

Short communication

ICAM-1/CD54 expression on conjunctival epithelium during pollen season

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Seasonal allergic conjunctivitis is frequently associated with rhinitis, and it is usually more severe than perennial allergic conjunctivitis caused by mites, as recently reported (5). During natural allergen exposure to pollen, the clinical features of seasonal allergic conjunctivitis are ocular redness and itching, lacrimation, and swelling of eyelids (6). Pathophysiologic features include release of allergic reaction mediators and inflammatory cell infiltrate, mainly comprising eosinophils and neutrophils. Furthermore, adhesion molecule expression has been detected on inflammatory cells and epithelium, and this expression has been assumed to be a marker of allergic inflammation (1). As recently shown, ICAM-1/CD54 expression occurs on nasal epithelium in patients with allergic rhinitis caused by pollen during natural exposure or on conjunctival and nasal epithelium in house-dust-mite-sensitive subjects continuously exposed to natural allergen, even during clinical latency (2). On the contrary, this molecule is not constitutively expressed in allergic subjects outside the pollen season, whereas it is expressed early after allergen conjunctival challenge (4). ICAM-1/CD54 expression on epithelium is of particular interest, because it is the natural ligand of LFA-1, an integrin expressed on leukocytes; in particular, on the eosinophil surface. In this manner, the typical

epithelial damage caused by eosinophil infiltration during allergic reaction may be explained.

In the present study, we assessed ICAM-1/CD54 expression on epithelial conjunctival cells during natural seasonal exposure, determining the possible correlation with clinical features and inflammatory cell involvement.

Material and methods

Fifteen patients (including eight women, mean age 31.7 years, range 18–40 years) were enrolled in the study on the basis of a history of signs and symptoms of allergic rhinoconjunctivitis during the pollen season (*Parietaria judaica*, March–November) for at least two seasons, a positive skin prick test to the allergen (greater than or equal to the histamine wheal), and a positive RAST to the allergen (greater than or equal to class III). All subjects had no other ocular diseases and did not wear contact lenses. No allergic subject had been treated with any topical or systemic drug in the preceding 6 weeks. Before the study, they had not received specific immunotherapy.

Fifteen healthy volunteers (including nine women; mean age 33.5 years; range, 19–43 years) were also studied as normal controls. They had no history of allergic diseases, did not smoke, and had negative

skin prick tests and RAST to a panel of common allergens.

All subjects gave their informed consent, and the trial was approved by the department ethics committee.

Study design

The subjects were studied twice: before and during the pollen season. Outside the pollen season, all subjects underwent a conjunctival scraping in January 1993, and were symptomless. During the pollen season, ocular and nasal signs and symptoms were evaluated, respectively, as hyperemia, burning-itching, lacrimation, and eyelid swelling, and sneezing, nose itching, rhinorrhea, and nasal obstruction. Scoring was recorded according to an arbitrary four-point rating scale, from 0 to 3, increasing with severity (0 = absent, 1 = mild, 2 = moderate, 3 = severe). A total symptom score was calculated as the sum of single symptom scores, and was expressed weekly on a diary card, as previously reported (5). When the conjunctival total symptom score was more than 7, patients underwent conjunctival scraping. Healthy subjects underwent conjunctival scraping at the peak of pollen counts.

According to our previously described protocol, a conjunctival scraping for cytologic assessment was obtained from both eyes (2–4). After topical anesthesia, the upper tarsal conjunctiva was scraped by a sterile Kimura spatula. Specimens were spread on glass slides, air-dried, stained with May-Grünwald Giemsa dye, and read by microscope (Leitz Laborlux D microscope, $\times 500$ focus). The number of inflammatory cells, i.e., neutrophils, eosinophils, lymphocytes, and monocytes, was counted individually and as a total number of cells in each microscope field; data were expressed as a mean of 10 fields. The immunoenzymatic alkaline phosphatase-monoclonal antialkaline phosphatase (APAAP) complex procedure was employed to assess ICAM-1/CD54 expression on epithelium. Specimens were air-dried and submitted to appropriate dilution of purified ICAM-1/CD54 monoclonal antibody (mAb) (1 mg/ml, 84H10, IgG1) (Immunotech, Marseille, France) or anticytokeratin mAb (α CK19, DAKO, Milan, Italy) as a positive control for epithelial cells. After they were washed, samples were incubated with rabbit antimouse Ig, followed by APAAP complex, and then with substrate solution containing basic new fuchsin, naphthol arsenic biphosphate, and levamisole (Sigma, St Louis, MO, USA). Control samples were set up with irrelevant isotype-matched mAbs. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators blind to the identity of the samples. ICAM-1/CD54 on epithelial cells was expressed according to a four-

point rating scale, from 0 to 4, as previously described (4).

The *P. judaica* pollen count was made weekly.

Statistical analysis

Statistical analysis of the results was done by the Wilcoxon test for intragroup comparison, the Mann-Whitney test for intergroup comparison, and the Spearman test for data correlation. A *P* value of 0.05 or less was considered to be statistically significant.

Results

Neither clinical events, cellular infiltrate, nor ICAM-1/CD54 expression on conjunctival epithelium was detectable in any sensitized patient outside the pollen season (i.e., in the absence of natural exposure to the allergen).

As expected, healthy volunteers did not show any clinical or cytologic reaction either outside or during the pollen season. On the contrary, during the *P. judaica* pollen season, all allergic patients showed acute conjunctivitis symptoms ($P < 0.001$, as compared with preseasonal values) (Table 1). The most common signs and symptoms were hyperemia and burning-itching (Table 1). Conjunctival scrapings showed an inflammatory cell infiltrate, mainly comprising neutrophils, eosinophils, and lymphocytes ($P < 0.01$, as compared with preseasonal values, and $P < 0.001$, as compared with healthy volunteers) and ICAM-1/CD54-positive conjunctival epithelial cells ($P < 0.001$, as compared with preseasonal values, and $P < 0.001$, as compared with healthy volunteers), as reported in Fig. 1. In particular, a statistically significant increase in eosinophil numbers was

Table 1. Seasonal clinical scores of allergic patients undergoing conjunctival scraping.
* Clinical signs and symptoms of rhinitis are reported as total score

Patient no.	HYP	B/I	LAC	ES	TES	TNS	TSS
1	3	3	2	1	9	8	17
2	2	3	1	1	7	8	15
3	3	3	2	1	8	8	16
4	3	3	2	2	10	11	21
5	3	2	2	2	9	9	18
6	3	2	1	1	7	7	14
7	3	3	2	1	9	8	14
8	3	3	2	1	9	10	13
9	3	3	1	1	8	9	11
10	3	2	1	1	7	8	15
11	3	2	1	1	7	7	14
12	3	2	1	1	7	7	14
13	3	2	2	1	8	8	17
14	3	2	2	1	8	9	17
15	3	3	2	1	9	7	16

HYP=hyperemia; B/I=burning-itching; LAC=lacrimation; ES=eye swelling; TES=total eye score; TNS=total nasal score; TSS=total symptom score.

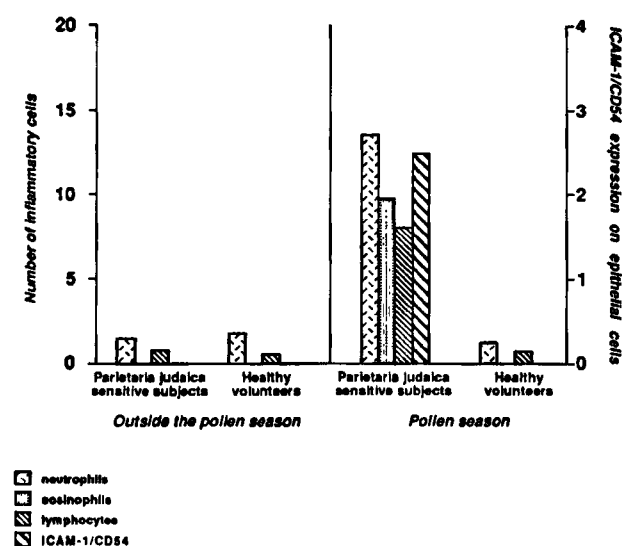


Fig. 1. Preseasonal and seasonal cytologic data in allergic patients. Data are expressed as median values.

shown in allergic patients ($P < 0.001$), statistically correlated with ICAM-1/CD54 expression on epithelium ($P < 0.03$) (Table 2). In addition, ICAM-1/CD54 expression was statistically correlated with conjunctival total symptom score ($P < 0.02$).

Discussion

During the pollen season, allergic conjunctivitis is characterized by relevant changes, being the clinical response accompanied by an infiltration of inflammatory cells (6). The inflammatory infiltrate also contains several pollen grains, whose granules frequently appear to be ruptured along the outermost

envelope, i.e., esine, thus releasing their cytoplasm content (5). The severity of clinical and cellular findings in pollen-sensitized subjects could be related to the constant presence of *P. judaica* granules in the conjunctiva, whereas no pollen granules can be detected in conjunctival scrapings of normal controls (5).

In the present study, neither healthy subjects nor *P. judaica*-sensitive subjects constitutively expressed ICAM-1/CD54 on conjunctival epithelium out of the pollen season. ICAM-1/CD54 expression on conjunctival epithelium occurred only in allergic subjects upon allergen stimulation. These findings indicate that ICAM-1/CD54 may play a central role in the allergic inflammation: in fact, ICAM-1/CD54 expression on epithelial cells caused by natural allergen exposure might suggest a direct involvement of epithelial cells in the inflammatory process: however, this phenomenon is presumably due to cytokines released in the microenvironment and is not related to a direct effect of the allergen on epithelial cells. Moreover, since the damage to epithelium proved to be a relevant event in bronchial asthma (7), it is likely that ICAM-1/CD54 expression makes the epithelial cells susceptible to the damage by offending cells such as eosinophils, which bear on the membrane LFA-1, the natural counterreceptor for ICAM-1/CD54.

Interestingly, no ICAM-1/CD54 expression has been detected in healthy subjects: this phenomenon underlines the specificity of the allergen exposure in eliciting ICAM-1/CD54 expression on conjunctival epithelium. New insights about the specific role of the allergen stimulation derive from a recent study in which histamine was shown to be unable to elicit by itself ICAM-1/CD54 expression on conjunctival epithelial cells of *P. judaica*-allergic subjects out of the pollen season (3).

The data of the present study fit very well with previous reports showing that ICAM-1/CD54 expression may be considered to be a marker of inflammatory changes at conjunctival, nasal, and bronchial levels, because its presence strictly correlates with the site and the severity of the inflammatory process (2).

In conclusion, pollen-induced conjunctivitis may provide a useful model for further investigation of the role of the CD54/LFA1 system in the allergic inflammatory process caused by natural exposure to specific allergen.

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Table 2. Preseasonal and seasonal cytologic scores in allergic patients

Patient no.	Preseasonal data					Seasonal data				
	TC	N	E	L	CD54	TC	N	E	L	CD54
1	3	2	0	1	0	14	8	3	3	2
2	1	1	0	0	0	18	12	4	2	2
3	1	1	0	0	0	21	9	8	4	2
4	0	0	0	0	0	21	9	6	6	2
5	2	2	0	0	0	22	12	6	4	2
6	2	1	0	1	0	10	5	3	2	1
7	2	2	0	0	0	31	15	10	6	2
8	0	0	0	0	0	28	15	7	6	2
9	3	3	0	0	0	12	7	3	2	1
10	1	1	0	0	0	25	10	8	7	2
11	1	1	0	0	0	19	10	3	6	2
12	1	0	0	1	0	20	8	4	8	2
13	3	3	0	0	0	29	12	10	9	2
14	1	1	0	0	0	33	14	12	7	2
15	2	2	0	0	0	30	16	10	4	3

TC = total cells; N = neutrophils; L = lymphocytes; E = eosinophils; M = monocytes; CD54 = ICAM-1/CD54 expression on epithelium.

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