

# Allergic subjects express intercellular adhesion molecule - 1 (ICAM-1 or CD54) on epithelial cells of conjunctiva after allergen challenge

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**Background:** Allergic inflammation can be experimentally reproduced in vivo in humans, by means of the conjunctival provocation test with allergen. The allergen stimulation triggers an early clinical response and an almost simultaneous cellular infiltrate. Among the factors that can contribute to the local cellular recruitment, we postulate a possible early involvement of CD54 in the development of inflammation caused by the allergic reaction.

**Methods:** We used a sensitive immunocytochemical immunoenzymatic alkaline phosphatase-monoclonal antialkaline phosphatase technique to detect the possible expression of CD54 molecule on epithelial cells of conjunctiva in 15 allergic subjects and in 15 healthy individuals in basal conditions and after allergen challenge (*Parietaria judaica*) during the off-pollen season.

**Results:** At baseline all studied individuals did not evidence CD54 expression on epithelial cells; 30 minutes after allergen challenge, all the allergic individuals showed a marked expression of CD54 on conjunctival epithelium, whereas none of healthy subjects showed any CD54 expression. First, CD54 expression on conjunctival epithelium after specific provocation test appeared as a specific phenomenon occurring only in sensitized subjects; moreover, it is an immediate event concomitant with the local inflammatory infiltrate. Therefore conjunctival epithelium unexpectedly appeared to be more than a bystander in the allergic reaction; it may be perceived as an active participant interacting with the inflammatory infiltrate.

**Conclusions:** These findings indicate that CD54 may play a central role in the allergic inflammation and strongly support the concept that maneuvers designed to interact with the adhesion machinery expressed on inflammatory cells and epithelium may be a helpful therapeutic approach. (*J ALLERGY CLIN IMMUNOL* 1993;91:783-92.)

**Key words:** Seasonal rhinoconjunctivitis, off-pollen season, conjunctival provocation test, *Parietaria judaica*, allergic inflammation, adhesion molecules, conjunctival epithelial cells

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The eye is a common target of allergic reaction either as the main focus of allergic disease or as a concomitant event of respiratory allergy, and the conjunctival mucosa is largely exposed to environmental allergens.<sup>1-3</sup> Moreover, as with other target organs (i.e., nose or bronchus) the eye represents an eligible model in humans to study the pathophysiologic mechanisms of the allergic reaction, both during acute inflammation and after provocation test.<sup>4-9</sup> Conjunctival provocation test (CPT) with allergen is safe, simple, reliable, and reproducible.<sup>10-12</sup> It is used to diagnose allergy, to study signs and symptoms associated with the allergic reaction, to collect cells and mediators of

**Abbreviations used**

|                     |                                                                          |
|---------------------|--------------------------------------------------------------------------|
| CPT:                | Conjunctival provocation test                                            |
| RAST:               | Radioallergosorbent test                                                 |
| SCPT:               | Specific conjunctival provocation test                                   |
| AUR/ml:             | Allergen unit by RAST (codified by DHS, Bayropharm, Milan, Italy)        |
| APAAP:              | Immunoenzymatic alkaline phosphatase-monoclonal antialkaline phosphatase |
| W-K:                | Wong-Kilburne human conjunctival cell line                               |
| <i>P. judaica</i> : | <i>Parietaria judaica</i>                                                |
| ICAM:               | Intercellular adhesion molecule                                          |

the allergic response, to evaluate the effectiveness of treatments, and to predict prognosis.<sup>3, 13-15</sup> The immediate conjunctival events consequent to CPT are characterized by a clinical response concomitant with a cellular infiltrate.<sup>3, 7, 14-16</sup> Several factors contribute to the local recruitment of inflammatory cells (i.e., neutrophils, lymphocytes, monocytes, and eosinophils).<sup>5, 8, 9, 17</sup> These are the release of cytokines in the microenvironment and the interaction between surface molecules expressed on inflammatory cells (receptors) and those on the potential target cells (ligand).<sup>18-21</sup>

A ligand for one of these adhesion receptors, LFA-1 (CD11a/CD18) is intercellular adhesion molecule-1 (CD54), a member of the immunoglobulin supergene family.<sup>22</sup> It is the main physiologic ligand of the leukocyte integrin LFA-1, widely expressed on mononuclear cells and granulocytes. The LFA-1/CD54 intercellular adhesion system is involved in immunologic reactions requiring intercellular contact.<sup>22</sup>

CD54 expression has been detected on inflamed endothelium and epithelium<sup>22,23</sup>; as to allergic inflammation, CD54 is expressed on epithelial cells and eosinophils of bronchial mucosa after allergen challenge in a primate model of asthma.<sup>20</sup>

The aim of the present study was to investigate the possible expression of CD54 on epithelial cells from conjunctival scrapings in basal conditions and after specific conjunctival challenge in healthy subjects and allergic patients.

## SUBJECTS AND METHODS

### Subjects

During off-pollen season we studied 30 subjects, 14 women and 16 men, between 20 to 45 years of age, selected in two groups, 15 subjects in each group. Fifteen subjects were healthy controls: they all had negative history of allergy and negative skin prick test and RAST.

Fifteen subjects suffered from seasonal rhinoconjunctiv-

itis as a result of *Parietaria judaica* (*P. judaica*). All allergic patients had positive history (for at least 2 previous years), positive skin prick test (more than + + +, histamine + + +), and RAST (more than class III) for specific allergens.

On admission all subjects did not suffer from any eye or respiratory disease and were not contact lens wearers. Allergic patients were not treated with any kind of drug, topical or systemic or both, in the preceding 6 weeks.

An informed consent according to the rules of our institution was obtained from each patient.

### Allergens

The *P. judaica* extract (the same used for skin prick test, provided by Bayropharm-DHS, Bayer Group, Milan, Italy) stored as a lyophilized powder at 4° C, was reconstituted in the diluent (saline human albumin) within 24 hours before the test.

The concentration was expressed as allergen unit by RAST (AUR)/ml; six concentrations (20, 60, 200, 600, 1200, and 2000 AUR/ml) were used.

### Specific conjunctival provocation test (SCPT)

Allergic patients underwent SCPT as follows: 10 µl of allergen was administered to the lower conjunctival fornix of the right eye. The initial allergen concentration used for ocular challenge was the same as the end point skin test. If no reaction was elicited every 20 minutes, the next more concentrated allergen solution (following the above-mentioned concentration) was used until an ocular clinical hypersensitivity reaction was elicited.<sup>14, 15</sup>

Healthy subjects underwent SCPT with use of the highest allergen concentration (2000 AUR/ml).

Ten microliters of saline albumin diluent was administered to the contralateral eye as control.

Clinical conjunctival evaluation and cytologic assessment were established in both eyes at baseline and 30 minutes after specific challenge.

### Clinical evaluation

The clinical reaction was assessed as total symptom score according to an arbitrary four-point rating scale, from 0 to 3 (0, absent; 1, mild; 2, moderate; 3, severe), increasing for severity in both eyes on the basis of conjunctival hyperemia, lacrimation, itching, and burning. Signs were separately evaluated by two physicians (G.C. and S.B.), and symptoms were spontaneously reported by the subject.<sup>14, 15</sup> Total symptom score after challenge of more than 7 was considered a positive response.

### Cytologic assessment

Conjunctival scrapings for cytologic assessment were obtained from both eyes at baseline and 30 minutes after challenge. After topical anesthesia (ossibuprocaine 4 mg/ml, one drop each eye), the upper tarsal conjunctiva was scraped with a sterile Kimura (Kimura Medical Instrument Co.,

**TABLE 1.** Clinical features of subjects

| Patient             | Age (yr) | Sex | Skin prick test to <i>P. judaica</i> | RAST to <i>P. judaica</i> (class) | Skin test endpoint (AUR/ml) | Conjunctival PD (AUR/ml) |
|---------------------|----------|-----|--------------------------------------|-----------------------------------|-----------------------------|--------------------------|
| Allergic patients   |          |     |                                      |                                   |                             |                          |
| 1                   | 24       | M   | ++++                                 | IV                                | 20                          | 600                      |
| 2                   | 21       | M   | ++++                                 | IV                                | 60                          | 600                      |
| 3                   | 34       | F   | ++++                                 | IV                                | 20                          | 600                      |
| 4                   | 30       | M   | ++++                                 | IV                                | 10                          | 600                      |
| 5                   | 20       | M   | ++++                                 | IV                                | 60                          | 1200                     |
| 6                   | 27       | F   | ++++                                 | IV                                | 20                          | 600                      |
| 7                   | 42       | F   | ++++                                 | IV                                | 3                           | 880                      |
| 8                   | 45       | F   | ++++                                 | IV                                | 10                          | 600                      |
| 9                   | 28       | M   | ++++                                 | IV                                | 60                          | 4080                     |
| 10                  | 27       | F   | ++++                                 | IV                                | 20                          | 600                      |
| 11                  | 41       | M   | ++++                                 | IV                                | 10                          | 600                      |
| 12                  | 35       | M   | ++++                                 | IV                                | 10                          | 880                      |
| 13                  | 38       | F   | ++++                                 | IV                                | 20                          | 600                      |
| 14                  | 24       | F   | ++++                                 | IV                                | 20                          | 600                      |
| 15                  | 21       | M   | ++++                                 | IV                                | 10                          | 600                      |
| Healthy individuals |          |     |                                      |                                   |                             |                          |
| 16                  | 26       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 17                  | 42       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 18                  | 20       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 19                  | 25       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 20                  | 34       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 21                  | 36       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 22                  | 37       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 23                  | 27       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 24                  | 28       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 25                  | 39       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 26                  | 40       | F   | Neg                                  | 0                                 | 0                           | 2000                     |
| 27                  | 20       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 28                  | 22       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 29                  | 27       | M   | Neg                                  | 0                                 | 0                           | 2000                     |
| 30                  | 31       | F   | Neg                                  | 0                                 | 0                           | 2000                     |

Tokyo, Japan) spatula. Specimens were immediately spread on glass slides and air dried.

Specimens were stained with May-Grünwald-Giemsa dye and read by microscope (Leitz Laborlux [Leitz KG, E, Hamburg, Germany] microscope  $\times 250$ ,  $\times 500$  focus). The total number of inflammatory cells including neutrophils, eosinophils, lymphocytes, and monocytes was counted for each microscopic field; the data are expressed as a mean of 10 fields. The slides were examined by two investigators (G.P.P. and B.V.) masked to the identity of the samples.<sup>14, 15</sup>

### Immunocytochemistry

Immunoenzymatic alkaline phosphatase-monoclonal antialkaline phosphatase (APAAP) complex procedure modified from Cordell et al.<sup>24</sup> and Bagnasco et al.<sup>25</sup> was used.

Specimens were air-dried at room temperature for 30 minutes and incubated with a 1:50 dilution of purified CD54

MAb (1 mg/ml, 84H10, IgG<sub>1</sub> - Immunotech, Marseille, France)<sup>26</sup> or a 1:100 dilution of anticytokeratin MAb ( $\alpha$ CK19, IgG<sub>1</sub>, DAKO, Milan, Italy) as a marker of epithelial cells.<sup>27</sup> After washing in phosphate-buffered saline pH 7.6, samples were incubated with rabbit antimouse-Ig, followed by APAAP complex. Afterward, specimens were incubated in substrate solution containing basic new fuchsin, naphthol as biphosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.). In control samples either MAb or antimouse Ig were omitted. As negative isotype control for CD54 staining on epithelial cells, an anti T lymphocyte (CD3) MAb OKT3, IgG<sub>1</sub>, (Ortho Diagnostic, Raritan, N.J.) at 1:20 dilution of the stock solution (provided by the manufacturer) was used. The dilutions were established on the basis of previous titration experiments. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators masked to the identity of the samples.

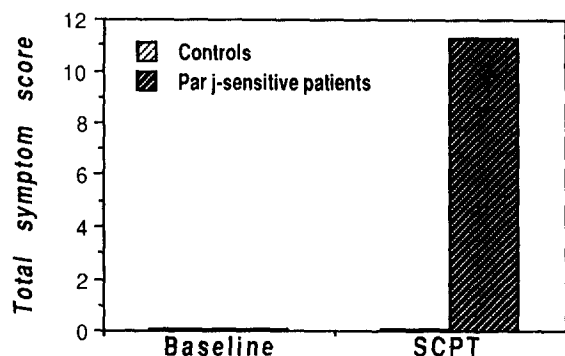


FIG. 1. Total symptom scores expressed as median value (score range from 9.6 to 12 in *P. judaica*-sensitive individuals in the two study groups ( $p < 0.001$  for *P. judaica*-sensitive individuals baseline vs SCPT).

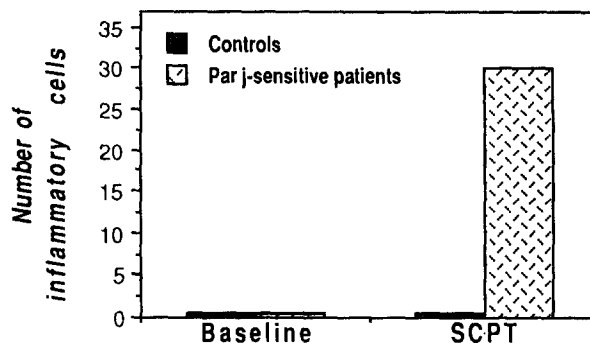


FIG. 2. Total number of inflammatory cells expressed as median value (score range from 27 to 33 in *P. judaica*-sensitive individuals) in the two study groups ( $p < 0.001$  for *P. judaica*-sensitive individuals baseline vs SCPT).

CD54 expression on epithelial cells was expressed according to a four-point rating scale from 0 to 4 where 0 represents no positive cells; 1, mild positivity on 25% of epithelial cells; 2, mild positivity on 75% of epithelial cells; 3, intense positivity on 75% of epithelial cells; 4, very intense positivity on all epithelial cells.

### Statistical analysis

Statistical analysis of results was performed by Wilcoxon rank sum test to test differences within groups.<sup>28</sup> A probability of 0.05 or less was considered statistically significant.

### RESULTS

We used a sensitive immunocytochemical APAAP technique to detect the possible expression of CD54 molecule on epithelial cells of conjunctiva in allergic patients and healthy controls, both in basal conditions and after challenge with *P. judaica* extract.

Table I reports clinical features of subjects included in the study. At baseline healthy subjects and patients with allergy caused by *P. judaica* did not evidence any type of inflammatory cells in conjunctival scrapings. The SCPT protocol we used proved to be effective and specific in eliciting a local response. On challenge, all allergic subjects had an immediate hypersensitivity reaction (allergen concentration 600 up to 2000 AUR/ml). The clinical reaction (hyperemia, lacrimation, itching, and burning) appeared approximately 5 minutes after allergen instillation, peaked at 30 minutes, and partially subsided within 1 hour (score from 9.6 to 12) (Fig. 1).

The scrapings from all allergic patients contained a significantly greater number of inflammatory cells, 72% neutrophils, 16% eosinophils, 12% lymphocytes, and 5% monocytes (score from 27 to 33) compared with baseline (Figs. 2 and 3).

On the other hand, healthy subjects did not evidence

any clinical and cellular reaction after allergen challenge. The contralateral eye did not show any clinical or cellular change in all challenged subjects after stimulation with control solution.

As far as the epithelium is concerned, a relevant number of epithelial cells were obtained by conjunctival scrapings (up to 15 to 30 cells in each microscopic field at  $\times 250$ ); all exhibited positive staining with anticytokeratin MAb. The median values of intercellular adhesion molecule (ICAM)-1 expression score are reported in Fig. 4. At baseline healthy subjects and patients with allergy caused by *P. judaica* did not show any CD54 expression on epithelium (Fig. 5).

After specific challenge, all allergic patients showed a remarkable CD54 expression ( $p < 0.001$ ) compared baseline (Fig. 6); conversely, no CD54 expression was elicited in healthy subjects.

The kinetics of appearance of CD54 expression is represented in Fig. 7. As clearly shown, relevant epithelial CD54 staining is apparent as early as 30 minutes after specific stimulus in allergic subjects.

The morphologic pattern of CD54 expression on conjunctival epithelium was quite similar to cytokeratin (Fig. 6); both cell membrane and cytoplasm were stained with a granular pattern, and similar patterns were observed on dispersed as well as clustered cells (Fig. 8). The intensity of staining was even higher than cytokeratin in many CD54 positive scrapings. In contrast, epithelial cells were not stained by isotype control OKT3 MAb.

### DISCUSSION

Several authors, including ourselves, have extensively described the relevant changes occurring after conjunctival challenge.<sup>4, 8, 13-16</sup> In particular, this model

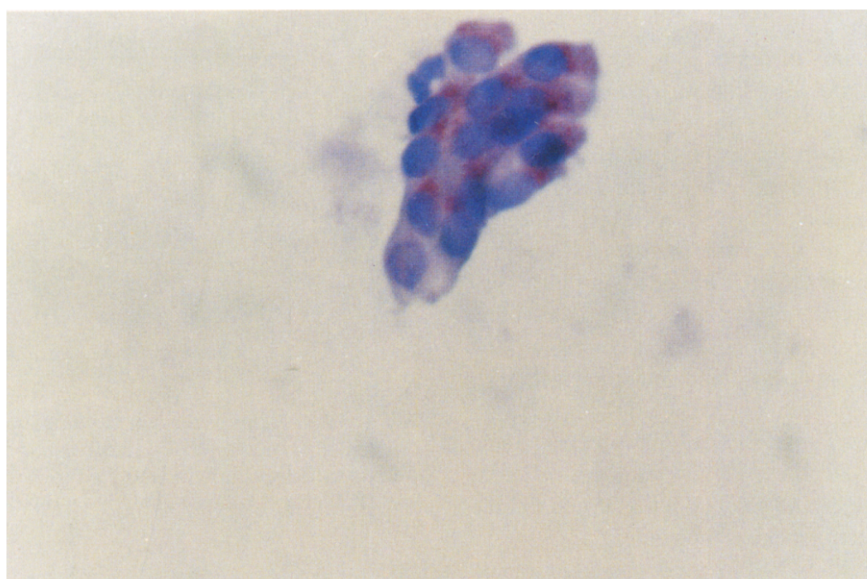


FIG. 3. Granulocytic infiltrate after specific CPT in a *P. judaica*-sensitive individual ( $\times 500$ ).

has enabled the study of the relationship between the clinical response and the infiltration of inflammatory cells.<sup>7, 11, 14, 15</sup>

As demonstrated in the asthma model, acute inflammation is a short-term process characterized by edema and migration of granulocytes (neutrophils and eosinophils) through the microvasculature to the site of allergic reaction.<sup>26</sup> In contrast, chronic inflammation has a longer time span, and granulocytic migration, mainly eosinophils, is accompanied by lymphocyte and monocyte and macrophage infiltration.<sup>6, 9, 29-31</sup>

Several factors contribute to the process, such as the IgE-dependent mediators released by mast cells,<sup>5, 8, 17</sup> the release of soluble cytokines,<sup>19</sup> and the interaction between adhesion molecules.<sup>20</sup>

In the present study we focused our attention on one of these molecules, CD54, and provided the first evidence of its expression on conjunctival epithelium in humans. CD54 is a surface molecule with immunoglobulin-like structure involved in intercellular adhesion via interaction with the  $\beta_2$  integrin LFA1.<sup>32-35</sup> It is deeply involved in the immune reactions requiring cell-to-cell contact,<sup>35-38</sup> as in leukocyte and endothelial cell interactions and in intrathymic maturation of T-cell precursors being expressed at high level on thymic epithelial cells.<sup>21, 35-38</sup>

As recently shown, the physiologic distribution of CD54 includes fibroblasts, endothelial cells, thymic epithelium, and astrocytes; it is weakly expressed on resting T cells, B cells, and monocytes, but increases

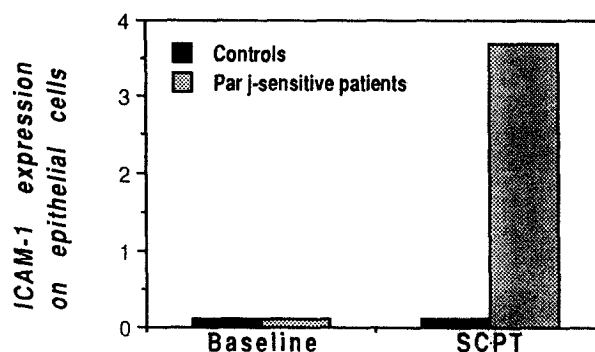


FIG. 4. ICAM-1 expression on conjunctival epithelial cells expressed as median value (score range from 3 to 4 in *P. judaica*-sensitive individuals) in the two study groups ( $p < 0.001$  for *P. judaica*-sensitive individuals baseline vs SCPT).

on activation.<sup>36-40</sup> Its expression is upregulated by interferon-gamma, tumor necrosis factor-alpha, and in some cases interleukin-1 alpha and beta,<sup>22, 23, 41</sup> all cytokines involved in the inflammatory process. During inflammation CD54 expression is rapidly induced. It is increased on hepatocytes in several forms of hepatitis, on keratinocytes in various dermatoses, on renal tubular epithelium during renal allograft rejection, on cerebral vascular endothelium in viral encephalitis and multiple sclerosis plaques, and on thyroid epithelial cells in autoimmune thyroiditis.<sup>42-47</sup>

Recently Leung et al.<sup>21</sup> demonstrated the expression of ELAM-1, another adhesion molecule on endothelial cells of the skin, when triggered with allergen

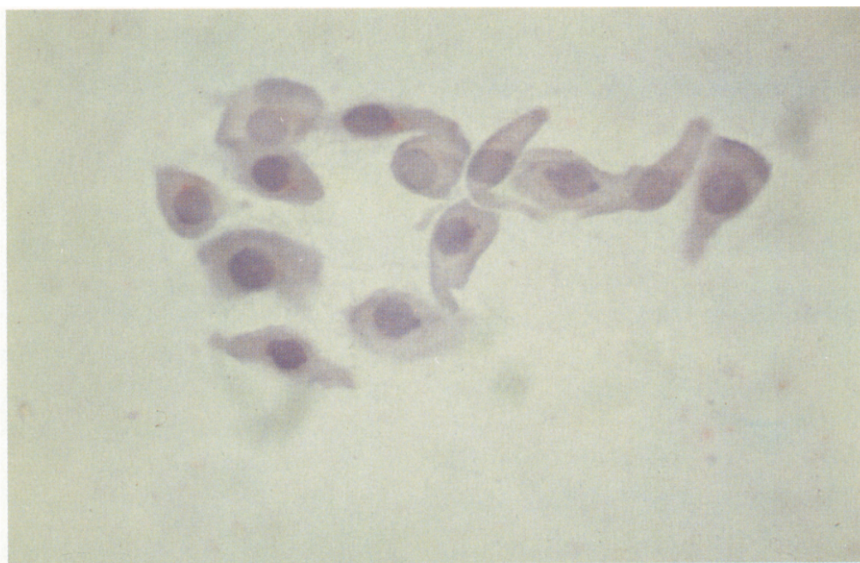


**FIG. 5.** Conjunctival epithelial cells in basal conditions in an allergic individual ( $\times 500$ )

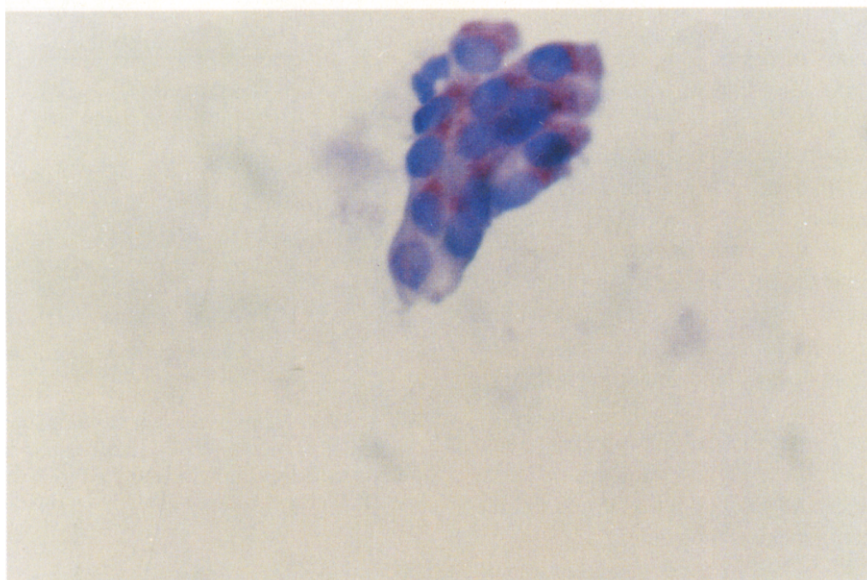


**FIG. 6.** Positivity for CD54 of conjunctival epithelium after allergen challenge in an allergic individual ( $\times 500$ ).

(ragweed or dust mites) in individuals with respiratory allergy. This finding further emphasized the role of adhesion molecules in the locomotion of inflammatory cells into the site of allergic reaction. The expression of CD54 on epithelial cells may play a role in allergic inflammation as well, by promoting the interactions between inflammatory cells and the target organ.<sup>36, 38</sup>

In the present study both healthy individuals and subjects sensitive to *P. judaica* do not constitutively express CD54 on conjunctival epithelium. Of note,

the study was performed in the off-pollen season, so that all allergic subjects were not exposed to the allergen, and neither clinical signs and symptoms nor cellular infiltration in conjunctival scrapings were apparent. After allergen challenge of allergic subjects, CD54 expression on conjunctival epithelium occurs as an early event, appearing within 30 minutes after specific stimulation and simultaneously with the inflammatory infiltrate (72% neutrophils, 16% eosinophils, 12% lymphocytes, and 5% monocytes) presum-

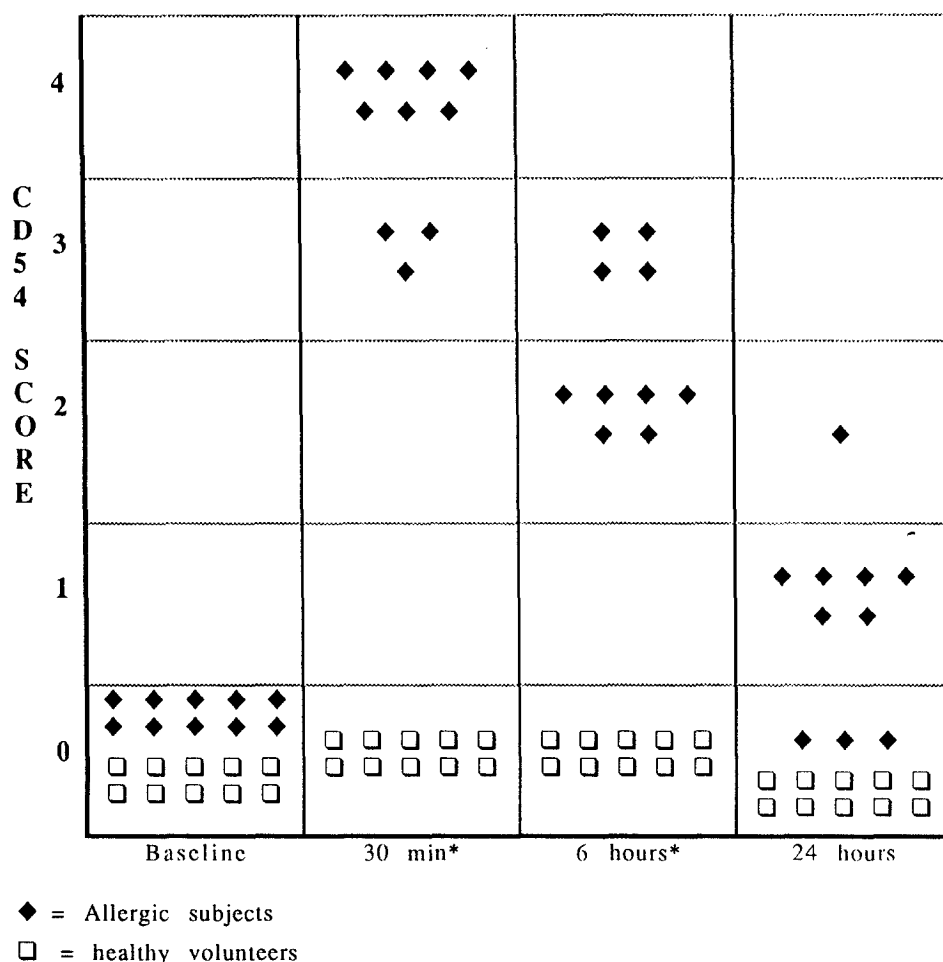


**FIG. 7.** CD54 expression on conjunctival epithelial cells after allergen challenge at different times. Results are expressed as positivity score (see text). \* $p < 0.001$  versus baseline for allergic subjects. No significant difference detected between 30 minutes and 6 hours of positivity.

ably because of local release of inflammatory mediators and/or cytokines from intraepithelial T lymphocytes and mast cells.

It is of note that the appearance of CD54 is very rapid (Fig. 7) compared with that on lymphocytes after mitogen stimulation.<sup>33, 38</sup> Nevertheless, we obtained experimental evidence that an identical phenomenon occurs on nasal epithelial cells after allergen-specific stimulation with the same kinetics (manuscript in preparation). A possible concern may arise about the appearance of CD54 on conjunctival epithelium as a result of endogenous synthesis or to soluble CD54 binding. However, we recently provided evidences for CD54 expression on the continuously cultured conjunctival cell line (W-K) in the absence of any additional cell in culture.<sup>48</sup> Moreover, Campbell et al.<sup>49</sup> have recently shown that bronchial epithelial cells in culture can express CD54, particularly after histamine addition. Altogether, these data indi-

cate the endogenous origin of CD54 in conjunctival epithelial cells.

The expression of CD54 after allergen exposure in sensitized subjects confirms recent report of the contribution of CD54 in the allergic reaction in a primate model of asthma where a MAb to CD54 was able to attenuate both eosinophil migration and airway responsiveness.<sup>20</sup>

These findings indicated that CD54 may play a central role in the allergic inflammation and suggest that maneuvers designed to decrease adhesion of inflammatory cells expressing LFA-1, such as lymphocytes, eosinophils, and the like, may be a useful therapeutic approach. Along these lines, Barnes<sup>50</sup> suggested cell adhesion blocker compounds as a new class of effective therapeutic agents in asthma. In vitro data have recently shown that deflazacort, a new corticosteroid compound, and cetirizine, a new third-generation antihistamine compound, exert a strong inhibitory effect

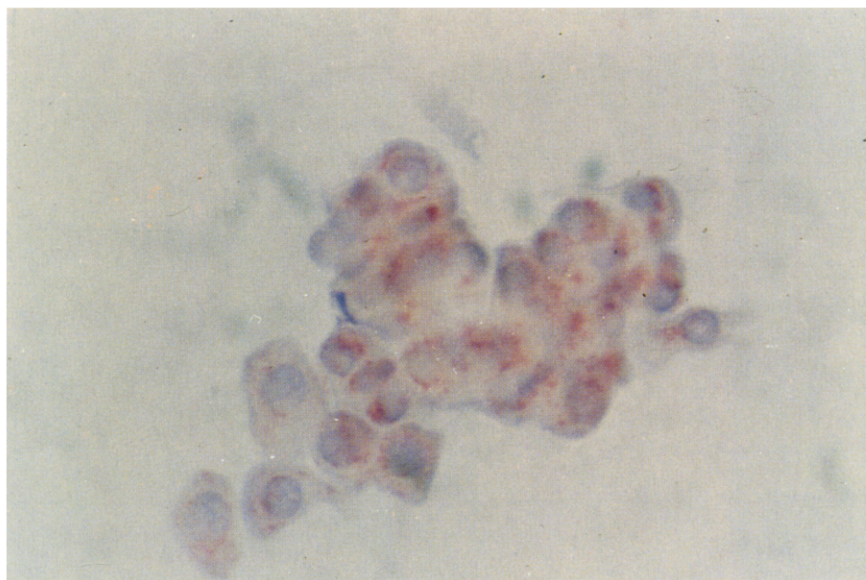


**FIG. 8.** Positivity for MAbαCK-19 of conjunctival epithelial cells: both cell membrane and cytoplasm appear stained with granular pattern ( $\times 500$ ).

on CD54 expression on epithelial cell lines in culture.<sup>48</sup>

The CD54 expression on epithelial cells as a result of allergen challenge 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 not to a direct effect of the allergen on epithelial cells. Moreover, since the damage of epithelium proved to be a relevant event in bronchial asthma, it is likely that CD54 surface expression makes the epithelial cells susceptible to the damage by offending cells such as eosinophils and other cytotoxic cells.

It is interesting to note that no expression was detected after allergen challenge in healthy subjects; this phenomenon underlines the specificity of the allergenic stimulus in eliciting CD54 expression on conjunctival epithelium. Also no CD54 expression was detected in the contralateral eye in all subjects after challenge. Previous reports showed an increase of neutrophils in scrapings taken from control subjects<sup>16</sup>; according to such authors, the recruitment of these cells might be related to the trauma of repeated scrapings. The present investigation shows that the trauma of repeated scrapings is not a sufficient stimulus for cellular recruitment and CD54 expression and, presumably, not able to induce an inflammatory response.

Our data fit very well with previous reports showing that CD54 expression may be considered a sensitive marker of inflammatory changes, because its presence strictly correlates with the site and the severity of the inflammatory process.<sup>26, 42-47</sup>

In conclusion, the conjunctival challenge test with allergen may provide a useful model for investigating further the role of CD54/LFA1 system in allergic inflammatory process. The test procedure has been demonstrated to be safe and always well tolerated by the patients, and can be easily repeated. Moreover, the immunocytochemical procedure, although semiquantitative, provides clear-cut and reproducible experimental data.

Further studies with use of the eye as an experimental model are required to define the following:

- (1) The kinetics of CD54 expression on conjunctival epithelium in the development of the ocular reactions after allergen challenge
- (2) The possible CD54 expression on conjunctival epithelium in allergic individuals during natural allergen exposure, that is, throughout the year for mite-sensitive subjects or during pollen season for pollen-sensitive subjects. In addition, it will be of interest to evaluate whether this parameter may be affected by topical or systemic treatment with steroid or antiallergic drugs.

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## Interleukin-2 and lymphocyte-induced eosinophil proliferation and survival in asthmatic patients

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**Background:** Eosinophils are increased in the airways and blood of asthmatic patients. However, the mechanism of regulation of eosinophilia is incompletely understood.

**Methods:** To study the potential effect of asthmatic lymphocytes on eosinophils, lymphocytes from the blood of asthmatic patients in exacerbation, or from healthy subjects, were isolated and cultured in medium alone (LC-CM) or with interleukin-2 (IL-2-CM) (125 U/ml), and the effect of supernatant obtained from these cultures on eosinophil proliferation from progenitors and survival was studied.

**Results:** IL-2-CM from asthmatic patients significantly increased eosinophil colony formation from asthmatic blood but had no effect on colony formation from the blood of healthy subjects. IL-2-CM from asthmatic patients also significantly prolonged the survival of eosinophils. IL-2 alone and IL-2-CM from healthy subjects had no effect on eosinophil proliferation and survival. Asthmatic lymphocytes had more IL-2 receptors (CD25) than normal lymphocytes, and this difference persisted even after culture in IL-2. However, upregulation of the CD25 receptor on normal lymphocytes by incubation with concanavalin A led to the production of IL-2-CM, which did not increase eosinophil proliferation from progenitors.

**Conclusions:** Lymphocytes from asthmatic patients but not from normal blood can significantly increase eosinophil proliferation and survival. The effects on eosinophil proliferation do not seem to be directly related to the presence of increased CD25 expression on lymphocytes. (*J ALLERGY CLIN IMMUNOL* 1993;91:792-801.)

**Key words:** IL-2, lymphocyte conditioned medium, eosinophil, asthma

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Asthma is a chronic disease characterized by reversible airway obstruction and increased airway responsiveness. The histologic finding of airway inflammation in association with the late airway response of asthmatic patients to inhalational challenge with allergen, and the link between late responses and airway hyperresponsiveness, have led to the hypothesis that inflammation is responsible for hyperresponsiveness.<sup>1,2</sup> Among the inflammatory cells, eosinophils seem to have an important role in asthma.<sup>3,4</sup> These cells are present in increased numbers in the blood, lung lavage fluid, and bronchial biopsies of asthmatic patients.<sup>5-8</sup> Eosinophils are activated in asthmatic patients and also can release enzymes and membrane-