

Involvement of Interleukin-16 (IL-16) in Allergic Conjunctivitis

¹Magda Mahran, ¹Maha Haggag and ²Ayman Shouman

¹Microbiology and Immunology and ²Ophthalmology Departments, Research Institute of Ophthalmology, Giza, Egypt

Abstract: IL-16 is a novel cytokine, which is chemoattractant for CD4 T cell, macrophages, and eosinophils. These cells are of considerable importance in asthma and allergic conjunctivitis. A strong association between allergic- conjunctivitis, rhinitis and asthma exist from a clinical and epidemiologic standpoint of view. To elucidate the mechanism of ocular surface allergic disease, we focused on IL-16 which is one of the key factors in up –regulating IgE production through chemo-attractant for CD4 T cells. Tear IgE has been considered to play an important role in allergic conjunctivitis and the measurement of tear IgE concentration can help to diagnose this condition, and the locally produced IgE level have been shown to be the largest contributor to the severity of allergic conjunctivitis. The aim of this study is to assess the level of IgE and IL-16 in tear fluid using automated quantitative Enzyme Linked Fluorescent Assay technique (ELFA) and quantitative sandwich enzyme immune-assay respectively, from 10 patients with allergic conjunctivitis , 10 patients with allergic rhino-conjunctivitis and 10 patients with allergic rhino-conjunctivitis in association with bronchial asthma. Results showed significant increase in the mean level of IgE in the tear fluid of the three groups of allergic patients (293.6 IU/ml=704.6 ng/ml) and significant increase in the mean level of IL-16 (239.5 pg/ml) in their tears. The highest mean levels were measured in patients suffering from rhino-conjunctivitis, associated with asthma. There was significant correlation between the increased levels of IgE and the increased levels of IL16. We recommend further studies to investigate the use of anti-IgE therapy and IL-16 antagonists for management of allergic conjunctivitis.

Key words: Interleukin-16, IL-16, Allergic Conjunctivitis

INTRODUCTION

Owing to the fact that the eye is one of the first organs to encounter environmental allergens, allergic eye disease has become a common ocular problem, estimated to affect about 20% of the population worldwide. Allergic conjunctivitis is typically divided into five types; seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal kerato-conjunctivitis (VKC), atopic kerato-conjunctivitis (AKC) and giant papillary conjunctivitis (GPC), the latter is an iatrogenic disease associated with foreign bodies on the eye (Manzouri *et al.*, 2006). The last decade brought a substantial progress in understanding the pathogenesis of allergic eye diseases, in addition to the well known effect induced by the cross-linking of IgE antibodies bound to high affinity IgE receptors by the antigen and the release of various preformed (histamine) and newly synthesized mediators those cause immediate mast cell de-granulation we have now learned that these cells produce and release several other chemotactic factors, cytokines and chemokines. The cytokines/growth factors detected in situ in human mast cells include Interleukin IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-16, tumor necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF), granulocyte/monocyte-colony forming factor (GM-CSF), stem cell factor (SCF), nerve growth factor (NGF), fibroblast growth factor (β -FGF) and MIP-1 α (Bonini *et al.*, 2003 and Bonini, 2005). IgE has been considered to play an important role in allergic reactions in the eye, thus it may be very important to be able to detect local allergic reactions so that they can be used in diagnosing allergic conjunctivitis. Locally produced IgE has been shown to be the largest contributor to the severity of the disease. Thus, tear IgE measurement could provide a much better way to diagnose allergic conjunctivitis (Nomura and Takamura, 1998). IL-16 formerly known as lymphocyte chemo-attractant factor or LCF, is a pro-inflammatory cytokine that is chemo-tactic for CD4⁺ T lymphocytes, monocytes, and eosinophils (Rand *et al.*, 1991; Cruikshank *et al.*, 2000). In addition to inducing chemotaxis, IL-16 can up-regulate IL-2 receptor and HLA-DR4 expression, inhibit T cell receptor (TCR)/CD3-dependant activation, and

Corresponding Author: Magda Mahran, Microbiology & Immunology Department, Research Institute of Ophthalmology, Giza, Egypt.
E-mail: magdamahran@hotmail.com

promote repression of HIV-1 transcription. IL-16 is a unique cytokine with no significant sequence homology to other well-characterized cytokines or chemokines. IL-16 has been characterized as a chemoattractant for a variety of CD4⁺ T cells. Several inflammatory diseases, including allergic disorders, have been reported to correlate with IL-16 (Karaki *et al.*, 2005). In the previous grant period, the investigators found that IL-16 is the major lymphocyte chemoattractant released early after segmental airway antigen challenge that the epithelium of atopic asthmatics preferentially expresses IL-16 mRNA and protein and the degree of epithelial expression of IL-16 correlates with the extent of CD4⁺ T cells accumulation and airway reactivity (Gordon and Snider, 2007). Likewise, IL-16 increases in the nasal mucosa of patients with allergic rhinitis after experimental or seasonal allergen exposures (Bandeira-Melo *et al.*, 2002).

Subjects:

This study included 30 patients and 10 normal healthy subjects which was the control group (group 4). All individuals were non smokers and they were referred to the Allergy Lab from Outpatient Clinics of Research Institute of Ophthalmology (RIO).

Patients were divided into three groups of tens according to their clinical diagnosis.

Allergic conjunctivitis patients (group1), allergic rhino-conjunctivitis patients (group 2) and allergic rhino-conjunctivitis associated with asthma (group 3).

All subjects gave their written consent after being fully informed about the purpose and nature of the study, which were approved by the Ethics Committee of the Institute.

MATERIALS AND METHODS

Twelve natural allergenic extracts were used for skin prick test (SPT). Except for mite and cockroach which were purchased from United Company for Chemical and Medical preparation (UCCMA), ten allergens were had been prepared in our Allergy Lab of RIO (Haggag, 2002) as follows:

1. Preparation of the extracting fluid which was coca solution as suggested by Ortolani *et al.* (1984) by dissolving 20 gm sodium chloride and 11 gm sodium bicarbonate in one liter distilled water in a sterilized flask.
2. Standardization of the allergenic extract by weight to volume ratio (w/v) method according to Fischer *et al.* (1988). Different allergen extracts had different concentrations according to the nature of the crude allergen; the ratio was the same for wool, hairs of cat, dog and goat, feathers mixture (duck, goose & hen) and the pollens of palm, orange and Bermuda grass (El-Negeel) which was 1:20 w/v the ratio for house dust was 1:10 w/v for rabbit hair it was 1: 40 w/v. The mixture was shaken thoroughly by electric shaker for more than two hours for two successive days.
3. Primary filtration by the usual filter paper and secondary filtration by Millipore Syringe filter (Nalgene) 0.2 µm into sterilized sealed glass bottles (vaccine bottle). (Fig.1) were performed, then checking for sterility by culturing on blood agar aerobically and anaerobically was done.



Fig. 1: filtration by Milipore syringe filter

Skin prick test (SPT) was done for all 40 subjects; ten in the control group and 30 allergic patients in the three other groups to diagnose the causative allergens (Bousquet & Michel, 1993). Reactions with wheal diameter of 3mm or more were considered to be positive (Bousquet & Michel, 1993 and Huss *et al.*, 2001).

Immunological studies:

Tear Sampling:

Tear samples were collected using the micro-capillary method without local anaesthesia and samples were taken from the temporal meniscus. During sampling the patients were requested to look in the opposite direction and not to blink their eye (Nomura & Takamura, 1998 and Vasanthi *et al.*, 2007). A new capillary tube was used for each tear sample. Each sample was transferred into 0.5 ml sterile microfuge tube and centrifuged for 10 minutes at 2.000 revolutions per minute (rpm) to separate the cells from the tear fluid. Supernatants were stored at – 70C within one hour, until assaying for IgE and cytokine.

Detection of IgE in Tear Fluid:

Total tear fluid IgE was assessed by automated quantitative Enzyme Linked Fluorescent Assay technique (ELFA), using the assay principle with the indirect immunoassay method with final fluorescent detection. The reaction steps were performed using the VIDAS apparatus “Bio Merieux France”. Quantitation of tear IgE were determined according to the manufacturer’s instructions. The minimum detectable concentration of IgE by the kit was 5.0 IU/ml. Twenty UL of tear fluid was pipette in each well in duplicate. The optical density was read in a microplate reader (Bio-RAD 550) at 450 nm. The IgE values in the samples were calculated using a standard plot and expressed as IU/ml of tear fluid.

Detection of IL-16 in Tear Fluid:

Quantitation of IL-16 protein in tear fluid was accomplished by commercially available performed quantitative sandwich enzyme immune-assay kit (Bio Source International k Inc, Camarillo, USA). The assay was according to the manufacturer instructions with the reagents provided. Results were expressed in pg/ml relative to a set of standard IL-16 protein provided with the test kit. Quantitation of IL-16 in tear fluid was accomplished by ELISA as described by Cruikshank *et al.* (1995). Briefly, anti IL-16 monoclonal antibody was coated directly onto 96 well ELISA plates at a concentration of 1ug/ml in coating buffer (0.1M sodium bicarbonate, PH 8.8) and incubated overnight at 4°C. To eliminate nonspecific binding by the primary antibody, the plates were blocked with 300ul of blocking buffer (phosphate-buffered saline PBS containing 10% fetal bovine serum (FBS) and 0.05% NaN₃) for 2h at ambient temperature. The plates were then washed twice with PBS Tween (PBS containing 0.05% Tween 20). A standard curve was generated using serial dilution of recombinant interleukin 16 (rIL-16). Tear fluid samples (100ul) were incubated in duplicate in the 96 –well plates(Nunc. Naperville, IL) at 37°C for 1h. After washing, 100 ul of rabbit polyclonal anti IL-16 antibody (10 ug/ml) diluted in PBS containing 0.05% Tween 20 was added to each well . The presence of IL-16 was then detected by incubation for 1 h with biotinylated goat anti-rabbit IgG diluted 1:500 in PBS. The lower limit of detection for the ELISA is routinely 10-15 pg/ml . Linearity was in the range of 12-500 pg/ml (coefficient of 0.993 in that range).

RESULTS AND DISCUSSION

Results of skin prick test (SPT) for all subjects in the control group (group 4) were negative for all the twelve allergens. Results of SPT for the 30 allergic patients in the other three groups are shown in Table (1).

Tear IL-16 and IgE mean levels were significantly increased in patients with allergic diseases compared to their mean levels in the control subjects (group 4). The highest mean levels of IL-16 and IgE were measured in tear fluid of patients who had rhino-conjunctivitis associated with asthma (group 3), while the lowest levels were met in pure conjunctivitis patients (group 1) Table (3) and Fig. (3).

Table 1: Results of SPT test.

Allergen	Group 1	Group 2	Group 3
Palm	9	6	8
Orange	7	7	6
Grass	7	5	7
Wool	8	6	4
Rabbit	5	4	2
Goat hairs	4	3	3
Cat	3	4	5
Dog	2	3	3
Feathers	5	4	6
House dust	5	6	7
HDM	3	5	7
Cockroach	0	2	5

Patients were differently sensitive to those causative allergens with multiple sensitizations were observed Table (2) and Fig (2).

Table 2: Prevalence of the causative allergen.

Allergen	No. of patients with +ve SPT	% out of 30
Palm	23	76.7
Orange	20	66.7
Grass	19	63.3
Wool	18	60
Rabbit	11	36.7
Goat	10	33.3
Cat	12	40
Dog	8	26.7
Feathers mix	15	50
HD	18	60
HDM	15	50
Cockroach	7	23.3

Table 3: Mean levels of IgE & IL-16 in tear fluid and that of IL-16 was 239.5pg/ml.

Group	Parameters	N	Minimum	Maximum	Mean	Std. Deviation
Group 1	IgE	10	136.9	242.3	204.180	32.424
	IL-16	10	165.3	316.3	210.210	49.032
	Valid N	10				
Group 2	IgE	10	152.7	332.3	237.890	57.738
	IL-16	10	156.9	340.5	226.120	59.245
	Valid N	10				
Group 3	IgE	10	249.3	649.3	438.720	140.285
	IL-16	10	215.5	349.8	282.240	44.273
	Valid N	10				
Group 4	IgE	10	65.1	131.8	96.000	21.210
	IL-16	10	16.9	41.3	28.760	7.752
	Valid N	10				

Mean level of IgE of the three groups of allergic patients together was 293.6 IU/ml = 704.6 ng/ml as One IU = 2.4 ng (Huss KRN et al., 2001).

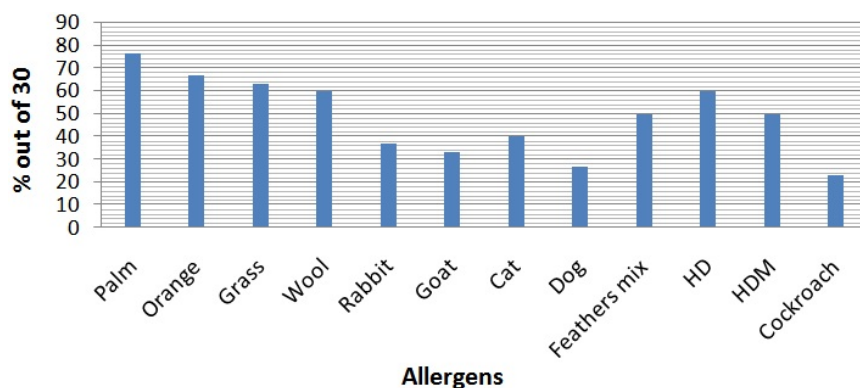


Fig. 2: Causative Allergens.

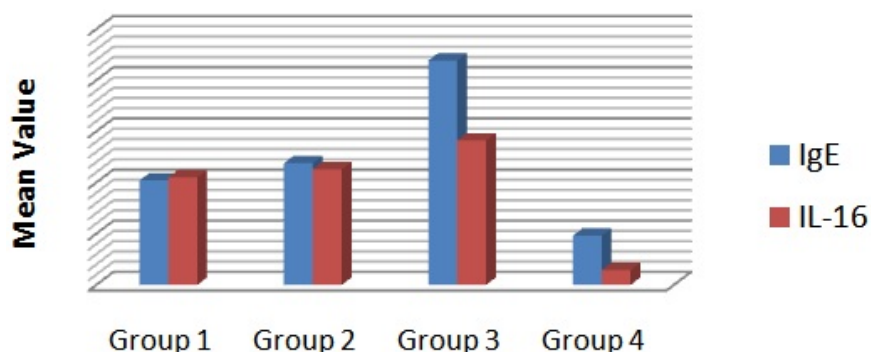


Fig. 3: Levels of IgE & IL-16 in Tears.

Descriptive statistics (Pearson Correlation) showed significant correlation between levels of IgE and IL-16 in all groups at the 0.05 level.

Table 4: Correlation between IgE & IL-16.

Groups	Parameters		IGE	IL 16
Group 1	IgE	Pearson Correlation	1.000	0.646
		Sig. (2-tailed)	.	0.044
		N	10	10
	IL_16	Pearson Correlation	0.646	1.000
		Sig. (2-tailed)	0.044	.
		N	10	10
Group 2	IgE	Pearson Correlation	1.000	0.715
		Sig. (2-tailed)	.	0.020
		N	10	10
	IL_16	Pearson Correlation	0.715	1.000
		Sig. (2-tailed)	0.020	.
		N	10	10
Group 3	IgE	Pearson Correlation	1.000	0.730
		Sig. (2-tailed)	.	0.017
		N	10	10
	IL_16	Pearson Correlation	0.730	1.000
		Sig. (2-tailed)	0.017	.
		N	10	10
Group 4	IgE	Pearson Correlation	1.000	0.633
		Sig. (2-tailed)	.	0.049
		N	10	10
	IL_16	Pearson Correlation	0.633	1.000
		Sig. (2-tailed)	0.049	.
		N	10	10

Discussion:

In the present study pollens especially that of palm tree was the most causative allergen gave positive skin prick test (SPT). Animal dander came next to pollens in which wool was the most offending one. Feathers, house dust and house dust mite also had high percentage among the allergens while cockroach had the lowest. Our results are more or less agree with Bielory (2000) who reported that pollens are the most common causative allergen associated with seasonal allergic conjunctivitis (SAC) while animal dander, house dust mite and feathers are the most common airborne allergens implicated in perennial allergic conjunctivitis (PAC) which is more likely than SAC to be associated with perennial rhinitis. Nguyen (2007) concluded that seasonal allergens are primarily pollens of tree, grass and weed while molds, house dust and animal dander are more frequently with the perennial allergens that are present throughout the year. He also concluded that a person does not need to own a pet to be exposed to dander such as cat dander and that rabbit dander is highly allergenic. Huss *et al.* (2001). in their study in childhood asthma management program they reported that for house dust mite and cockroach allergens, the higher the level of allergen exposure the more likely patients were to have positive allergy skin test responses especially for mite, it was found that even with very low level of exposure it had a significant risk factor for sensitization. In contrast to mite and cockroach allergens, cat, dog or mold allergens was not associated with currently measured home exposures. Regarding the causative allergens in the present study, multiple sensitizations were a common finding in our previous studies (Haggag, 2007 and Haggag & Hamed, 2008) The high percentage of palm pollens as a causative allergen coincide with the nature of our environment in Egypt. Also the high percentage of animal dander in general and wool in special and also feathers can be explained by the high percentage of patients that are coming from country side to the Institute moreover urban patients and/or their neighbors had some kind of domestic animals and birds. However Friedlaender (1993) concluded that the most important allergens vary from one location to another. In the present study the mean level of IgE in tears of normal subjects was 96 IU/ml, it was increased in all patients suffering from allergy being highest 438.7 IU/ml in cases of rhino-conjunctivitis associated with asthma next was cases of rhino-conjunctivitis 237.9 IU/ml and the lowest was 204.2 IU/ml that measured in pure conjunctivitis patients and hence it was significantly increased in tears of the three groups of allergic patients, when compared with normal subjects. Sen *et al.* (1978) concluded that if the estimation of IgE in tears is to serve as diagnostic tool it is important to initially estimated its level in normal individuals of a given population to serve as control, their study involved 31 normal Indian individuals and the mean level of IgE in tears was 282.2 ± 189.2 IU/ml (range 25 to 780 IU/ml). Allen smith *et al.* (1976) determined IgE level with mean value 61 ng/ml in tears of 10 normal American subjects. McClellan *et al.* (1973) reported the detection and measurement of IgE in tears of 22 Navajo children with average value was 250 ng/ml (range 60-700 ng/ml). In a study made in Tokyo by Nomura & Takamura (1998), the level of IgE in tears of 18 normal controls was 52.1 ± 9.7 ng/ml and its level in 70 patients with (SAC) was 194.7 ± 21.7 ng/ml and its level in 21 patients with (PAC) was 134.8 ± 23.1 ng/ml and its highest level was 322.2 ± 45.7 ng/ml in patients with vernal keratoconjunctivitis (VKC) and hence their results showed significant increase in the level of tear

IgE in the three groups of allergic conjunctivitis when compared with their control group, however they suggested that measuring tear IgE concentrations can help to diagnose allergic conjunctivitis. Bielory (2000) reported that elevated serum IgE had been noted in 78% of (SAC) where tear fluid IgE was present in almost all 96% tear fluid samples from (SAC) and (PAC) patients.

There has been no study to the best of our knowledge that demonstrated the presence of IL-16 in tear fluid. In the present study IL-16 was detected in the tear fluid in the control group with mean value 28.8 pg/ml and in patients with pure allergic conjunctivitis with mean value 210.2 pg/ml and was 226.1 pg/ml in allergic rhino-conjunctivitis patients while the highest mean level was 282.2 pg/ml in patients suffering from allergic rhino-conjunctivitis associated with asthma. And hence IL-16 was significantly increased in tears of allergic conjunctivitis whether it was the only manifestation of allergy or if it was associated with rhinitis or was also associated with asthma, with significant correlation between the increased levels of IL-16 and IgE was found. Hessel *et al.* (1998) had a study in murine model of asthma they reported the presence of IL-16 immuno-reactivity in the airways after specific allergen sensitization, and with repeated inhalation of that specific allergen, IL-16 immuno-reactivity was markedly increased and IL-16 was detectable in the broncho-alveolar lavage fluid and also up-regulation of IgE was proved. Furthermore treatment *in vivo* with monoclonal antibodies to IL-16 suppresses the up-regulation of allergen specific IgE during allergen challenge and inhibit the development of airway hyper-responsiveness. In a study made by Krug *et al.* (2000), IL-16 concentration was significantly elevated in the bronchoalveolar lavage of 13 patients with asthma after allergen challenge and the range was 38-362 pg/ml. Taha *et al.* (2001) reported, increased IL-16 level in the bronchial mucosa and sputum of patients with atopic asthma. Pullerits *et al.* (2001) evaluated the effect of natural allergen exposure during a grass pollen season on IL-16 expression and number of CD4+ cells in nasal mucosa of patients with allergic rhinitis, they reported increased IL-16 expression and CD4+ cells with significant correlation. Their study also involved treatment with glucocorticoids and their data suggested that inhibition of IL-16 expression can be one of the mechanisms by which nasal glucocorticoids achieve their anti-inflammatory effect in allergic rhinitis. Likewise in atopic asthma Gordon and Snider (2007), reported that glucocorticoids inhibit IL-16 expression in epithelium and that IL-19, IL-13 and histamine induce IL-16 expression and secretion in human epithelial cells *in vitro*.

Recently, in a study made by Akiyama *et al.* (2009) in a murine experimental allergic rhinitis model they concluded that IL-16 was both systemically and locally up-regulated and was significantly inhibited after treatment with anti-allergic drugs.

Conclusion and Recommendations:

Tear IL-16 was significantly increased in patients with allergic conjunctivitis whether it was pure conjunctivitis or rhino-conjunctivitis or also associated with asthma, patients in the last group had the highest mean level of IL-16 in their tears. IgE in tear fluid was also significantly increased and significant correlation between the elevated levels of IL-16 and IgE in tears of allergic patients was observed. We recommend further studies to investigate the use of Anti-IgE therapy or IL-16 antagonists for management of allergic conjunctivitis.

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