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Cytologic Changes in Tears During the Late Type of Secondary Conjunctival Response Induced by Nasal Allergy

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1. Introduction

Allergic conjunctivitis (AC),a disorder of the conjunctiva in which an allergic component plays a key role, affects approximately 15-25% of the adult and pediatric population [1-8]. Estimates in the literature indicate that the seasonal allergic conjunctivitis (SAC) occurs most frequently, followed by atopic keratoconjunctivitis (AKC), vernal keratoconjunctivitis (VKC), perennial allergic conjunctivitis (PAC), whereas giant papillary conjunctivitis (GPC) occurs only sporadically [1-9]. However, according to our clinical experience, the PAC occurs most frequently, followed by SAC, AKC and VKC, whereas the GPC represents only 0.5- 1% of all AC cases [10-17].

The allergic conjunctivitis (AC) can be divided into two basic forms with respect to the localization of the antigen-antibody or antigen-sensitized Th1 cell interaction with subsequent steps (allergic reaction) [10-16] In the primary form of AC, the allergic reaction due to the direct exposure of conjunctiva to an external allergen takes place primarily in the conjunctiva,. In this case, the conjunctiva is the primary site of the allergic reaction which results in the development of the primary (or classical) AC form. The secondary form of AC is induced by the allergic reaction occurring primarily in the nasal mucosa due to the exposure of nasal mucosa to an external allergen via various possible mechanisms which are described in the discussion. Moreover, the initial allergic reaction in the nasal mucosa usually can, but does not necessarily, cause also the concomitant nasal response characterized by nasal mucosal edema resulting in nasal obstruction, hypersecretion and sneezing [10, 12].

Various hypersensitivity types, such as immediate type (type I, IgE-mediated), late type (type III) or delayed type (type IV, cell-mediated) can participate both in the primary and in the secondary form of AC.[1, 3, 5, 6, 10-36] The involvement of various hypersensitivity mechanisms in AC may result in development of three basic types of conjunctival response (CR), an immediate (ICR), a late (LCR) or a delayed (DYCR) type, and two supplementary types of CR, such as dual late (DLCR), being a combination of an immediate and a late CR, and a dual delayed (DDYCR), a combination of an immediate and a delayed CR.[1, 6, 8, 9, 10-16, 19, 20, 24, 25, 28, 36- 40] Additionally, the non-specific hyperreactivity resulting from direct stimulation of mucosal, glandular, or neurogenic receptors/elements in the nasal mucosa and/or conjunctival tissue by non-specific agents might also participate in the conjunctivitis complaints/response, however, usually to a lesser degree.[3, 12, 41]

There is a dearth of information concerning both the role of an allergic reaction occurring initially in the nasal mucosa in the conjunctiva and possible induction of the secondary conjunctival and corneal response [10-16]. Moreover, no data are available to illustrate the appearance of the individual cell types and the cytologic changes in the tears accompanying the particular types of secondary CR [12, 13].

The purpose of this study, which is a continuation of our earlier work,[10-16] was to: (1) investigate the appearance of particular cell types and the changes in their counts in the tears during the secondary late conjunctival response, (2) to evaluate the possible significance of the individual cell types and their count changes in the tears for the diagnostic approach as well as for the clarification of the mmunologic mechanism(s) underlying this CR.

2. Material and methods

2.1 Patients

One hundred sixty–nine patients suffering from allergic conjunctivitis for more than 3 years and responding insufficiently to the topical ophthalmologic treatment, had been referred to our Department of Allergology & Immunology (Institute of Medical Sciences "De Klokkenberg", Breda, The Netherlands) for more extensive diagnostic and therapeutical analysis Thirty-five of 169 patients, developing the secondary late conjunctival response (SLCR) to the nasal provocation tests with allergen (NPT) performed as a part of the routine diagnostic procedure, volunteered to participate in this study.

These patients, 15 males and 20 females, 19-43 years of age, suffering from SAC (n=16) or PAC (n=19) showed subjective symptoms and objective signs of conjunctivitis and positive skin tests to various inhalant allergens. In 6 of these patients also positive specific IgE in the serum (RAST) to Dermatophagoides pteronyssinus or farinae and/or grasspollen was recorded. All these patients had normal intraocular pressure. None of them suffered from other ocular disorders, infections, systemic disease or immunodeficiency. Twelve patients had previously undergone 17 conjunctival provocation tests CPT) with various inhalant allergens, which were negative. They had previously been treated with topical and systemic H1-receptor antagonists, topical ocular cromolyn, topical ocular glucocorticosteroids, decongestants, topical vasoconstrictors and some of them also with NSAID drugs, however, without any substantial improvement of their conjunctival complaints.

Patients underwent a routine diagnostic procedure consisting of: a detailed disease history, general examination to exclude systemic or other disorders, basic laboratory tests, bacteriological screening of the tears and nasal secrections, basic and supplementary skin tests with inhalant and food allergens, X-ray of the paranasal sinuses in Water's projection, nasoscopy and cytologic examination of the nasal secretions, and ophthalmologic examination including ophthalmoscopy, slit-lamp evaluation, vital staining with fluorescein and cytologic examination of the tears.

The diagnostic procedure revealed a positive history for nasal allergy, positive skin tests with various inhalant allergens,, hyperaemic and edematous nasal mucosa, increased eosinophil and neutrophil counts in the nasal secretions, conjunctival hyperaemia and tearing to a slight degree, appearance of incidental eosinophil and/or conjunctival epithelial cell in the tear specimens and non-increased nasal responsiveness to histamine. In 4 patients a significant blood eosinophilia was also recorded. No other abnormalities were detected.

In these 35 patients, 47 nasal provocation tests (NPT) with various inhalant allergens (Table 1), with respect to the positive skin tests and/or suspect disease history and 35 PBS

(phosphate-buffered saline) control tests were performed by means of rhinomanometry [10-16, 42-47] combined with recording of the ocular signs and symptoms. [10- 14] The patients were investigated in a period without acute ocular and nasal complaints, without symptoms of an acute infection, outside the allergen-relevant season and during hospitalization.

Long-acting H1-receptor antagonists and topical (nasal) glucocorticosteroids were withdrawn 6 weeks, topical and oral short- acting H1-receptor antagonists, topical decongestants and other treatments were withdrawn 48 hours before each of the NPTs. In these 35 patients, 35 NPTs with the same allergens producing the secondary late CR and 35 PBS control challenges (Table 1) were repeated 2 weeks later. The repeated SLCR and PBS controls (Fig. 1C) were supplemented with collection of the tears for the cytologic examination. A 4-day interval was always inserted between the end of the preceding test and the begin of the following test to prevent the carry-over effects and to allow for patient recovery. The study protocol was approved by the local ethical committee and informed consent was obtained from all study participants.

Allergen	Concentration	Nasal responses positive (n=35)	Conjunctival responses (n=35)	
			SAC (n=16)	PAC (n=19)
Dermatophagoides pteron	1000 BU/mL	6		6
Animal danders				
-dog	3000 BU/mL	3		3
-cat	2000 BU/mL	4		4
-hamster	2000 BU/mL	2		2
Feathers				
-canary	3000 BU/mL	1		1
-parrot	3000 BU/mL	2		2
Aspergillus fumigatus	1000 BU/mL	1		1
Pollen				
-grass mix I	1000 BU/mL	5	5	
-grass mix II	1000 BU/mL	2	2	
-flower mix	5000 BU/mL	3	3	
-tree mix	3000 BU/mL	1	1	
-weed mix	1000 BU/mL	1	1	
-birch	1000 BU/mL	4	4	

Grasspollen mix I= *Dactylis glomerata, Lolium perenne, Phleum pratensis, Poa pratensis;*
Grasspollen mix II=*Festuca pratensis, Holcus lanatus, Agrostis alba, Anthoxanthum odoratum*
Flower pollen mix=*Dahlia variabilis, Solidago virgaurea, Primula variabilis, Forsythia suspensa*
Tree pollen mix= *Betula pendula, Corylus avellana, Juniperus communis, Salix alba*
Weed pollen mix=*Artemisia vulgaris,Plantago lanceolata, Rumex acetosa, Taraxacum officinale*

Table 1. Survey of the allergens used for nasal challenge

2.2 Allergens

Dialyzed and lyophilized allergen extracts (Allergopharma, Reinbek, Germany) were diluted in phosphate-buffered saline (PBS) and used for skin tests in concentrations of 100-500 BU/mL and for NPTs in concentrations of 1000-5000 BU/mL (Table 1), as recommended by the manufacturer. If indicated, higher dilutions of the allergen extracts were used both for the skin tests and for the NPTs.

2.3 Skin tests

Scratch tests with allergenic extracts in concentrations of 500 BU/mL were performed and the results evaluated after 20 minutes. If the results were negative, then intracutaneous tests in concentrations of 100 BU/mL and 500 BU/mL were performed and evaluated 20 minutes and 6, 12, 24, 36, 48, 56, 72 and 96 hours after the intradermal injection. A skin wheal (>7.0 mm in diameter) occurring 20 minutes after the intracutaneous injection was qualified as a positive immediate skin response, the skin infiltration appearing between 6 and 12 hours as a late skin response, and the skin induration recorded later than 48 hours as a delayed skin response.[10-17, 42-47]

2.4 Nasal provocation tests (NPTs)

Nasal challenges with allergens were performed by means of rhinomanometry, already described in our previous studies.[10-16, 42-47] The nasal mucosa response (nasal obstruction due to the nasal mucosa edema) was evaluated by means of nasopharynx- nostril pressure gradient (NPG) parameters, which are the pressure differences (ΔP) between the nasopharyngeal cavity and the outside air, expressed in cm H_2O. NPTs were performed by the following schedule: (1) baseline values recorded at 0, 5 and 10 minutes before the challenge; (2) PBS control values recorded at 0, 5 and 10 minutes after a 3-minute application of PBS to the nasal mucosa of the non-intubated nasal cavity by means of a saturated wad of cotton wool on a nasal probe inserted under the concha media; (3) post-challenge values recorded after a 3-minute challenge with allergen, carried out in the same manner as the challenge with PBS, at 0, 5, 10, 20, 30, 45, 60, 90 and 120 minutes, and subsequently every hour up to the 12th hour, and then every second hour during the time-periods between the 24th-38th and 48th – 56th (60th) hour [12, 42-47]. The allergens used for the NPTs were chosen with respect of the disease history and positive skin tests (Table 1). The nasal response (NR) was assessed to be positive when the post-challenge mean NPG values increased by at least 2.0 cm H_2O (1.2 ± 0.3, mean± SE) with respect to the mean baseline values, recorded at least at three consecutive time intervals[12, 42-47]. The NPG changes recorded within 60-120 minutes after the allergen challenge were considered to be an immediate nasal response (INR), those recorded within 4-12 hours to be a late nasal response (LNR), and the changes measured later than 24 hours to be a delayed nasal response (DYNR) [10-15, 42-47].

2.5 Control tests with phosphate-buffered saline (PBS)

The control nasal challenge with PBS was performed in each patient studied following the same schedule as that used for the NPTs with allergen, however, 3 days later.

2.6 Conjunctival response

The objective conjunctival signs and relevant subjective symptoms were registered before and during all NPTs with allergens and PBS at the same time-points as the nasal NPG

values. The features of the conjunctiva were assessed by ophthalmoscopy including a slit lamp. The conjunctival signs, such as hyperaemia (injection), chemosis, hyperlacrimation, and palpebral edema, and the subjective symptoms, such as itching (burning), blurred vision and photophobia, were registered and evaluated by means of the scale suggested previously by Abelson [48-51], but modified by us (Pelikan's scale). [10, 11, 13, 14] The evaluation criteria of the individual signs and symptoms were as follows: 0=absent, 1=mild (present to a slight degree), 2=moderate, 3= pronounced (moderately severe), 4=severe (Table 2). Differences in total sign score of 4 points or more (3 ± 1, mean ± SE), recorded at least at three consecutive time-intervals, were found to be statistically significant (p<0.05).[13, 14]

	Abelson I*	Abelson II**	Our score
I. OBJECTIVE SIGNS			
-Hyperemia (injection, redness)	0 - 4	0 - 3	0 - 4
-Chemosis		0 - 3	0 - 4
-Hyperlacrimation (tearing)		0 - 3	0 - 4
-Palpebral edema			0 - 4
II. SUBJECTIVE SYMPTOMS			
-Itching (burning)	0 - 4	0 - 4	0 - 4
-Photophobia			0 - 4
-Blurred vision			0 - 4

* = References 48-50; ** = References 51

Abelson's grading scale: 0=None; 1=Mild (intermittent); 2=Moderate; 3=Severe; 4=extremely severe or "incapacitating "itching; [Significant threshold: ≥+2]

Our evaluation scale: 0=Absent; 1=Mild (present to a slight degree or intermittent); 2=Moderate; 3= Pronounced (moderately severe); 4=Severe; [Significant difference: ≥4 points (p<0.05), with respect to the pre-challenge value, recorded at least at 3 consecutive time-points].

Table 2. Survey of Abelson's and our "modified" conjunctivitis grading scale and symptom score

2.7 Collection and processing of tears

The tear specimens were collected from each of eyes separately by means of micropipette from the fornix (corner) before, then after 30 and 60 minutes and every second hour up to 12 hours and 24 hours after the allergen challenge. The specimens were divided into 3 portion transferred to the microscopic slides and spread out on the slide surface using a glass probe. The first series of the air-dried specimens was fixed by polyethylene glycol and stained by Hansel's method modified by us.[12, 42- 44] The second air-dried series was stained by May-Grünwald-Giemsa, modified by us.[12, 42- 44] The third series fixed by methanol was stained by toluidine blue method.[12, 42-44] Specimens were dehydrated by methyl alcohol, mounted in Canada balsam and scanned microscopically.[12, 42-44]The absolute numbers of the individual cell types have been counted per microscopic field at magnification x250 and means were

calculated from 20 fields, per each eye separately. The mean values from both the eyes were finally calculated.
Doubtful cells were re-examined under oil immersion at magnification x1200. The appearance of particular cell types was evaluated by the following scale: - = no appearance; ± = sporadical; + =slight; +± =moderate; ++ =pronounced; ++± =distinct; +++ = large; ++++ = very large appearance. The statistically significant magnitude of changes in the count of particular cell types in tears between two consecutive count degrees (mean ±SD) for secondary CRs was as follows: eosinophils 4 (4.15±0.38); neutrophils 5(4.61±0.72); basophils 1 (0.58±0.30); mast cells 1 (0.65±0.41); lymphocytes 2 (1.83±0.26); monocytes 1(0.53±0.39); epithelial cells 5 (4.97±0.51). The lowest statistically significant magnitude of the cell count is expressed as + (slight).

2.8 Control group

Eleven young adults suffering from allergic rhinitis, confirmed by positive history, skin tests and NPTs with inhalant allergens, but without history of any ocular disease and with normal ophthalmologic findings, volunteered to participate as control subjects. In these patients 11 positive late nasal responses (LNR) to inhalant allergens were repeated and supplemented with registration of the conjunctival features, subjective symptoms and cytologic examination of the tears.

2.9 Statistical analysis

The dynamic course of the nasal as well as the conjunctival responses were statistically evaluated by means of generalized multivariate analysis of variance model (MANOVA)[52.] The polynomials were fitted to the mean curves over time(8 time points within 120 minutes, 18 time points between 2 and 12 hours and 8 time points between 24 an 52 hours after the allergen challenge), and the appropriate hypotheses were tested by the modified MANOVA computerized system. [52]

1. The mean NPG values and the mean total conjunctival score values of the same type of response were compared with corresponding PBS control values at each of the time-points and analyzed by the Mann-Whitney *U* test.
2. The changes in the count of particular cell types in tears during the NPTs as well as the PBS control challenges were analyzed by the Wilcoxon matched-pair signed rank test, comparing the post-challenge values with the mean pre-challenge (baseline) values at each of the time-points. Statistical evaluation of the CR was performed separately for each of the eyes and then the mean from both the eyes was always calculated. A p value <0.05 was considered to be statistically significant.

3. Results

3.1 Nasal responses (NRs)

In the 35 patients 47 nasal provocation tests (NPTs) with various inhalant allergens (Table 1) and 35 PBS control challenges were performed. The 35 patients developed 35 late nasal responses (LNRs; $p<0.001$) and 12 negative nasal responses (NNRs; $p>0.1$). The LNR began between 4-6 hours, reached its maximum between 6-8 hours and resolved within 12 hours after the nasal challenge with allergen.
The 35 PBS control tests were all negative ($p>0.2$) No significant differences were found in the appearance of the LNRs with respect to the individual allergens ($p>0.1$). In 6 patients

positive IgE in the serum to various inhalant allergens and in 4 patients increased blood eosinopil count were found. The LNRs were associated with significant changes ($p<0.05$) in the counts (mostly temporary increase) of the neutrophils, eosinophils, epithelial and goblet cells, and to a lesser degree of the lymphocytes, in the nasal secretions. The counts of basophils, mast cells, monocytes and plasma cells were relatively low and mostly without significant changes.

The repeated NPTs resulted in the development of a similar and statistically significant LNRs comparing the post-challenge with the pre-challenge (baseline) values ($p<0.01$) as well as with the PBS control values ($p<0.001$) (Fig. 1C). No statistical significant differences were found between the initial and the repeated LNRs ($p>0.1$).

3.2 Conjunctival responses (CRs)

The 35 positive LNRs, recorded in 35 patients, were associated with significantly positive secondary conjunctival responses of the late type (SLCR; $p<0.01$). The positive SLCR began between 5-6 hours, reached its maximum between 8-10 hours and resolved usually within 12, sometimes within 24 hours after the allergen challenge. The SLCR was represented by significant changes in the objective conjunctival signs ($p<0.01$) well as subjective symptoms ($p<0.05$) (Table 3). No SLCR has been accompanied by significant corneal signs. No significant conjunctival changes were recorded during the 12 negative nasal responses ($p>0.05$) or during the 35 PBS control challenges ($p>0.1$). No significant differences in the conjunctival changes were observed between the right and left eye ($p>0.1$).

The repeated NPTs have induced similar and statistically significant SLCRs, both comparing the post-challenge with the pre-challenge (baseline) values ($p<0.01$) and comparing with the PBS control challenge ($p<0.01$) (Fig. 1B). No statistically significant difference were found between the initial and the repeated SLCRs ($p>0.2$).

	SLCR Total ocular symptom score (TOSS)											
	minutes			hours								
	0	30	60	2	3	4	6	8	10	12	24	28
ILNR	-	-	-	-	-	*	***	***	***	*	-	-

SLCR= Secondary late conjunctival response (total mean conjunctival score values);
ILNR=Isolated late NR (NPG values);
- = $p>0.05$; * = $p<0.05$; **= $p<0.01$; ***= $p<0.001$

Table 3. Significance of the correlation between positive SLCR and positive ILNR

3.3 Cytologic changes in the tears during SLCRs

The repeated SLCRs were accompanied with low cellular counts in tears as compared with counts observed in tears during the primary types of allergic conjunctivitis.[11] The SLCRS were associated with significant changes in the count of eosinophils and neutrophils, but not of other cell types (Fig. 1A, Table 4). Before the NPTs the eosinophils as well as the neutrophils appeared in tears only sporadically. The counts of eosinophils increased between 6 and 8 hours ($p<0.05$), then they decreased and disappeared from the tears at 10

hours after the allergen challenge. The counts of neutrophils increased significantly ($p<0.05$) between 8 and 10 hours, decreased at 12 hours and persisted to a slight, non-significant, degree up to 24 hours after the allergen challenge. The counts of epithelial cells increased slightly between 10 and 12 hours after the allergen challenge, but they did not reach a significant degree ($p>0.05$). The other cell types, such as basophils, mast cells, lymphocytes and monocytes, appeared in tears during the SLCRs only sporadically. The cells appearing in tears were intact and they did not demonstrate any changes of their cytoplasmic granules. During the 35 PBS control tests as well as during the 12 negative nasal responses (NNRs) only sporadic epithelial cells and no other cell types were observed. No significant differences in results were found between both the eyes.

	Before the challenge	After the challenge (hours)														
		½	1	2	3	4	5	6	7	8	9	10	11	12	24	28
Eosinophils																
- *SLCR*	1	0	2	1	2	0	3	2	5*	6*	7*	5*	3	2	0	0
- *PBS*	1	1	1	0	0	1	0	0	1	1	0	1	0	0	1	0
Neutrophils																
- *SLCR*	0	1	0	0	0	2	1	1	3	7*	8*	8*	9*	4	1	0
- *PBS*	0	0	1	2	2	0	0	0	1	0	0	3	0	1	0	0
Mast cells																
- *SLCR*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
- PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Basophils																
- *SLCR*	0	0	0	0	0	0	0	0	0	0	1+	0	0	0	0	0
- PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymphocytes																
- *SLCR*	0	0	0	0	1	1	3+	3+	0	1	0	3+	2	1	0	0
- *PBS*	0	0	1	0	0	1	1	0	2	0	1	1	0	0	0	0
Monocytes																
- *SLCR*	0	0	0	0	0	0	0	0	0	1+	1+	0	0	0	0	0
- PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Goblet cells																
- *SLCR*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
- PBS	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epithelial cells																
- *SLCR*	1	1	2	1	3	2	4	0	2	2	3	6*	6*	7*	3	1
- PBS	1	0	0	2	0	1	0	2	0	0	1	1	1	0	1	1

SLCR = Secondary late conjunctival response; PBS = Phosphate-buffered saline; Significance with respect to the baseline (before the challenge): + = $p<\pm0.05$; * = $p<0.05$;

Table 4. Mean numbers of particular cell types in tears during the positive SLCR and PBS control challenge

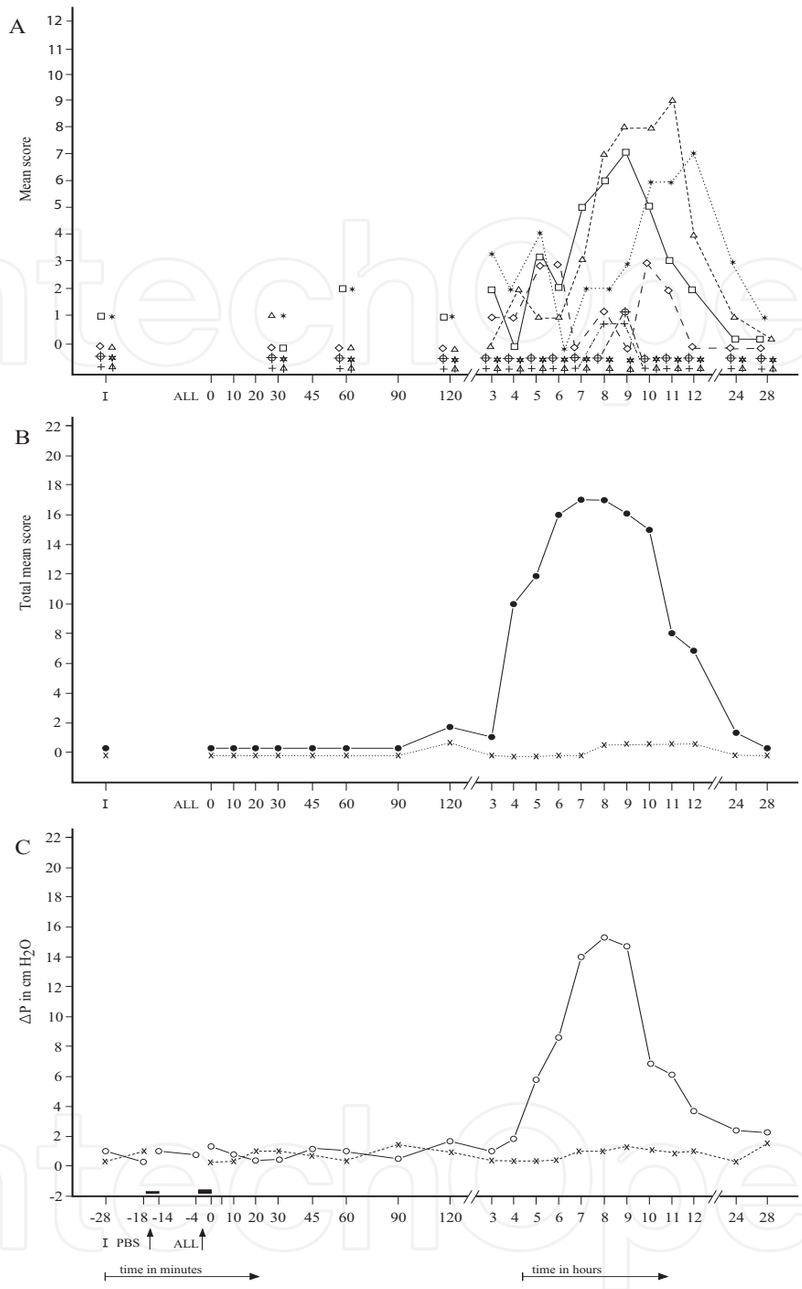

Fig. 1. The secondary late conjunctival responses (SLCRs; n=35) accompanying the isolated late nasal responses (ILNR; n= 35). **A.** The mean score of particular cell counts during the SLCR : □ = Eosinophils, Δ = Neutrophils; ⌖ = Basophils, ⍋ = Mast cells, ◇ = Lymphocytes, + = Monocytes, ✲ = Goblet cells, ✶ = Conjunctival epithelial cells. **B.** The mean total score of conjunctival signs and symptoms during the SLCR (●) and PBS (**x**). **C.** The mean rhinomanometric values (NPG) recorded during ILNR (○) and PBS (x). I = Initial (baseline) values, PBS = Phosphate buffered saline; ALL = Allergen challenge

4. Discussion

The allergic conjunctivitis (AC) can occur in two forms, in a primary form caused by a direct exposure of conjunctiva to an allergen leading to the development of an allergic reaction (antigen-antibody or antigen-sensitized Th1 lymphocytes) in the conjunctival tissue, or in a secondary form, where the allergic reaction taking place primarily in the nasal mucosa, due to its exposure to an external allergen, induces secondarily a conjunctival response. The existence of the secondary form of AC has already been demonstrated by us in patients suffering both from SAC and PAC as well as VKC and AKC. [10-16]

The allergic component involved in all AC entities, SAC, PAC, VKC, AKC and GPC, may be due to different hypersensitivity mechanisms, such as immediate type (IgE-mediated type I), late (type III) or delayed type (C cell-mediated type IV).[1, 3, 5, 6, 10-36] The involvement of various hypersensitivity mechanisms in AC may then result in three types of conjunctival response (CR), immediate (ICR), late (LCR) and delayed (DYCR), of the primary as well as of the secondary AC forms.[1, 6, 8-16, 19, 20, 25, 28, 36-40, 53-55]

Additionally, the non-specific hyperreactivity resulting from direct stimulation of mucosal, glandular and/or neurogenic receptors either in the conjunctivae (in the primary CR form) or in the nasal mucosa (in the secondary CR form) by non-specific agents might participate in the conjunctival complaints, although usually to a low and unimportant degree.[3, 12, 41]

The pathogenesis underlying the particular types of allergic conjunctivitis, such as SAC and PAC on the one hand, and keratoconjunctivitis types, such as VKC and AKC on the other hand and finally GPC, differs substantially, as it has already been reported in the literature.[1-16, 18, 22, 24, 26, 27, 33, 35, 39, 53, 54] Clinically, allergic conjunctivitis (SAC, PAC) is characterized by a number of objective conjunctival signs and subjective symptoms, related almost exclusively to the conjunctiva, whereas the keratoconjunctivitis (VKC, AKC) features include both the objective conjunctival and corneal signs and the subjective symptoms. [1-15, 26, 33, 35, 55] Generally, the SAC and PAC represent more functional and almost fully reversible process, whereas the VKC and AKC (and to a certain extent also GPC) may be characterized as a functional-morphologic process associated with usually reversible damage of the corneal surface (epithelium), and sometimes also with temporary damage of limbus and/or papillae, and incidentally also with other complications, such as formation of corneal scars, lacrimal way contractures and uveitis. [1, 6, 8, 9, 18, 26, 33- 35] The objective conjunctiva-related signs typical for allergic conjunctivitis (SAC, PAC) include hyperaemia (injection) of the conjunctiva, chemosis, tearing (hyperlacrimation, watery discharge) and sometimes palpebral oedema. The subjective conjunctiva-related symptoms consist of itching, burning and sometimes blurred vision and photophobia. These objective conjunctiva-related signs and subjective symptoms have been recorded during all three types (immediate, late, delayed) of CR, both in their primary and in their secondary form.[10-16, 19, 20, 26, 33, 36, 39, 48-51, 53-55] However, the appearance and participation of individual objective signs and subjective symptoms in the clinical picture exhibited some differences with respect to the particular CR types (immediate, late, delayed), to the individual CR forms (primary vs. secondary) and to the particular clinical AC entities, such as SAC, PAC, VKC, AKC and GPC. [10-17, 19, 20, 26, 28, 29, 33, 36, 39, 51, 53-55]

The objective signs and subjective symptoms of AC and their appearance during the conjunctival provocation tests (CPTs) as well as during the nasal provocation tests (NPTs) can be evaluated by a scoring (grading) system. Various scoring systems have been proposed in the literature up to date.[1, 3-9, 19, 33, 37, 53-56] One of the widely used scoring system

has been suggested by Abelson et al.[48-52] However, the proposed scoring systems differ with respect to the parameters registered and the numbers of the grading points. Moreover, in the proposed scoring systems, including that suggested by Abelson et al, not all conjunctival and corneal signs and subjective symptoms typical for AC forms are included. Therefore, we have developed an improved and modified scoring (grading) system (Pelikan's scoring system)[10, 11, 13- 15], including additionally conjunctival chemosis, palpebral edema, burning, blurred vision and photophobia (Table 2).

In our system, predominantly the relative values, which means the difference between the post-challenge mean signs and symptoms and their pre-challenge values, as well as their dynamic course have been considered to be basic parameters for the assessment of the CSs. Using our model, the statistically significant differences in the total signs and symptoms have been calculated to be at least 4 points ($p<0.05$; Fig.1B). We belief that evaluation of the CRs by means of repeated comparison of the relative values of particular clinical parameters (conjunctival signs and symptoms) in their time-course may increase the credibility of the results.

The relationship between the conjunctivae and lacrimal ways on one hand and the nasal mucosa on the other hand is effectuated both on the anatomic and on the functional level.[1, 3, 5-31, 33-36, 55-66] Both the levels include connection of the conjunctiva with the nasal cavity through the naso-lacrimal duct, facilitating the tear drainage into the nasal cavity, but allowing also retrograde migration of factors from the nasal cavity into the conjunctivae, and connection of both the organs through the blood vessels, lymphatic tissue and the neurogenic network, all showing some links and sharing some common properties.

An allergic reaction occurring initially in the nasal mucosa can affect the conjunctiva in different manners upon involving of different mechanisms: (1) This reaction leads to release of a number of mediators, immunoglobulins, cytokines, adhesion molecules, chemotactic and other factors, which can then reach the conjunctiva via a retrograde penetration through the naso-lacrimal duct and system; [5, 6, 15, 18-31, 35, 55, 63, 64] (2) The released mediators and factors can be transported by the bloodstream through the local blood vessel system, e.g. *arteria maxillaries-pars pterygopalatina, vena facialis and plexus pterygoideus*;[5, 6, 12-15, 19, 35, 65] (3) This reaction can stimulate the local neurogenic network (sensory nerves, sympathetic and parasympathetic fibers) and released neuropeptides may reach conjunctiva along and/or through the particular nerves, such as *nervus trigeminus, nervus nasociliaris and ganglion pterygopalatinum*; [63, 64] (4) Various cell types participating in the allergic reaction occurring in the nasal mucosa, such as mast cells, basophils, eosinophils, neutrophils, plasma cells, monocytes , thrombocytes, B- and particular sub-sets of T-lymphocytes (Th1, Th2, Th 17, T-regulatory and natural killer cells), dendritic cells and macrophages can migrate through the bloodstream and/or the lymphatic stream into the ocular tissue;[11-14, 33, 61] (5) The allergic reaction occurring in the nasal mucosa and released factors can stimulate the local nasal mucosal lymphatic system "*nose-associated lymphatic tissue*" [NALT], expressing manifold mutual communication with the lymphatic network both of the lacrimal system, such as " *tear duct-associated lymphatic tissue*"[TALT], "*lacrimal drainage-associated lymphoid tissue*" [LDALT], and that of conjunctiva, called "*conjunctiva-associated lymphatic tissue*" [CALT]and "*eye-associated lymphatic tissue*" [EALT].[12-16, 57-62, 65, 66] In this way not only the transmission of certain intercellular and cellular-tissue receptor signals but also cellular traffic of various cell types , e.g. of T-lymphocytes (Th1, Th2),antigen-presenting cells (APC) and B-cells (plasma cells) can be realized.[10, 12-15, 19, 29, 30, 35, 39, 62, 65, 66] An additional mechanism playing also a role

in the cellular traffic among the particular organ-associated lymphatic tissues , under certain circumstances, is the defective "homing mechanism" of the B- and T-lymphocytes, controlled by a number of homing factors.[10, 12-15, 29, 61, 62, 65, 66]

The allergy reaction in the conjunctival tissue, similarly to the nasal mucosa, is a dynamic process caused by a certain external allergen in which various types of cells are involved in various steps of this process.[1-16, 18-40, 42-44, 54-56, 61, 62, 65-67] This is also an exfoliative process leading to release and migration of various cell types, usually after finishing of their active involvement, into the particular fluids(media), such as nasal secretions or tears.[1-19, 24-26, 28, 29, 32-35, 38-40, 42-44, 53-56, 58-62, 65, 67, 68] These fluids may therefore be considered not only as a conditioning means of the particular organ, but also as waste media serving for drainage and removal of the exhausted and no longer active cells migrating from the mucosal membrane or being eliminated by the mucosal tissues after finishing their active participation in the allergic reaction. [8, 9, 12, 19, 24, 35, 42-44, 61, 67]

The numbers as well as the stage and condition of the eliminated cells can also indicate the qualitative as well as quantitative aspects of the running and/or passing allergic reaction in the particular mucosal membrane, in this case conjunctival tissue and/or nasal mucosa. [11, 12, 14] However, the current or passing involvement of the individual cell types in the allergic reaction can only be characterized by comparing their counts and conditions before and repeatedly after a well-defined intervention, which is a challenge with a certain allergen in a certain dose during a certain interval of time.[10-16, 19, 33, 37, 39, 42-49, 51, 56, 68] The cytologic examination of tears, as with the nasal secretions, is a relatively easy and valuable technique for evaluation of changes in the particular cell types appearing in tears during the allergic reaction. [19, 22, 34, 35, 57, 59] However, this method is limited only to tears and does not allow full evaluation of the cellular changes in the mucosal membrane itself. This may only be derived from a biopsy of the mucosal (conjunctival) tissue only.[19, 29, 31, 38, 68]

The cytologic examination of the conjunctiva can be performed by means of various techniques, such as brush, impression and scraping technique and classical biopsy technique.[4, 19, 24, 28, 31, 35, 37-39, 46, 54, 55, 65, 67-74] The brush and impression techniques may be considered to be in fact already semi-invasive methods, whereas scraping and biopsy are typical invasive methods. Each of these methods has its advantages and disadvantages. The major disadvantage of these techniques, except the brush method, is the use of anesthetics. Certain traumatizing effects of the conjunctival (ocular) tissue, stimulation/irritation of the tissue and blood capillary neurogenic receptors causing some of the undesirable reflexes and their unsuitability for the serial application, may also be qualified as a disadvantage. These techniques cannot be performed repeatedly on the same conjunctival location owing to traumatizing effects and tissue repair and *vice versa* the results attained from different localities are not fully comparable. Recently, a new promising technique, the confocal laser scanning microscopy, has been introduced. [71] On the other hand, the cytologic examination of the tears, collected by means of aspiration with a micropipette, is a very simple and easy method, which can be repeated almost endlessly, requires no anesthesia, does not traumatize the ocular tissue and is most similar to the natural clearance of the eye and drainage of tears. [19-23, 27, 30-34, 37, 39, 54, 64] The tear specimens can be processed and stained applying various methods and techniques, such as Hansel's stain, Wright's stain, May-Grünwald-Giemsa, Leishman stain, Winkler-Schultze method, Kardoz-Gemisch technique, Romanowski stain, Shorr's stain, Alcian blue-Safranin method, Astra blue, Azure A stain, Toluidine blue, Papanicolaou stain or

Carnoy's fixation-Periodic-Acid-Schiff (PAS).[4, 37, 39, 54, 55, 67, 69, 72-74] We have employed May-Grünwald-Giemsa staining as a basic method, and the Hansel's and toluidine blue staining as a supplementary technique, analogically to the staining technique a of the nasal secretions. [11, 12, 42-44]

Studies concerning the cytologic examination of tears in patients with allergic conjunctivitis, especially the appearance of particular cell types in tears and their changes, are not numerous. [39, 69, 73, 75, 76] The appearance of particular cell types has been investigated by means of a single tear cytogram , revealing increased numbers of eosinophils, mast cells, epithelial cells and sometimes neutrophils in tears.

The data gathered by brush, impressive or scrapping methods as well as by conjunctival biopsy performed in AC patients demonstrated some variations in presence of particular cell types as well as their numbers both in the epithelial and sub- epithelial layers of the conjunctiva. Mostly eosinophils and neutrophils, sporadically mast cells and lymphocytes were found in conjunctival epithelium and subepithelial layers. [19, 20, 31, 37, 55, 67- 69, 73, 76] However, since these methods are not fully suitable to be performed repeatedly on the same location, the course of cellular changes remain to be unknown.

Nevertheless, there is a dearth of data documenting dynamic course of the changes of individual cell types in tears during particular types of the primary conjunctival response to the allergen challenge using conjunctival provocation tests (CPTs).[19, 37, 55, 68, 73, 76] Moreover, no information has been found by us in the literature concerning the cytologic changes in the tears during the secondarily induced conjunctival responses by the primary nasal response to allergen challenge. Our results cannot be therefore compared with other data.

Our findings of relatively low counts of all cell types in tears during the SLCRs and only slightly increased eosinophil and neutrophil counts at the peak of the SLCRs , 8-10 hours after the nasal allergen challenge, may suggest the following hypotheses:

1. The primary allergic reaction occurring in the nasal mucosa induces the secondary conjunctival response of the late type;
2. The cells, especially the eosinophils and neutrophils, appearing in the tears during the SLCR did not probably participate directly in the allergic reaction either in the conjunctival tissue or in the nasal mucosa. These cell did not originate primarily from either conjunctival tissue or from nasal mucosa during the early stages of allergic reaction, but they probably migrated from the dilated conjunctival capillaries during the later stages of conjunctival response as a consequence of effects of the mediators and other factors released during the primary allergic reaction in the nasal mucosa and subsequently penetrating into the conjunctival tissue. This hypothesis may be supported by the intact condition of these cells, which cytoplasmic granules were not degranulated;
3. The discrepancy between the low counts of eosinophils and neutrophils in the tears during the SLCR and their relatively high counts in the nasal secretions during the primary late nasal response, would oppose the possible migration of these cells from the nose into the conjunctival tissue;
4. The SLCR may probably be induced by mediators released primarily in the nasal mucosa and subsequently penetrating into the conjunctivae.

Nevertheless, the exact manner and route by which these factors penetrate and achieve the conjunctivae is not yet clarified and will need more concurrent studies comparing levels of particular mediators in the nasal secretions, lacrimal ways and tears.

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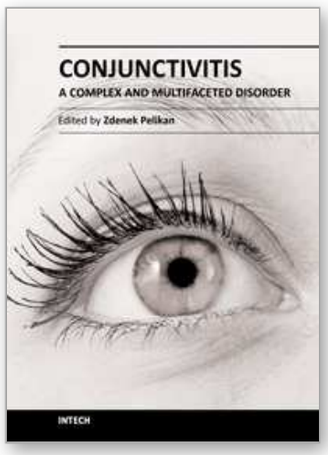

Conjunctivitis - A Complex and Multifaceted Disorder
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This book presents a number of interesting and useful aspects and facets concerning the clinical features, properties and therapeutical management of this condition. Dr. H. Mejía-López et al. present an interesting survey of the world-wide epidemiologic aspects of infectious conjunctivitis. Dr. U. Ubani evaluates conjunctival symptoms/signs participating in the clinical features of this disorder. Dr. A. Robles-Contreras et al. discuss immunologic aspects underlying possibly the conjunctivitis. Dr. Z. Pelikan presents the cytologic and concentration changes of some mediators and cytokines in the tears accompanying the secondary conjunctival response induced by the nasal challenge with allergen. Dr. S. Sahoo et al. summarize the treatment and pharmacologic control of particular clinical forms of conjunctivitis in general practice. Dr. S. Leonardi et al. explain the basic pharmacologic effects of leukotriene antagonists and their use for the treatment of allergic conjunctivitis. Dr. J.A. Capriotti et al. evaluate the therapeutical effects of various anti-adenoviral agents on the acute conjunctivitis caused by adenovirus. Dr. V. Vanzzini-Zago et al. assess the prophylactic use and efficacy of "povidone-iodium solution", prior the ocular surgery. Dr. F. Abazi et al. present the clinical features, diagnostic and therapeutical aspects of "neonatal conjunctivitis". Dr. I.A. Chaudhry et al. review the special sub-form of conjunctivitis, being a part of the "Trachoma". Dr. B. Kwiatkowska and Dr. M. Maślińska describe the clinical, pathophysiologic and immunologic features of conjunctivitis. Dr. S. Naem reviews the conjunctivitis form caused by Thelazia nematodes, occurring principally in animals.

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