Clinical Role of Soluble Adhesion Molecules During Immunotherapy for Perennial Allergic Rhinitis

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Background: Soluble forms of intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) have recently been identified in serum samples from atopic patients, but their clinical significance in the treatment of allergic diseases remains to be established.

Objective: To study the clinical roles of serum sICAM-1 and sVCAM-1 during immunotherapy for perennial allergic rhinitis.

Design: Our study included 30 nonatopic volunteers and 60 patients with perennial allergic rhinitis due to Dermatophagoides farinae. The 60 patients had been treated for variable periods (7.3±3.0 years [mean±SD]) with immunotherapy using a standardized D farinae antigen. Serum samples were collected from each patient before and after immunotherapy to determine sICAM-1 and sVCAM-1 with sandwich enzyme-linked immunosorbent assays.

Results: Serum levels of sICAM-1 in the patients before immunotherapy were higher than those in the nonatopic volunteers (P<.001). The levels of sICAM-1 in the patients’ serum samples were decreased significantly after immunotherapy (P<.001), and the percentage of the decrease in the sICAM-1 levels was significantly correlated with the duration of immunotherapy (P=.04) and with the percentage of the decrease in symptom scores (P<.001). The levels of sVCAM-1 in the serum samples from the patients with severe symptoms were significantly higher before immunotherapy than those in the nonatopic volunteers (P=.002) and were significantly decreased after immunotherapy (P=.05). However, the percentage of the decrease in the sVCAM-1 levels was not correlated with the duration of immunotherapy (P=.89) or with the percentage of the decrease in symptom scores (P=.89).

Conclusion: Decrease in serum sICAM-1 levels during immunotherapy is probably involved in the working mechanisms of immunotherapy, but modulation of serum sVCAM-1 levels is not likely related to the clinical effect of immunotherapy.


IT IS BECOMING increasingly apparent that the pathogenesis of respiratory allergic diseases, including asthma and allergic rhinitis, is closely linked to the presence of chronic inflammation. Leukocyte-endothelial cell interaction is an early step in the cascade of events leading to the development of airway allergic inflammation. Important adhesion molecules expressed on endothelial cells include intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin. Montefort et al compared the expression of endothelial cell adhesion molecules in nasal biopsy specimens from subjects with perennial allergic rhinitis and from normal controls. They found enhanced expression of ICAM-1 and VCAM-1, but not E-selectin, in the mucosa of subjects with allergic rhinitis. In a primate model of asthma, pretreatment with monoclonal antibody to ICAM-1 decreased eosinophil infiltration, a finding that suggests a potentially important part for ICAM-1 in allergic inflammatory process.

In vitro studies have shown that eosinophils use ICAM-1-, VCAM-1-, and E-selectin–dependent pathways to adhere to vascular endothelial cells. By contrast, neutrophils cannot use VCAM-1–dependent pathways, because of the lack of VLA-4 expression on their cell surface. In persons with asthma, the endothelial expression of VCAM-1 was positively correlated with the number of eosinophils, but not neutrophils, in the bronchial submucosa. Nakajima et al found that in vivo blocking of VCAM-1 and VLA-4, but not of ICAM-1 or LFA-1, prevented antigen-induced eosinophil infiltration into the mouse trachea and that VCAM-1–VLA-4 interaction is functionally predominant over ICAM-1–LFA-1 interaction in controlling antigen-induced T-cell recruitment into the tissue. These findings suggest that adhesion molecules such as ICAM-1 and VCAM-1 play a central role in the allergic inflammatory event and, especially, that the induction of VCAM-1 expression on the endothelium may play a key part in the selective recruit-

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PATIENTS AND METHODS

PATIENTS

The study design followed the principles outlined in the Declaration of Helsinki. The study included 60 patients (38 women, 22 men; age range, 19-55 years) with perennial allergic rhinitis due to Dermatophagoides farinae who gave informed consent for participation. All patients in the rhinitis group were selected from our outpatients who satisfied all of the following conditions: (1) a well-documented history of perennial allergic rhinitis and no seasonal aggravation during pollen season; (2) no history of asthma; (3) a positive skin test result and a positive reaction to nasal provocation with D farinae antigen before immunotherapy; (4) eosinophilia in nasal smears before immunotherapy; (5) undertreatment with immunotherapy using a standardized D farinae antigen (Holister Steir, Miles Inc, Spokane, Wash) for more than 3 years; and (6) the serum sample was previously collected before the initiation of immunotherapy and stored at −60°C.

The nasal symptoms and serum concentrations of sICAM-1 were determined twice in each case, once before and once sometime during the course of immunotherapy. To avoid seasonal fluctuations of house dust mite levels, the assessments of nasal symptoms and serum concentrations of sICAM-1 were performed at the beginning of the same month before and after immunotherapy. The intervals between the examinations were 7.3±3.0 years (mean±SD) (range, 3-14 years). The nasal symptoms were evaluated according to our criteria using daily symptom diary cards. The details of the method used in this study have been described elsewhere. In brief, all the patients were asked to complete daily symptom diary cards in the 14 days before the assessment of nasal symptoms. The daily symptom card includes the number of sneezing attacks, the number of nose blows, and the degree of nasal obstruction. A careful survey of the diary card graded the 3 nasal symptoms (sneeze, rhinorrhea, and obstruction) on a scale of 0 to 3, depending on severity. The average daily scores of the sum total of the 3 nasal symptom scores were used as the symptom scores (maximum score, 9).

To serve as normal controls, 30 nonatopic healthy volunteers (19 women, 11 men; age range, 20-64 years) matched for age and sex who gave informed consent for participation were chosen on the basis of the following criteria: (1) no history of allergic disease; (2) no physical findings indicative of atopic disease; and (3) negative serum IgE antibodies specific to the major allergens in Japan, such as house dust mites, Japanese cedar pollen, ragweed, Japanese cypress pollen, and molds.

MODE OF IMMUNOTHERAPY

The patients in the rhinitis group were each given a subcutaneous injection of standardized D farinae antigen after what the immunotherapy entailed was explained to them. The details of the immunotherapy used in this study were described elsewhere. The 60 patients in the rhinitis group received scheduled maintenance-dose subcutaneous injections at the follow-up examinations. The maximum tolerated dose was 3000 allergenic units in 54 of the 60 patients. The patients agreed not to take any concomitant medication that might affect their nasal symptoms during the study period.

DETERMINATION OF SERUM CONCENTRATION OF sICAM-1 AND sVCAM-1

All serum samples were simultaneously used for the determination of sICAM-1 and sVCAM-1 concentrations. The concentrations of sICAM-1 in the serum samples were determined with a sandwich enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minn) using 2 antibodies to distinguish different epitopes of ICAM-1 molecules, according to the manufacturer’s instructions. The concentrations of sVCAM-1 in the serum were also determined with an enzyme-linked immunosorbent assay (R & D Systems). In the present study, all the assays were run in duplicate. The percentage of the decrease in symptom scores and the sICAM-1 and sVCAM-1 concentrations were calculated using the following formulas:

Percent Decrease in Symptom Scores = [(Symptom Scores Before Immunotherapy−Symptom Scores After Immunotherapy)/(Symptom Scores Before Immunotherapy)]×100

Percent Decrease in sICAM-1 = [(sICAM-1 Before Immunotherapy−sICAM-1 After Immunotherapy)/(sICAM-1 Before Immunotherapy)]×100

Percent Decrease in sVCAM-1 = [(sVCAM-1 Before Immunotherapy−sVCAM-1 After Immunotherapy)/(sVCAM-1 Before Immunotherapy)]×100

STATISTICAL ANALYSIS

For comparisons between different groups, the Mann-Whitney U test was used. For comparisons of paired values, the Wilcoxon signed-rank test was used. Differences were considered significant when P<.05. The Spearman correlation coefficient (r_s) was calculated to determine the degree of correlation between 2 different parameters, and significant correlation was accepted when P<.05 on Spearman rank correlation analysis.

ment of eosinophils and T cells into allergic inflammatory lesions.

Recently, soluble forms of ICAM-1 (sICAM-1) and VCAM-1 (sVCAM-1) have been identified in samples of peripheral blood and other body fluids from normal subjects and patients with various inflammatory diseases, although the cells of origin and the mechanisms for release of these adhesion molecules are not clear. The serum levels of these adhesion molecules were also determined in patients with allergic disorders and were reported to be increased in patients with some allergic conditions. Higher levels of these adhesion molecules in serum samples from atopic individuals may reflect the up-regulation of cell surface ICAM-1 and VCAM-1 expression in allergic inflammation.

Immunotherapy is an effective form of treatment for perennial allergic rhinitis. Therefore, immunotherapy might modulate activation of endothelial cells and possibly decrease serum concentrations of sICAM-1 and sVCAM-1. The aim of our study was to investigate levels...
of sICAM-1 and sVCAM-1 in serum samples from patients with perennial allergic rhinitis, with special reference to the possible changes during and after immunotherapy.

RESULTS

SYMPTOM SCORES

The levels of symptom scores were 7.3±1.1 (mean±SD) before immunotherapy and 2.0±1.9 after immunotherapy. Every patient in the rhinitis group demonstrated somewhat of a decrease in the symptom scores after immunotherapy, and the symptom scores after immunotherapy were significantly smaller than those before immunotherapy (P<.001). The percentage of the decrease in symptom scores was significantly correlated with the percentage of the decrease in sICAM-1 concentrations (rS =0.721, P <.001) (Figure 1). The serum concentrations of sICAM-1 were significantly higher in the rhinitis group than in the nonatopic group (before immunotherapy, P<.001). The serum levels of sICAM-1 in the rhinitis group were significantly lower (238.9±110.2 ng/mL) after immunotherapy than before immunotherapy (P<.001, Figure 1), and the percentage of the decrease in sICAM-1 concentrations was 25.8%±19.9% (range, −17.9% to 64.2%). However, after immunotherapy, the serum levels of sICAM-1 were still significantly higher in the rhinitis group than in the nonatopic group (P=.02, Figure 1). A weak but significant correlation was recognized between the percentage of the decrease in sICAM-1 concentrations and the duration (in years) of immunotherapy (r2=0.273, P=.04). The levels of sICAM-1 (218.8±96.0 ng/mL) in the serum samples from 46 patients treated with immunotherapy for 5 or more years were not significantly different from those in the nonatopic group (P=.11). The percentage of the decrease in sICAM-1 concentrations was strongly correlated with the percentage of the decrease in symptom scores (r2=0.721, P<.001) (Figure 2).

SERUM sICAM-1

The serum levels of sICAM-1 in the rhinitis group (321.5±108.6 ng/mL) before immunotherapy were significantly higher than those in the nonatopic group (181.9±57.1 ng/mL, P<.001) (Figure 1). The serum concentrations of sICAM-1 were lower after immunotherapy in 53 of the 60 patients. The serum levels of sICAM-1 were significantly lower (238.9±110.2 ng/mL) after immunotherapy than before immunotherapy (P<.001, Figure 1), and the percentage of the decrease in sICAM-1 concentrations was 25.8%±19.9% (range, −17.9% to 64.2%). However, after immunotherapy, the serum levels of sICAM-1 were still significantly higher in the rhinitis group than in the nonatopic group (P=.02, Figure 1). A weak but significant correlation was recognized between the percentage of the decrease in sICAM-1 concentrations and the duration (in years) of immunotherapy (r2=0.273, P=.04). The levels of sICAM-1 (218.8±96.0 ng/mL) in the serum samples from 46 patients treated with immunotherapy for 5 or more years were not significantly different from those in the nonatopic group (P=.11). The percentage of the decrease in sICAM-1 concentrations was strongly correlated with the percentage of the decrease in symptom scores (r2=0.721, P<.001) (Figure 2).

SERUM sVCAM-1

The serum level of sVCAM-1 in the nonatopic group was 501.4±57.7 ng/mL. The serum levels of sVCAM-1 in the rhinitis group were 335.7±96.5 ng/mL before immunotherapy and 503.4±104.1 ng/mL after immunotherapy. The serum levels of sVCAM-1 before as well as after immunotherapy in the rhinitis group were not significantly different from those in the nonatopic group (before immunotherapy, P=.06; after immunotherapy, P=.46) (Figure 3). Serum concentrations of sVCAM-1 decreased after immunotherapy in 32 patients, and those of sVCAM-1 increased after immunotherapy in the remaining 28 patients. The serum levels of sVCAM-1 in the rhinitis group did not differ significantly before and after immunotherapy (P=.05, Figure 3). The percentage of the decrease in sVCAM-1 concentrations (4.9%±17.6%; range, −26.6% to 53.5%) was not signifi-
as well as before or after, the pollen season. In addition, a significant increase in serum sICAM-1 levels was observed following bronchoprovocation. In our present study, serum sICAM-1 levels were higher in patients with perennial allergic rhinitis than in nonatopic normal controls. If these lines of evidence are taken together, an increase in serum sICAM-1 concentration is likely to reflect the ongoing inflammatory response in the inflamed site, and determination of sICAM-1 levels could be useful in the investigation and monitoring of disease activity and inflammatory reactions in allergic disorders.

It is of clinical interest whether serum levels of sICAM-1 could be modulated by treatment. However, this question remains to be answered. In 1 study, it was shown that after an acute asthmatic attack, serum levels of sICAM-1 remained elevated for at least 28 days, even with aggressive pharmacotherapy using bronchodilators and systemic corticosteroids. This suggests that serum sICAM-1 levels, at most, are under limited steroid control. Since immunotherapy is an active form of treatment, unlike conventional drug therapies, it might modulate immunologic activation and inflammatory events occurring in allergic diseases to decrease the serum levels of sICAM-1. Indeed, the seasonal increase in sICAM-1 levels in the serum samples from patients with seasonal allergic rhinitis was suppressed by immunotherapy.

Our goals, therefore, were to find out whether the serum sICAM-1 levels in perennial allergic rhinitis could be decreased by immunotherapy and whether this decrease in sICAM-1 could be correlated with the duration and clinical efficacy of immunotherapy. Although serum levels of sICAM-1 in children decrease with age, studies in adults suggest that serum levels of sICAM-1 are constant between the ages of 18 and 65 years. We thus observed possible changes in sICAM-1 levels in the serum samples from patients who were between the ages of 19 and 55 years. The serum levels of sICAM-1 in the rhinitis groups were significantly decreased after immunotherapy (P < .001). The serum levels of sICAM-1 after immunotherapy were still higher in the nonatopic subjects than in those in the nonatopic group (severe rhinitis group vs nonatopic group, P = .002; mild rhinitis group vs nonatopic group, P = .77; Figure 3). The serum levels of sVCAM-1 after immunotherapy were 489.9±107.7 ng/mL in the mild rhinitis group and 519.8±98.9 ng/mL in the severe rhinitis group, respectively. The serum levels of sVCAM-1 were significantly decreased after immunotherapy in the severe rhinitis group (P = .05) but not in the mild rhinitis group (P = .35, Figure 3). The sVCAM-1 levels after immunotherapy in the severe rhinitis group were not significantly different from those in the nonatopic group (P = .14, Figure 3). However, the percentage of the decrease in sVCAM-1 concentrations was not significantly correlated with the percentage of the decrease in the symptom scores in either group (mild rhinitis group, r = 0.024, P = .97; severe rhinitis group, r = 0.076, P = .76).

**COMMENT**

Serum levels of sICAM-1 in persons with asthma have previously been determined to be higher during acute exacerbations and even during periods of stability than those in normal control subjects. Similarly, serum levels of sICAM-1 in patients with seasonal allergic rhinitis were also higher than those in normal control subjects, during severe rhinitis group vs nonatopic group, P = .77; Figure 3). The serum sICAM-1 levels after immunotherapy were 507.0±96.6 ng/mL in the remaining 33 patients (mild rhinitis group) whose symptom scores before immunotherapy were 7 or less (P < .01, Figure 3). The serum sVCAM-1 levels before immunotherapy were 489.9±107.7 ng/mL in the mild rhinitis group and 570.7±85.6 ng/mL in the 27 patients (severe rhinitis group) whose symptom scores before immunotherapy were 8 or 9 were significantly higher than the sVCAM-1 levels (507.0±96.6 ng/mL) in the remaining 33 patients (mild rhinitis group) whose symptom scores were 7 or less (P = .033, P = .89).

However, the levels of sVCAM-1 before immunotherapy were significantly correlated with the levels of symptom scores (r = 0.479, P = .001) (Figure 4). The sVCAM-1 levels (570.7±85.6 ng/mL) in the 27 patients (severe rhinitis group) whose symptom scores before immunotherapy were 8 or 9 were significantly higher than the sVCAM-1 levels (507.0±96.6 ng/mL) in the remaining 33 patients (mild rhinitis group) whose symptom scores were 7 or less (P = .14, Figure 3). The serum levels of sVCAM-1 before immunotherapy were significantly higher in the severe rhinitis group but not in the mild rhinitis group than those in the nonatopic group (severe rhinitis group vs nonatopic group, P = .002; mild rhinitis group vs nonatopic group, P = .77; Figure 3). The serum levels of sVCAM-1 after immunotherapy were 489.9±107.7 ng/mL in the mild rhinitis group and 519.8±98.9 ng/mL in the severe rhinitis group, respectively. The serum levels of sVCAM-1 were significantly decreased after immunotherapy in the severe rhinitis group (P = .05) but not in the mild rhinitis group (P = .35, Figure 3). The sVCAM-1 levels after immunotherapy in the severe rhinitis group were not significantly different from those in the nonatopic group (P = .14, Figure 3). However, the percentage of the decrease in sVCAM-1 concentrations was not significantly correlated with the percentage of the decrease in the symptom scores in either group (mild rhinitis group, r = 0.024, P = .97; severe rhinitis group, r = 0.076, P = .76).
found a large increase in sVCAM-1 concentrations in bronchoalveolar lavage fluid samples from atopic persons with asthma after segmental allergen challenge, and the levels were correlated with eosinophil influx and the late phase response. Therefore, sVCAM-1 levels might be an objective parameter of ongoing allergic inflammation, not only in bronchoalveolar lavage fluid but also in serum. To the best of our knowledge, however, the information that has been published regarding the serum levels of sVCAM-1 in patients with allergic rhinitis is extremely limited. In our previous study, the serum levels of sVCAM-1 in patients with perennial allergic rhinitis were not different from those in nonatopic subjects, but sVCAM-1 levels were elevated only in patients with severe perennial allergic rhinitis. In our present study, serum levels of sVCAM-1 in the rhinitis group before immunotherapy were not significantly different from those in the nonatopic group (P = .06), but sVCAM-1 levels in the rhinitis group were significantly correlated with their symptom scores (P = .001). Also, the sVCAM-1 levels in the severe rhinitis group were significantly higher than those in the nonatopic group (P = .002). Measurement of serum concentrations of sVCAM-1 is therefore likely to be of limited diagnostic use, but it may be a useful tool for investigating the severity of allergic rhinitis and underlying inflammatory reactions.

It is also of clinical interest whether higher levels of sVCAM-1 in patients with severe nasal symptoms could be used for active or appropriate medications. We therefore studied possible changes in serum sVCAM-1 levels resulting from immunotherapy in patients with perennial allergic rhinitis, especially patients with severe nasal symptoms. In the present study, all patients were 19 years of age or older, because serum sVCAM-1 levels in children might decrease with age. A decrease in sVCAM-1 levels after immunotherapy was significant in the severe rhinitis group (P = .05) but not in the mild rhinitis group (P = .35). The sVCAM-1 levels in the severe rhinitis group after immunotherapy were not different from those in the nonatopic group (P = .14). However, the percentage of the decrease in sVCAM-1 levels was not correlated with the percentage of the decrease in symptom scores in either group (mild rhinitis group, P = .97; severe rhinitis group, P = .76). Therefore, immunotherapy decreased higher levels of serum sVCAM-1 in patients with severe perennial allergic rhinitis, but this decrease in sVCAM-1 levels was not likely to be related to the clinical efficacy of immunotherapy. Further studies will be necessary to establish the clinical or therapeutic roles of sVCAM-1 in the serum of patients with perennial allergic rhinitis.

In conclusion, immunotherapy can decrease elevated sICAM-1 levels in the serum of patients with perennial allergic rhinitis, and this suppressive effect becomes more apparent with more prolonged immunotherapy. The decrease in sICAM-1 levels is probably involved in the working mechanisms related to the clinical effect of immunotherapy. Therefore, it is likely that the serum sICAM-1 level in patients with perennial allergic rhinitis is a useful marker for monitoring the effect of immunotherapy. On the other hand, immunotherapy decreases elevated sVCAM-1 levels in patients with severe nasal symptoms, but this decrease may not be related to the clinical effect of immunotherapy because it was not correlated with the clinical effect of immunotherapy.

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