperemia and mild upper tarsal papillary response in all patients; they were diagnosed as having a chronic form of seasonal allergic conjunctivitis. All patients had received topical cromolin sodium or topical corticosteroids drops at some time, but none had been prescribed topical ocular medication for at least 1 month prior to (FCM) analysis. Control subjects were 22 volunteers, ophthalmologically normal except for refractive errors, with no ocular or systemic allergic symptoms. The 15 men and seven women ranged in age from 25 to 45 years (mean 31.2 ± 2.2 SD).

Conjunctival samples were obtained from both eyes of all patients and controls using calcium alginate swabs; smears were prepared and reacted with Clamidiazyme (Abbott Laboratories, North Chicago, IL, USA). Conjunctival samples from all eyes were also cultured in blood and thyoglycolate agar. Any patients or controls with purulent or mucopurulent discharge showing known pathogen microorganism growth, or a positive clamidiazyme test, were excluded from the study.

Without stimulating tear production, tear samples (minimum 100 μL; mean 120 μL ± 14.8 μL) were obtained from patients and controls over a period of 20 minutes. Each specimen was divided into four tubes of 25 μL each; 20 μL of isotopic antibody (Cytostat Coulter Clone, Coulter Immunology, Hialeah, FL, USA) conjugated with fluoroisothiocyanate (FITC) was added to the control tubes. CD 4 RD 1 (conjugated with rhodamine) and CD 8 FITC (conjugated with FITC) (Coulter), to identify T helper and T suppressor lymphocytes, respectively (20 μL); CD 3 FITC and HLA DR RD 1 (Coulter), to identify pan T lymphocytes and cells carrying class II histocompatibility antigen (20 μL); and CD 23 FITC (Coulter) to identify activated B lymphocytes (20 μL), were added, separately, to three tubes of tear samples of each patient and control. Following incubation of all tubes at room temperature for 10 minutes, the samples were fixed with sodium azide 1% and paraformaldehyde 1%, and examined with the Epics-Elite EST analyzer. We used PMT-log 2 and PMT-log 3 parameters with forward/side scatter (FS, SS). Cells restricted in a fixed area by the FS and SS scatter were then studied (minimum: 500 cells). Results were obtained graphically in quadrants, defined by CD 4+/CD 8+ ratios, and percentages of CD 3+, HLA DR+, and CD 23+ cells. Patient and control groups were compared using the student's *t*-test.

Results

Analysis of tears from chronic allergic conjunctivitis patients, using flow cytometry, reveals that 21–58% (mean: 48.7% ± 11.0% SD) of conjunctival cells found in the tears carry HLA DR receptor on the cell membrane; in tears of the control group, the results were 3.2 to 6.7% (mean: 3.8% ± 1.1% SD), a statistically significant difference ($P < 0.001$). The number of CD 23+ and HLA DR+ cells in patients' tears was significantly higher than in the controls' tears. T lymphocytes are significantly predominant in the patient group, which has higher T helper/T suppressor ratios than the control group (Table 1).

Discussion

FCM, using fluorochrome-labeled specific antibodies, is used in the differential diagnosis of malignant lymphoma and reactive lymphoid hyperplasia to identify the presence or absence of specific antigens on the surface of the lymphoid cells.[7] Although use of FCM currently involves measurement of at least 10 000 cells,[7–9] the technique is also suitable for detecting surface receptors on a small number of cells.[10]

Tears contain many cells originating from corneal-conjunctival epithelial tissue, subepithelial lymphoid tissue, and conjunctival vessels. Lymphocytes are the most abundant, but large numbers of conjunctival

Table 1. Flow Cytometry Results of Tear Analysis

	Percentage in Tears (Mean ± SD)			
	HLA DR$^+$	CD 23$^+$	CD 3$^+$	CD 4$^+$/CD 8$^+$ Ratio
Patients	21–58	22.8–56	14.2–28.7	1.5–2.4
	(48.7 ± 11.0)	(43.7 ± 6.8)	(21.2 ± 5.2)	(1.58 ± 0.20)
Controls	3.2–6.7	1.1–7.2	6.9–12.7	0.51–0.83
	(3.8 ± 1.1)	(2.8 ± 1.7)	(9.8 ± 2.1)	(0.74 ± 0.11)
P values (Student's test)	$P < 0.001$	$P < 0.001$	$P < 0.005$	$P < 0.005$

epithelial cells and polymorphic nuclear leukocytes are also found.[11] In normal conditions, tears may indicate the cellular components of conjunctival and subepithelial lymphoid tissue in healthy conjunctivas. However, cell components in tears may represent different processes in a pathologic condition. Conjunctival hyperemias, like those present in our patient group, might enhance cellular leakage from the conjunctival vessels, though we cannot assume that permeability of any specific cell type is altered.

Identification of the cellular components in tears during an inflammatory event may provide clues to the processes involved in the disease. In tears from our chronic allergic conjunctivitis patients, we found an abnormally high number of T cells, CD 23+, HLA DR+ cells, activated B cells (CD 23+), and increased T helper/T suppressor (CD 4+/CD 8+) ratios. This was not found in the control group. T lymphocyte infiltration was shown previously in an animal model of allergic conjunctivitis.[12] Foster et al[13] reported abnormal numbers of T cells, T helper cells, and macrophages in the conjunctival epithelium affected by active atopic keratoconjunctivitis. In both of these studies, however, conjunctival biopsies were studied using immunofluorescence techniques. We believe that FCM allows detection of more cell surface receptors and cell types without requiring any surgical intervention.

Our results also demonstrate that the immunopathogenic mechanism of chronic allergic conjunctivitis is much more complex than a simple Gell and Coomb's type I hypersensitivity "allergic" reaction. An abundance of T cells, and increase in the T helper/T suppressor ratio, and an increase in activated B and HLA DR+ cells indicates clearly that there is much more than a simple mast cell degranulation involved in this disease. The increase in T helper cells may enhance IgE production of B cells because IgE and eosinophil-mediated immune reactions are dependent on the activation of CD 4+ helper 2 (Th 2) subsets of T helper cells.[3] These Th 2 cells secrete interleukin-4 (IL-4), which is required for isotype switching to IgE and promotes eosinophil recruitment, and IL-5, which activates eosinophils. Accumulations of Th 2 cells have been demonstrated at sites of immediate sensitivity reactions in the skin and bronchial mucosa.[14] Atopic individuals have larger numbers of the allergen-specific IL-4-secreting Th 2 cells in their circulation than nonatopic individuals. Once IgE production increases, signs and symptoms are predominantly provoked by the inflammatory mediators liberated from mast cell degranulation through antigen-IgE interaction. A similar mechanism was previously shown in atopic keratoconjunctivitis.[13] Observation of enhanced CD 23+ cells supports this hypothesis: CD 23 is a cell surface receptor with activated B cells, acting as a low affinity receptor to IgE;[15] its expression, enhanced by mediators of inflammation such as platelet activating factor, indicates an inflammatory event.[16]

HLA DR expression on the surface of conjunctival cells indicates immune activation;[17] it is a prerequisite for the immune response because B lymphocyte—T lymphocyte and T lymphocyte—macrophage interactions could not occur without HLA DR receptors on the cell surfaces.[11] Immunohistochemical studies of enhanced HLA DR expression of conjunctival cells has been reported previously in patients who have used eyedrops containing preservative for a long period.[13] Our findings of a large number of HLA DR+ conjunctival cells in chronic allergic conjunctivitis confirms the immune nature of the disease.

Although further clarification of the immunopathologic mechanism(s) of chronic allergic conjunctivitis are necessary, FCM analysis of tears may offer valuable information. FCM may, in fact, be superior to immunohistochemical techniques because it gives more quantitative data and does not require an invasive approach.

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