Clin Exp Immunol 1995; **102**:395–398

Measurement of IL-4 in tears of patients with seasonal allergic conjunctivitis and vernal keratoconjunctivitis

H. FUJISHIMA*†, T. TAKEUCHI‡, N. SHINOZAKI*, I. SAITO§ & K. TSUBOTA* *Department of Ophthalmology, Tokyo Dental College, Chiba, †Department of Ophthalmology, Keio University School of Medicine, Tokyo, ‡Department of Internal Medicine, Saitama Medical Centre, Saitama Medical School, Saitama, and §Division of Immunological Diseases, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan

(Accepted for publication 3 July 1995)

SUMMARY

To elucidate the mechanism of ocular surface allergic disease, we focused on IL-4, which is one of the key factors in regulating IgE production, and thus determined the concentration of IL-4 in tears. IL-4 concentration was determined in the tears of 15 patients with seasonal allergic conjunctivitis, 15 vernal keratoconjunctivitis (VKC), 10 giant papillary conjunctivitis (GPC), 10 patients with non-allergic conjunctivitis and post-cataract surgical conjunctivitis as intermediate conjunctivitis, and 10 normal subjects using a highly sensitive sandwich ELISA. The mean level of IL-4 in normal controls was low, and seasonal allergic conjunctivitis, VKC and GPC showed a significant elevation ($P < 0.05$), respectively. IL-4 of VKC and GPC were also significantly higher than allergic conjunctivitis, and non-allergic conjunctivitis and post-cataract surgical conjunctivitis were not higher than normal. These results raise the possibility that the increased level of IL-4 in tears could play a role in allergic disease and its severity in patients.

Keywords allergic conjunctivitis vernal keratoconjunctivitis tears IL-4 sandwich ELISA

INTRODUCTION

The eye, particulary the conjunctiva, is a common target of allergic reaction, since it is exposed to environmental allergens [1]. The serum and tear levels of IgE were reported to be very high in allergic conjunctivitis or vernal keratoconjunctivitis (VKC) or giant papillary conjunctivitis (GPC) [2–9]. IL-4 is one of the key factors regarding IgE production of B cells [10], and secreted by activated helper T cells, especially Th2 cells [11,12], epithelial cells, and mast cells [13]. IgE stimulates the mast cells and then, for example, histamine is secreted [14]. Cytological study has shown the presence of mast cells [15] and IgE in tears [2–8,16], in allergic conjunctivitis and VKC, but cytokines such as IL-4 in tears have not yet been determined. Recently a sandwich ELISA system was developed to allow us to detect tiny amounts of protein [17–20]. In this study to elucidate the mechanism of the overproduction of IgE in tears from patients with seasonal allergic conjunctivitis, VKC and GPC, we targeted the presence of IL-4 in the tears, and compared the levels in tears and clinical severity of patients with seasonal allergic conjunctivitis, VKC, and GPC with other forms of conjunctivitis using a new sensitive ELISA system.

Correspondence: Hiroshi Fujishima MD, Department of Ophthalmology, Tokyo Dental College, 5–11–13 Sugano, Ichikawa, Chiba, Japan 272.

PATIENTS AND METHODS

Patients

IL-4 was determined in tears of 60 subjects, 32 males and 28 females, aged 23.7 ± 13.1 years (mean $\pm$ s.d.), range 5–83 years, who visited the Department of Ophthalmology, Tokyo Dental College, Chiba, Japan, between February and May of 1993 and 1994 when allergic patients with cedar pollen increase in number in Japan. Diagnoses included seasonal allergic conjunctivitis (15 subjects, 28.7 ± 17.6 years), VKC (15 subjects, 15.0 ± 6.1 years), GPC (10 subjects, 24.7 ± 12.7 years), non-allergic conjunctivitis (10 subjects, 28.4 ± 15.4 years), and post-cataract surgical conjunctivitis (10 subjects, 67.7 ± 7.3 years). Tears from 10 healthy individuals served as controls (six males and four females aged 12–36 years, mean 23.7 ± 6.9 years). Allergic conjunctivitis was diagnosed according to clinical symptoms as ocular itching, redness, tearing, or ocular pain, slit lamp examinations showing filamentous or mucous discharge, chemosis, hyperaemia or papillae of the palpebral conjunctiva, and positivity for serum antigen-specific IgE by the multiple antigen simultaneous test 16 (MAST16) [21].

VKC was also diagnosed according to slit lamp examinations showing typical cobblestone excrescences (giant papillary conjunctivitis of the upper tarsal conjunctiva which is more than 1 mm in size) [3,7], and the very severe clinical symptoms of more serious ailments than allergic conjunctivitis, including

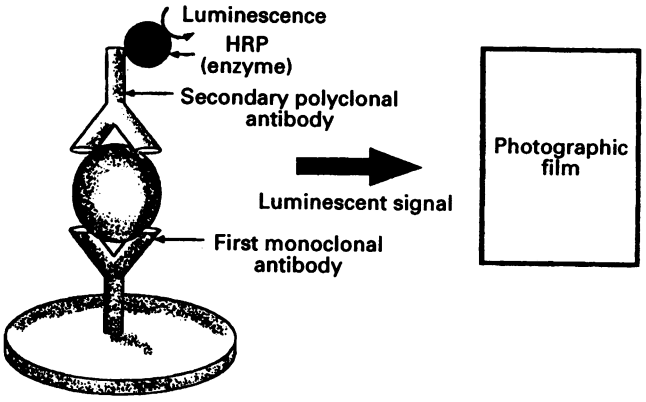

Fig. 1. Schema of the sandwich ELISA. Sandwich ELISA is based on the concept that the addition of 0·1% bovine serum albumin (BSA) amplifies the luminescent signal and improves the detection limit for alkaline phosphatase by approximately one order of magnitude under certain conditions. This assay is due to the presence of a hydrophobic microenvironment provided by the enhancer which 'stabilizes' the dephosphorylated 3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD) emitter. We observed that certain macromolecules enhance the luminescence of AMPPD. The simplicity and sensitivity of this chemiluminescent readout allowed the development of rapid clinical assays.

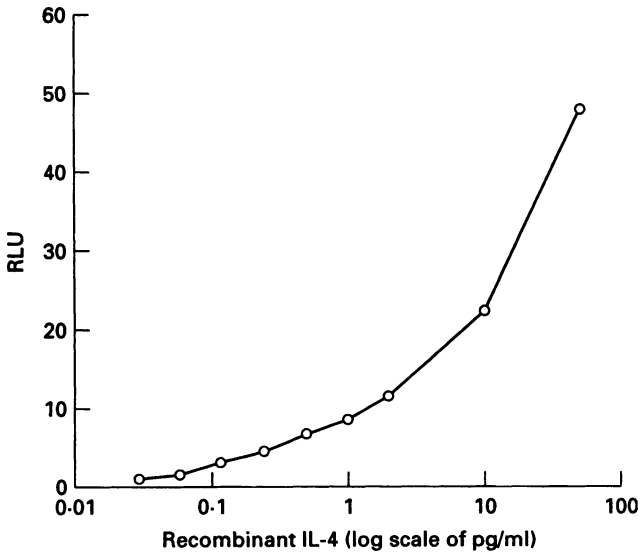

Fig. 2. Dose-response curve generated in this ELISA. IL-4 level is shown on the abscissa by log scale of pg/ml, and relative luminescence units (RLU) is shown on the ordinate.

keratitis and positivity for serum antigen-specific IgE as allergic conjunctivitis. GPC was diagnosed according to slit lamp examinations showing especially giant papilla of the upper tarsal conjunctiva which was about 1 mm in size and had fewer clinical symptoms than allergic conjunctivitis. Their mean level of IgE was 112·2 ± 89·3 U/ml (normal level < 250 U/ml). Control subjects had no symptoms or signs of allergic conjunctivitis and were negative for serum antigen-specific IgE antibodies. Patients with non-allergic conjunctivitis were diagnosed from their clinical history and results of clinical examination. The diagnosis included four bacterial conjunctivitis, two foreign body-induced conjunctivitis, and four epidemic keratoconjunctivitis. Tears from post-cataract surgical patients were collected the day after their cataract operation. All subjects gave their informed consent for participation in this study.

Sample preparation and assay

A volume of tears exceeding 30 μl was collected with a micropipette without the use of solutions [22]. Tears were stored at −20°C until assayed. The tears were diluted 11 times, and the concentration of IL-4 in the tears was assayed by sandwich ELISA (SRL Inc., Tokyo, Japan) [17,18] with a high sensitivity and rapid reactivity. The sandwich ELISA is based on the concept that the addition of 0·1% bovine serum albumin (BSA) amplifies the luminescent signal and improves the detection limit for alkaline phosphatase by approximately one order of magnitude under certain conditions. This effect is due to the hydrophobic microenvironment provided by the enhancer, which stabilizes the dephosphorylated 3-(2′-spiroadamantane)-4-methoxy-4-(3″-phosphoryloxy)phenyl-1,2-dioxetane (AMPPD) emitter [17–20]. Using this emitter we could detect 0·01 attomole quantities of alkaline phosphatase immobilized on membrane supports and imaged on photographic film and, in solution, measured in a luminometer (Fig. 1). Anti-human IL-4 MoAb (5 μg/ml; Genzyme, Boston, MA) was used for the first antibody and 3 μg/ml rabbit anti-human IL-4 polyclonal antibody (Genzyme) was used for the second antibody [23]. The dose-response curve generated in this ELISA and its detection limit are shown in Fig. 2.

Statistical analysis

Each disease was compared with the other diseases listed (a total of 10 comparisons). For example: allergic conjunctivitis ($n = 15$) *versus* VKC ($n = 15$), allergic conjunctivitis ($n = 15$) *versus* GPC ($n = 10$), allergic conjunctivitis ($n = 15$) *versus* normal ($n = 10$), etc. Data were reported as mean ± s.d. pg/ml and were analysed using the unpaired two-tailed Student's *t*-test, with a level of $P < 0·05$ accepted as statistically significant.

RESULTS

One main characteristic of VKC is that the patients are young (mid-teens), and of cataract that the patients are older (late 60s). There was significant difference in age, i.e. patients with seasonal allergic conjunctivitis were older than VKC ($P = 0·008$). The mean level of IL-4 in the tears of patients with seasonal allergic conjunctivitis ($n = 15$) was 3·51 ± 2·53 pg/ml (mean ± s.d.), VKC ($n = 15$) 23·95 ± 22·52 pg/ml, and GPC ($n = 10$) 18·00 ± 18·96 pg/ml, while those in normal controls ($n = 10$), non-allergic conjunctivitis ($n = 10$), and post-cataract surgical conjunctivitis ($n = 10$) were 0·92 ± 0·59 pg/ml, 1·84 ± 1·60 pg/ml, and 2·36 ± 2·11 pg/ml, respectively (Fig. 3). IL-4 in tears in patients with seasonal allergic conjunctivitis was significantly higher than those from normal controls ($P = 0·02$), but was lower than those with VKC ($P = 0·002$) and GPC ($P = 0·007$). IL-4 in tears in patients with VKC and GPC was also significantly higher than in normal controls (VKC, $P = 0·01$; GPC, $P = 0·03$) and those with non-allergic conjunctivitis (VKC, $P = 0·005$; GPC, $P = 0·02$). The level of IL-4 in patients with VKC and GPC was extremely high.

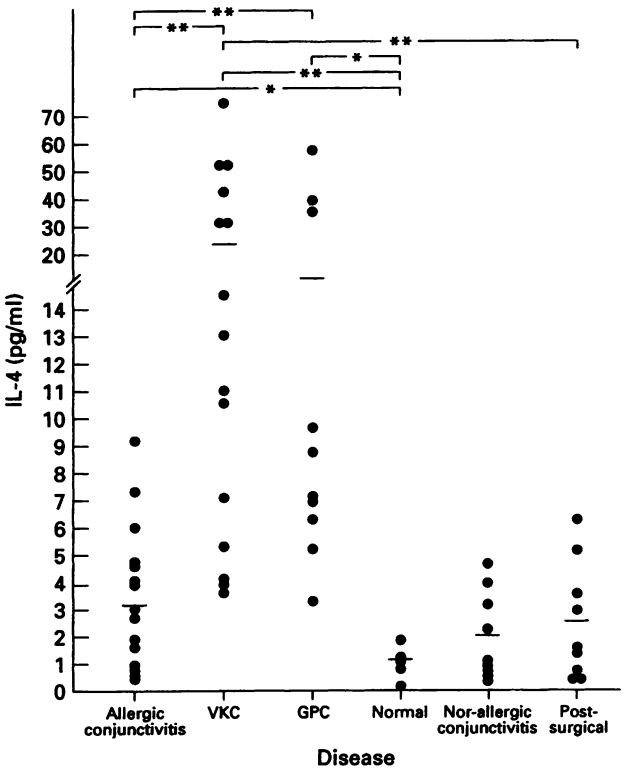

Fig. 3. Concentration of IL-4 in tears of patients with four forms of conjunctivitis, and normal subjects. IL-4 in tears in patients with seasonal allergic conjunctivitis ($P = 0{\cdot}02$), vernal keratoconjunctivitis (VKC) ($P = 0{\cdot}01$), and giant papillary conjunctivitis (GPC) ($P = 0{\cdot}03$) were significantly higher than those of normal controls. IL-4 in tears in patients with VKC ($P = 0{\cdot}002$) and GPC ($P = 0{\cdot}007$) were also significantly higher than in allergic conjunctivitis.

DISCUSSION

As with past investigations of IgE in the tear fluid of patients with allergic conjunctivitis and VKC [2–8,16], we found IL-4 in the tears collected from patients with seasonal allergic conjunctivitis, VKC, and GPC, which was significantly increased compared with normal subjects or patients with other forms of conjunctivitis. This increase was not age-related but disease-related. Moreover, the VKC was thought to be the severe type of allergic conjunctivitis; there was significant change between seasonal allergic conjunctivitis and VKC regarding the IL-4 level in tears, just as in clinical severity.

We recruited GPC patients as controls originally, because the GPC is believed to be induced by mechanical rubbing or irritation, not only by contact lens but also exposed sutures [24]. Their mean level of IgE was not increased. However, our results showed increased levels of IL-4 in this group. The alternative explanation of development of GPC includes the relationship with allergy [9], and our result may support the concept that GPC may be induced by allergic reactions.

Recent studies on the identification and cloning of T cell lymphokines have revealed that IL-4, a product of activated helper T cells, plays an essential role in the growth and differentiation of B cells [12–15]. IL-4 enhances the production of IgE antibody by B cells. There have been reports of IgE in tears, but none about related cytokines such as IL-4 in tears [2–8]. In basic research, it was reported that IgE production was IL-4-dependent, increasing linearly with IL-4 concentrations between 0·2 and 2·5 ng/ml and plateauing at concentrations of 5 ng/ml or more [26]. However, to our knowledge, no study concerning IL-4 in tears has ever been reported, possibly due to the difficulty of using small volumes of tears and of detecting the very low concentration of IL-4 in tears [16,23].

A newly developed sandwich ELISA using the chemiluminescent enzyme substrate, AMPPD, is useful in measuring small amounts of protein [17,18,20,23]. The serum level of IL-4 in patients with atopic dermatitis was found by this method to be significantly higher than those in normal controls. Previous reports using sandwich ELISA found the serum level of IL-4 to be $1{\cdot}50 \pm 1{\cdot}36$ pg/ml in patients with atopic disease, and $0{\cdot}79 \pm 0{\cdot}62$ pg/ml in normal subjects [23]. We demonstrated that the amount of IL-4 in the tears of normal subjects was similar to that in serum, but in patients with allergic conjunctivitis, the level in tears exceeded that in serum. These results introduce a possibility that the allergic reaction can occur locally at the ocular surface itself.

A volume of tears of 30 μl was sufficient for quantifying IL-4, but a lower volume may give unreliable results [5,22]. Tear collection in cellulose sponge, capillary tubes, or aspiration are the more frequent methods, and there was no statistically significant difference in volume of collection, contamination, and reflex lacrimation [22]. The s.d. level for all groups, and especially clinically diagnosed patients, was high in this study. If the conjunctivitis groups have elevated tear production due to irrigation, tear flow rates and volumes would strengthen the argument that IL-4 would be high in spite of dilution. As Tuft pointed out, any technique has several sources of error that may affect the derived value of tears, and dilution or concentration of the tear during collection are not clear [7,22].

We found increased levels of IL-4 in tears collected from patients with seasonal allergic conjunctivitis, VKC, and GPC, but we have no evidence that the source of IL-4 in tears is T cells. It has been shown that mast cells [13] or epithelial cells [16,27] are also considered to produce IL-4. As there was a huge increase in T cell population in VKC and GPC, and increase in IL-4 in tears in these two diseases, we thought that the source of the IL-4 was likely to be T cells. Future study must explore what cells in brush cytology samples produce IL-4. Inflammation, such as non-allergic or post-cataract surgical, increased slightly the level of production of IL-4, but high IL-4 production was related to allergic reactions. Th2-like helper T cells play a role in allergic disease [11]. It is known that activated CD4 cells increase in VKC. A high level of IL-4 suggests that the Th2 cell may play a role in the pathogenesis of allergic conjunctivitis, VKC, and GPC, as observed in patients with other allergic reactions [16]. Most T cell clones specific for allergens exhibit a Th2-like cytokine profile, whereas T cell clones that are specific for mycobacterial antigen, *Borrelia burgdorferi*, nickel, and streptokinase, usually exhibit a Th1-like profile [28,29]. IL-4 would turn the T cells in the conjunctiva into Th2 cells [30,31]. Manipulation of IL-4 expression might become a new mode of treatment for certain ocular allergy diseases.

ACKNOWLEDGMENTS

We thank SRL Inc. for preparation of sandwich ELISA, and Kirin

Brewery Company Ltd. and Santen Pharmaceutical Co., Ltd. for their financial support.

REFERENCES

1 Friedlaender MH. Current concepts in ocular allergy. Ann Allergy 1991; **67**:5–13.

2 Matsuki S, Ando F. Aging alteration of immunoglobulin E level in human tear fluid. Folia Ophthalmol Jpn 1985; **36**:1494–7.

3 Allansmith MR, Hahn GS, Simon MA. Tissue, tear and serum IgE concentrations in vernal conjunctivitis. Am J Ophthalmol 1976; **81**:506–11.

4 Tuft SJ, Kemeny DM, Dart JK, Buckley RJ. Clinical features of atopic keratoconjunctivitis. Ophthalmology 1991; **98**:150–8.

5 Insler MS, Lim JM, Queng JT, Wanissorn C, Mcgovern JP. Tear and serum IgE concentrations by Tandem-R IgE immunoradiometric assay in allergic patients. Ophthalmology 1987; **94**:945–8.

6 Kari O, Salo OP, Bjorksten F, Backman A. Allergic conjunctivitis, total and specific IgE in the tear fluid. Acta Ophthalmol 1985; **63**:97–99.

7 Ballow M, Mendelson L. Specific immunoglobulin E antibodies in tear secretions of patients with vernal conjunctivitis. J Allergy Clin Immunol 1980; **66**:112–8.

8 Liotet S, Warnet VN, Arrata M. Lacrimal immunoglobulin E and allergic conjunctivitis. Ophthalmologica 1983; **186**:31–34.

9 Donshik PC, Ballow M. Tear immunoglobulins in giant papillary conjunctivitis induced by contact lenses. Am J Ophthalmol 1983; **96**:460–6.

10 Noma Y, Sideras P, Naito T *et al.* Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. Nature 1986; **319**:640–6.

11 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986; **136**:2348–57.

12 Pene J, Rousset F, Briere F *et al.* IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons γ and δ and prostaglandin E2. Proc Natl Acad Sci USA 1988; **85**:6880–4.

13 Plaut M, Pierce JH, Watson CJ, Nordan RP, Paul WE. Mast cell lines produce lymphokines in response to cross-linkage of FcεRI or to calcium ionophores. Nature 1989; **339**:64–67.

14 Ishizaka K. Basic mechanisms of IgE-mediated hypersensitivity. Curr Opin Immunol 1989; **1**:625–9.

15 Tsubota K, Takamura E, Hasegawa T, Kobayashi T. Detection by brush cytology of mast cells and eosinophils in allergic and vernal conjunctivitis. Cornea 1991; **10**:525–31.

16 Maggi E, Biswas P, Prete GD *et al.* Accumulation of Th-2-like helper T cells in the conjunctiva of patients with vernal conjunctivitis. J Immunol 1991; **146**:1169–74.

17 Bronstein I, Edwards B, Voyta JC. 1:2-Dioxetanes: novel chemiluminescent enzyme substrates. Applications to immunoassays. J Biolum Chemilum 1989; **4**:99–111.

18 Tsuji A, Maeda M. Chemi- and bioluminescent immunoassays. Dojin News 1993; **65**:11–15.

19 Bronstein I, Voyta JC, Thorpe GHG, Kricka LJ, Armstrong G. Chemiluminescent assay of alkaline phosphatase applied in an ultrasensitive enzyme immunoassay of thyrotropin. Clin Chem 1989; **35**:1441–6.

20 Matsumoto K, Taki F, Miura M, Matsuzaki M, Takagi K. Serum levels of soluble IL-2R, IL-4, and soluble FcεRII in adult bronchial asthma. Chest 1994; **105**:681–6.

21 Fujishima H, Yagi Y, Toda I, Tsubota K, Takamura E. Evaluation of allergen-specific IgE antibodies by multiple antigen simultaneous test. Atarashii Ganka (in Japanese) 1992; **9**:2097–101.

22 Tuft SJ, Dart JKG. The measurement of IgE in tear fluid: a comparison of collection by sponge or capillary. Acta Ophthalmol 1989; **67**:301–5.

23 Kasamatsu M, Tsuji T. A method for the quantification of interleukin-4 in serum (sandwich ELISA) and IL-4 levels in patients with atopic dermatitis. Jpn J Allergol 1993; **42**:878–82.

24 Nirankari VS, Karesh JW, Richards RD. Complications of exposed monofilament sutures. Am J Ophthalmol 1983; **95**:515–9.

25 Tepper RI, Levinson DA, Stanger BZ, Torres JC, Abbas AK, Leder P. IL-4 induces allergen-like inflammatory disease and alters T cell development in transgenic mice. Cell 1990; **62**:457–67.

26 Claassen JL, Levine AD, Buckley RH. Recombinant human IL-4 induces IgE and IgG synthesis by normal and atopic donor mononuclear cells. J Immunol 1990; **144**:2123–30.

27 Bernauer W, Wright P, Dart JK, Leonard JN, Lightman S. Cytokines in the conjunctiva of acute and chronic mucous membrane pemphigoid: an immunohistochemical analysis. Graefes Arch Clin Exp Ophthalmol 1993; **231**:563–70.

28 Parronchi P, Macchina D, Piccinni MP *et al.* Allergen and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. Proc Natl Acad Sci USA 1991; **88**:4538–42.

29 Prete GFD, Carli MD, Mastromauro C *et al.* Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen (s) of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991; **88**:346–50.

30 Maggi E, Parronchi P, Manetti R *et al.* Reciprocal regulatory effects of IFN-γ and IL-4 on the *in vitro* development of human Th1 and Th2 clones. J Immunol 1992; **148**:2142–7.

31 Swain S, Weinberg A, English M, Huston G. IL-4 directs the development of TH2-like helper effectors. J Immunol 1990; **145**:3796–806.