

Influence of hyposensitization on soluble interleukin-2 receptor, eosinophil cationic protein, *in vitro* lymphocyte proliferation, *in vitro* lymphocyte adhesion, and lymphocyte membrane markers in childhood asthma

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Soluble interleukin-2 receptor (sIL-2R), eosinophil cationic protein (ECP), the lymphoproliferative response to house-dust mite (HDM), adhesion to human umbilical vein endothelial cells (HUVEC), and lymphocyte membrane markers were studied in three groups of children: healthy children, asthmatic children without hyposensitization (HS), and asthmatic children with HS. HS was associated with significantly lower numbers of peripheral blood eosinophils (PBE) and lower sIL-2R serum levels and with a tendency to lower ECP serum levels and lymphoproliferative response to HDM. There were no changes in the T-lymphocyte phenotypic markers CD4 and CD8 among the three groups. The interleukin-2 receptor (IL-2R, CD25) on HDM-stimulated T lymphocytes increased over unstimulated T lymphocytes in the three groups. The CD25 expression was higher on HDM-stimulated lymphocytes in both asthmatic groups than in healthy children. Adhesion of lymphocytes on HUVEC increased significantly after HDM stimulation in asthmatic children without HS, whereas no change was observed in the two other groups. However, there was no change in the expression of adhesion molecules CD29 and CD11a on lymphocytes in either of the groups. This study provides further evidence that HS can modify lymphocyte and eosinophil functions.

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The beneficial effects of hyposensitization (HS) in asthma have been shown by several studies (reviewed in 2). Although the exact underlying mechanisms of HS are largely unknown, one constant observation, reported by several groups, is its inhibition of the late asthmatic reaction (LAR) (19, 31, 36). The LAR, being an inflammatory response to an inhaled allergen characterized by infiltration of various inflammatory cells in the bronchial wall, is considered to be an appropriate model of naturally occurring asthma (6, 35). The central role of T lymphocytes in regulating the cellular interactions during the LAR has been accepted (14, 34). Furthermore, various studies suggest that T lymphocytes act as target cells for HS (12, 24, 29). Therefore, it can

be assumed that the anti-inflammatory effects of HS may be due to a specific action of HS on T lymphocytes, resulting in decreased T-cell activation. An early event in the inflammatory process is the adhesion to and the migration through endothelial cells of inflammatory cells regulated by the expression of specific adhesion molecules (7, 17, 26). It can be hypothesized that HS might influence this process.

In the present study, soluble interleukin-2 receptor (sIL-2R) and eosinophil cationic protein (ECP) in serum and the lymphoproliferative response to house-dust mite (HDM) were measured. The adhesion of cultured lymphocytes, unstimulated and stimulated by HDM, to human umbilical vein endothelial cells (HUVEC), unstimulated and stimulated by

IL-1, was determined. The lymphocyte phenotypic markers (CD4 and CD8), the lymphocyte adhesion molecules (CD29 and CD11a), and a lymphocyte activation marker (CD25) were studied.

Material and methods

Subjects

The present study was conducted, with a parallel-group design, on three groups of 10 subjects: healthy children (median age and range: 13, 7–17 years), asthmatic children without HS (non-HS asthmatic; 10.5, 5–15 years), and asthmatic children with HS (HS asthmatic; 15, 10–17 years) for at least 8 months (8–60 months). The healthy children had no personal or family history of respiratory or atopic disease. Furthermore, they had a low total IgE and no specific IgE against HDM. The two groups of asthmatic children suffered from perennial asthma and were allergic only to HDM, as proven by both a positive skin test and positive specific IgE against HDM. All subjects were in stable clinical condition without symptoms for at least 4 weeks and had normal lung function tests. Before blood sampling, between 0830 and 0930, β_2 -agonists were stopped for 24 h, and inhaled corticosteroids and disodium cromoglycate for 48 h. Both groups of asthmatic children were comparable according to asthma severity and use of antiasthmatic drugs. Whereas controls did not differ in age from both patient groups, the difference in age between hyposensitized and non-hyposensitized children was significant. However, none of the variables studied were correlated with age. Therefore, we will not report on age differences in the study.

The selection of a child to receive HS was based on the willingness of the child and the parents to accept this treatment, and not on asthma severity. Before the start of this study HS was proposed to all subjects. The HS asthmatic children received monthly injections of 5000 allergy units HDM extract (Allerset, HAL, Haarlem, The Netherlands). HS was started with a semirush procedure, as previously described (27).

Lymphoproliferative response to HDM

Peripheral blood mononuclear cells (PBMC) were separated from 20 ml heparinized peripheral blood by Ficoll-Paque (Pharmacia LKB Biotechnology, Brussels, Belgium) density gradient centrifugation (density 1.077 g/ml). An amount of 10^5 PBMC in 150 μ l serum-free AIM-V medium (Gibco Life Technologies, Ghent, Belgium) was incubated with 10 μ g/ml HDM extract (*Dermatophagoides pteronyssinus*, HAL) or with medium as control in 96-well, round-bottomed microtiter plates (Falcon Becton

Dickinson Benelux, Erembodegem, Belgium) (3). The optimal concentration and incubation period were determined by preliminary experiments. The plates were incubated at 37°C in a humidified 5% CO₂ incubator (Jouan, Saint Herblain, France). After 6 days of culture, 20 μ l of methyl-³H-labeled thymidine (20 μ Ci/well; specific activity 25 Ci/mmol, Amersham Life Sciences, Amersham, UK) was added for 24 h. The cells were harvested with an automatic cell harvester (Automash 2000, Dynatech Laboratories, Chantilly, VA, USA) on fiberglass filter paper (MN GF-6, Machery-Nagel, Düren, Germany). The incorporated radioactivity was measured with a liquid scintillation counter (Packard Tricarb, Packard Instruments, Meriden, CT, USA). Results were expressed as a stimulation index (SI), i.e., the number of disintegrations per minute of the lymphocytes stimulated with HDM divided by the number of disintegrations per minute of the lymphocytes cultured with only medium. An SI of greater than 2 was considered to be a positive proliferative response to HDM.

Culture of lymphocytes for adhesion experiments on HUVEC

For the adhesion experiments, PBMC were separated as described above. Lymphocytes (10^6 /ml) were incubated in 24-well culture plates (Costar, Cambridge, MA, USA) with 10 μ g/ml HDM or with medium. After 3 days, the cultured cells were collected for adhesion experiments to HUVEC and for determination of lymphocyte subtypes, adhesion molecules, and activation markers. Before adhesion to HUVEC, the cultured lymphocytes were stained with 5-(and 6-)carboxyfluorescein diacetate (CFDA, 0.3 mg/ml) (Sigma, St Louis, MO, USA). After 15-min incubation at 37°C, cells were washed twice in CO₂-independent medium with 1% bovine calf serum (Gibco Life Technologies, Ghent, Belgium), penicillin (100 U/ml), and streptomycin (100 μ g/ml). The labeled cells were concentrated at 10^6 cells/ml and used for adhesion to HUVEC.

Adhesion assay

Culture of HUVEC. HUVEC were isolated from the veins of human umbilical cords by collagenase-dispase (Boehringer-Mannheim, Brussels, Belgium) digestion, by the method of Jaffe et al. (13) with minor modifications. The HUVEC were cultured in M199 medium (Gibco Life Technologies) containing 20% fetal calf serum (Gibco Life Technologies), penicillin (100 U/ml) and streptomycin (100 μ g/ml), endothelial cell growth supplement (30 μ g/ml) (Sigma), and sodium heparin (10 U/ml) (Sigma). HUVEC were seeded at a density of 3000 cells/cm²

in culture flasks (Falcon) pretreated for 30 min with gelatin 2% (Sigma) in water, and cultured in a humidified 5% CO₂ incubator at 37°C for 2–5 days until confluence was observed. The confluent monolayered cells were detached with 0.125% trypsin-EDTA in modified Puck's saline A (Gibco Life Technologies). The cells were identified as HUVEC by staining for factor VIII-related antigen.

Adhesion of lymphocytes to HUVEC. From the second to fourth passage, HUVEC were transferred on gelatin-coated, 96-well, flat-bottomed microtiter plates (Falcon), at a concentration of 35 000 HUVEC per well. After overnight culture, a confluent monolayer was obtained and used in the adhesion experiments. HUVEC were stimulated for 6 h at 37°C in 5% CO₂ with 100 µl IL-1 (Janssen Biochimica, Beerse, Belgium), 20 U/ml in the CO₂-independent medium, or with 100 µl CO₂-independent medium as control. Afterwards, HUVEC were washed twice with the CO₂-independent medium. IL-1-stimulated and unstimulated HUVEC were incubated with 100 000 CFDA-labeled cells in 200 µl per well at 37°C in 5% CO₂ for 30 min. After incubation, the total fluorescence (F_{tot}) was measured with a Cytofluor 2300 fluorometer (Millipore). The nonadherent cells were removed by washing four times with the prewarmed CO₂-independent medium. The remaining fluorescence (F_{adh}) was measured, and adhesion was expressed as the percentage of the total fluorescence:

$$\% \text{ adhesion} = (F_{\text{adh}} \times 100) / F_{\text{tot}}$$

Flowcytometric analysis

The following monoclonal antibodies were used: CD3, CD4, CD8, and CD25 from DAKO (Glostrup, Denmark); CD29 and CD11a from IMMUNOTECH (Marseille, France). Double-labeling immunofluorescence was performed on the cultured lymphocytes with fluorescein isothiocyanate and phycoerythrin. After incubation of lymphocytes with HDM or medium as control for 3 days, the following subsets were determined: CD4 + CD29 + and CD8 + CD29 + (T cells with expression of the β_1 -integrin VLA/CD29); CD4 + CD11a + and CD8 + CD11a + (T cells with expression of the β_2 -integrin CD11a/CD18 or LFA-1); and the CD3 + cells with expression of the IL-2R or CD25 (CD3 + CD25 +). Flowcytometric analysis was done with a Fac-scan (Becton Dickinson) standardized with fluorescent beads (Fluoresbrite intensity kit, Polysciences, Warrington, PA, USA) (25). Fluorescence of 5000 cells was evaluated, and the percentage of positive cells was determined. The mean density was determined with the mean fluorescence intensity (FI) for each marker expressed as the mean

channel number (25). If more subpopulations were recognized, the percentage of positive cells of each subpopulation was determined, and the mean density was calculated as the mean FI of the combined subpopulations (15).

Determination of activation markers in serum

ECP in serum was determined by a double antibody radioimmunoassay kindly provided by Pharmacia, Brussels, Belgium (32). Serum was centrifuged at 1350 g for 10 min after coagulation of the whole blood for 1 h at room temperature. Serum was frozen and stored at –20°C until analysis. The concentration of serum ECP was expressed as microgram per liter (µg/l). The range of the standard curve was 2–200 µg/l.

sIL-2R was determined with a sandwich enzyme-linked immunosorbent assay (ELISA) with two monoclonal antibodies recognizing two different epitopes of the IL-2R, kindly provided by Eurogenetics, Tessenderloo, Belgium. The concentration of sIL-2R was expressed in units per milliliter (U/ml). The intra-assay variation was <8% at 50 U/ml and <7% at 500 U/ml. The minimum detectable concentration of sIL-2R was 12.5 U/ml.

Statistical analysis

The Mann-Whitney U-test, the Kruskal-Wallis analysis, the Wilcoxon signed-rank test, and the Spearman rank test were used where appropriate. A *P* value of less than 0.05 was considered to be significant. All results were expressed as median and range. Ages were compared by the unpaired Student's *t*-test.

Results

Lymphoproliferative response to HDM

The median SI was significantly higher in both the non-HS asthmatic group (6.7, 2.2–23.7) and the HS asthmatic group (3.5, 2.5–11.1) than in healthy children (1.5, 0.8–2.7) (*P* = 0.0002 for both groups). Patients with HS had a lower median SI than non-HS patients although this difference was not statistically significant (*P* = 0.23).

Peripheral blood eosinophils (PBE) and serum levels of ECP and sIL-2R (Table 1)

The number of PBE was significantly higher in non-HS asthmatic children than healthy children and HS asthmatic children. The number of PBE was not different between healthy and HS asthmatic children. Serum ECP levels were higher in non-HS asthmatic than in healthy children (*P* = 0.003). ECP

Table 1. Number of peripheral blood eosinophils (PBE) and serum values of ECP and sIL-2R in the three groups (median and range)

	Healthy children	non-HS asthmatic group	HS asthmatic group
PBE ($10^9/l$)	0.20 (0.07–0.30)	0.66 (0.25–10.8)**/ ^o	0.29 (0.05–1.20)
ECP ($\mu g/l$)	30.9 (7.2–66.1)	67.9 (26.3–134.9)*	43.4 (7.2–128.8)
sIL-2R (U/ml)	93 (40–168)	146.5 (70–159) ^{oo}	65.5 (47–140)

* $P < 0.005$ and ** $P < 0.0005$ between non-HS asthmatic group and controls.

^o $P < 0.05$ and ^{oo} $P < 0.005$ between non-HS asthmatic and HS asthmatic group.

was also higher in non-HS asthmatic than in HS asthmatic children, but the difference was not statistically significant ($P = 0.23$).

The sIL-2R level was higher in non-HS asthmatic than HS asthmatic patients ($P = 0.002$), and it was also higher in non-HS asthmatic than in healthy children ($P = 0.05$).

There was no significant correlation between the serum ECP and the serum sIL-2R levels for all subjects ($r = 0.25$; $P = 0.06$).

Adhesion of unstimulated and HDM-stimulated lymphocytes to HUVEC (Fig. 1)

The lymphocyte adhesion on unstimulated and IL-1-stimulated HUVEC was similar for the three groups for control and HDM-stimulated lymphocytes. In the group of non-HS asthmatic children, there was a small but consistent increase of adhesion to IL-1-stimulated HUVEC of HDM-incubated lymphocytes over lymphocytes incubated with medium alone (18.0, 5.1–26.2% and 14.3, 4.9–23.8%, respectively; $P = 0.02$). This was not found for the healthy children (13.0, 3.8–25.9% and 8.9, 5.7–29.7%, respectively; $P = 0.5$) nor for the HS asthmatic children (15.5, 4–34% and 9.3, 5.5–22.0%, respectively; $P = 0.3$).

Lymphocyte subtypes, adhesion molecules, and activation markers

The total CD4+ and CD8+ populations of the cultured lymphocytes did not differ among the three study groups. There was also no change after stimulation with HDM.

There was no change in percentage or in mean density of the LFA-1 (CD11a) on the CD4+ or on the CD8+ cells before and after HDM stimulation in the three groups. Two populations of CD29 on CD4+ and CD8+ cells, low and high density, were recognized with equal percentages in the three groups before and after HDM stimulation. Moreover, the mean density of the VLA (CD29) on the CD4+ and the CD8+ lymphocytes did not change before and after stimulation with HDM in the three groups. There was also no change in the percentage of CD25

% adhesion to IL-1 stimulated HUVEC

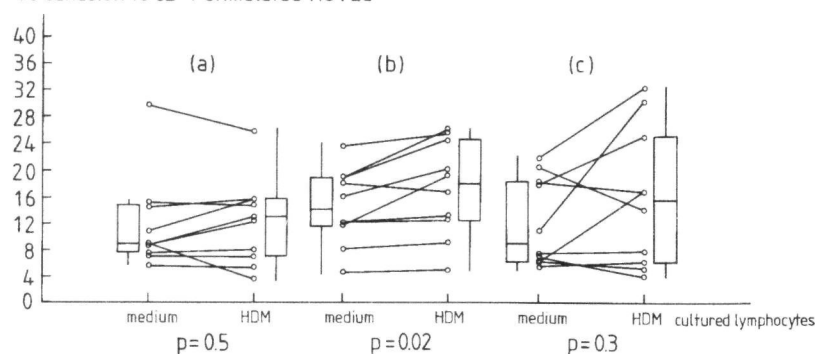


Fig. 1. Percent adhesion to IL-1-stimulated HUVEC of lymphocytes incubated with medium or HDM for 3 days: (a) healthy children; (b) non-HS asthmatic children; (c) HS asthmatic children. Individual values and box plots are shown.

density of CD25 on CD3+ lymphocytes

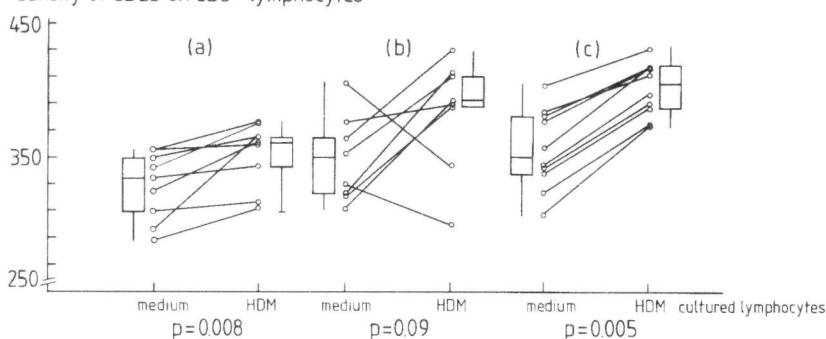


Fig. 2. Density of CD25 on CD3+ cells expressed as mean channel number, after incubation for 3 days with medium or HDM: (a) healthy children; (b) non-HS asthmatic children; (c) HS asthmatic children. Individual values and box plots are shown.

on the CD3+ lymphocytes before and after HDM stimulation in the three groups (data not shown).

The mean density of CD25 on unstimulated CD3+ lymphocytes did not differ in the three groups, but it became significantly higher on HDM-stimulated lymphocytes in both asthmatic groups than in the healthy children ($P = 0.02$ for non-HS asthmatic and $P = 0.0005$ for HS asthmatic children) (Fig. 2).

A correlation was found between the increase in expression of CD25 after HDM stimulation and the lymphoproliferative response to HDM for all children ($r = 0.52$; $P = 0.004$). The expression of CD25 on the cell surface increased significantly on HDM-stimulated CD3+ lymphocytes over the control culture in the healthy and the HS asthmatic children ($P = 0.008$ and $P = 0.005$, respectively), whereas there was no significant increase in the hyposensitized patients.

There was also an increase of the CD25 expression in the non-HS asthmatic group, but the increase was not statistically significant ($P = 0.09$) (Fig. 2).

Discussion

In this parallel-group study, differences of lymphocyte and eosinophil activity were found among

healthy children, non-HS asthmatic children, and HS asthmatic children. Since both groups of asthmatic subjects were comparable for asthma severity, sensitivity to HDM, and antiasthmatic therapy, the observed differences between the two patient groups were probably due to the HS treatment.

Asthmatic children with HS had lower levels of PBE and tended to have lower serum levels of ECP than non-HS asthmatic children. In the HS asthmatic children, both variables were indistinguishable from data found in healthy children, suggesting inactivation of eosinophils by HS. These results agree with studies showing decreased eosinophil activity in patients treated with HS (9, 21, 22). Furthermore, one study showed that serum ECP did not increase after natural allergen exposure in patients treated with HS, unlike patients who did not receive HS (20). Increased ECP serum levels as well as high numbers of PBE might reflect disease activity (8, 28, 33). However, some studies have shown that the ECP serum levels seem to be more related to disease activity than the number of eosinophils (1,10).

Conflicting results have been published on the effects of HS upon the allergen-induced lymphocyte proliferation (4, 5, 11). Asthmatic children with HS had significantly lower levels of sIL-2R than non-HS asthmatic children, suggesting lymphocyte inactivation by HS (12, 30). Another interesting observation was that non-HS asthmatic children tended to have higher levels of sIL-2R than normal children, suggesting ongoing subclinical inflammation.

The percentage of the CD25 + lymphocytes in the HDM-stimulated cultures did not differ from the control culture, as was also found by Kus (16). However, in the present study, the mean density of CD25 on HDM-stimulated lymphocytes increased in healthy as well as in asthmatic children, suggesting that natural exposure to HDM primes lymphocytes and that they can be activated by HDM *in vitro*. However, it was found that lymphocytes of asthmatic patients became more activated than lymphocytes of healthy children. Moreover, this was reflected by the correlation between the lymphoproliferative response to HDM and the increase in the mean density of CD25 after HDM stimulation.

Recently, adhesion studies of different cell types making up the inflammatory reaction have become the subject of intense research. To the best of our knowledge, adhesion of cultured lymphocytes has never been compared in asthmatic children with HS and non-HS asthmatic children. We found that HDM-stimulated lymphocytes from non-HS asthmatic children had a significantly higher adhesion to IL-1-stimulated HUVEC than lymphocytes incubated in medium containing no HDM. In contrast, HDM-stimulated lymphocytes from both normal and HS asthmatic children did not show an in-

creased adhesion to IL-1-stimulated HUVEC. Although preliminary, these findings suggest that HDM stimulation can enhance adhesion of lymphocytes to inflammatory endothelium in non-HS asthmatic children and that HS might be able to modify this process. These data agree with clinical studies showing inhibition by HS of the LAR evoked by allergen inhalation (19, 31, 36). Therefore, the inhibitory effects of HS upon the development of the LAR might be due to a decreased capacity of lymphocytes to adhere to endothelial cells, resulting in a decreased inflammatory reaction. The increased adhesion of HDM-stimulated lymphocytes to IL-1-stimulated HUVEC in non-HS asthmatic children was not reflected in a change in the adhesion molecules, LFA-1 and VLA.

The expression of CD25 on the cell surface increased significantly on HDM-stimulated CD3 + lymphocytes over the control culture in the healthy and the HS asthmatic children, whereas there was no significant increase in the nonhyposensitized patients. We have no explanation for this observation. In controls, CD25 expression is almost exclusively found in the memory CD4 T-cell subset (18). In allergic diseases, the T helper-1 cell, secreting preferentially IL-2 and interferon-gamma (IFN- γ), might be less important than the T helper-2 subtype, which produces IL-4 and IL-5, but no IL-2 or IFN- γ (23). Since all children inhale HDM antigens, CD25 might increase on the cell surface of all groups after HDM stimulation. However, the observation that there is no significant increase in nonhyposensitized asthmatic children could be due to the presence in these children of more T helper-2 cells, which, after stimulation, synthesize IL-4 and IL-5. On the contrary, normal children have a preponderance of the T helper-1 subtype, releasing more IFN- γ and IL-2. One of the effects of HS might be that in allergic patients antigen injection induces a shift from T helper-2 to T helper-1 cells. Further experiments are necessary to confirm this hypothesis.

In conclusion, this study provides further evidence that HS can modify lymphocyte and eosinophil functions, including adhesion of lymphocytes. These results might explain, at least in part, the inhibitory effects of HS on the LAR.

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References

1. ADELROTH E, ROSENHALL L, JOHANSSON SA, LINDEN M, VENGE P. Inflammatory cells and eosinophil activity in asthmatics investigated by bronchoalveolar lavage. *Am Rev Respir Dis* 1990; 142: 91–9.
2. BOUSQUET J, HEJJAOU A, MICHEL F-B. Specific immunotherapy in asthma. *J Allergy Clin Immunol* 1990; 86: 292–305.
3. BRIDTS CH, VAN BEVER HP, DE RIJCK TE, SCHUDDINCK L, DE CLERCK LS, STEVENS WJ. Importance of serum-free medium in the lymphocyte transformation test (LTT) with *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1991; 87: 195.
4. CAVAILLON J-M, FITTING C, GUINNEPAIN M-T, RASSEMONT R, DAVID B. Lymphocyte proliferative responses to the purified *Dermatophagoides farinae* major allergen in untreated and hyposensitized atopic patients. *Allergy* 1988; 43: 146–51.
5. DE CLERCK LS, DE RIJCK TE, BRIDTS CH, VAN BEVER HP, MOENS MM, STEVENS WJ. Hyposensitization (HS) to house dust mite (HDM) decreases the additional stimulatory effect of IL2 on lymphoproliferative responses to allergen (HDM) and mitogen (phytohemagglutinin (PHA)). *J Allergy Clin Immunol* 1992; 89: 342.
6. DE MONCHY JGR, KAUFFMAN HF, VENGE P, et al. Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. *Am Rev Respir Dis* 1985; 131: 373–6.
7. DINTHER-JANSSEN AC, HORST E, KOOPMAN G, et al. The VLA-4/VCAM-1 pathway is involved in lymphocyte adhesion to endothelium in rheumatoid synovium. *J Immunol* 1991; 12: 4207–10.
8. DURHAM SR, LOEGERING DA, DUNNETTE S, GLEICH GJ, KAY AB. Blood eosinophils and eosinophil-derived proteins in allergic asthma. *J Allergy Clin Immunol* 1989; 84: 931–6.
9. FURIN MJ, NORMAN PS, CRETICOS PS, et al. Immunotherapy decreases antigen-induced eosinophil migration into the nasal cavity. *J Allergy Clin Immunol* 1991; 88: 27–34.
10. GRIFFIN E, HÅKANSON L, FORMGREN H, JORGENSEN K, PETERSON CH, VENGE P. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991; 87: 548–57.
11. HIRATANI M, MUTO K, OSHIDA Y, ITO S, KASEI M. Lymphocyte responsiveness to *Dermatophagoides farinae* extract in mite-sensitive patients: effect of immunotherapy on cellular proliferative response and specific immunoglobulin E antibody (RAST score). *J Allergy Clin Immunol* 1981; 68: 205–11.
12. HSIEH KH. Decreased production of interleukin-2 receptors after immunotherapy to house dust. *J Clin Immunol* 1988; 8: 171–7.
13. JAFFE EA, NACHMAN RL, BECKER CG, MINICK CR. Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J Clin Invest* 1973; 52: 2745–56.
14. KAY AB. T lymphocytes and their products in atopic allergy and asthma. *Int Arch Allergy Appl Immunol* 1991; 94: 189–93.
15. KÜRZINGER K, REYNOLDS T, GERMAIN RN, DAVIGNON D, MARTZ E, SPRINGER TA. A novel lymphocyte function-associated antigen (LFA-1): cellular distribution, quantitative expression, and structure. *J Immunol* 1981; 127: 596–601.
16. KUS J. Lymphocyte activation by house dust allergen in asthma: analysis with monoclonal antibodies. *Clin Exp Allergy* 1990; 20: 165–70.
17. LEFF AR, HAMANN KJ, WEGNER CD. Inflammation and cell-cell interactions in airway hyperresponsiveness. *Am J Physiol* 1991; 260: L189–206.
18. MAURER D, FELZMANN T, HOLTER W, PETERA P, SMOLEN J, KNAPP W. Evidence for the presence of activated CD4 T cells with naive phenotype in the peripheral blood of patients with rheumatoid arthritis. *Clin Exp Immunol* 1992; 87: 429–34.
19. METZGER WJ, DONNELLY A, RICHESON HB. Modification of late asthmatic responses (LAR) during immunotherapy for *Alternaria*-induced asthma. *J Allergy Clin Immunol* 1983; 71: 119.
20. RAK S, LOWHAGEN O, VENGE P. The effect of immunotherapy on bronchial hyperresponsiveness and eosinophil cationic protein in pollen-allergic patients. *J Allergy Clin Immunol* 1988; 82: 470–80.
21. RAK S, HÅKANSON L, VENGE P. Immunotherapy abrogates the generation of eosinophil and neutrophil chemotactic activity during pollen season. *J Allergy Clin Immunol* 1990; 86: 706–13.
22. RAK S, BJÖRNSON A, HÅKANSON L, SORENSON S, VENGE P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during pollen exposure. *J Allergy Clin Immunol* 1991; 88: 878–88.
23. RICCI M, ROSSI O, BERTONI M, MATUCCI A. The importance of Th2-like cells in the pathogenesis of airway allergic inflammation. *Clin Exp Allergy* 1993; 23: 360–9.
24. ROCKLIN RE, SHEFFER AL, GREINER DK, MELMON KL. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980; 302: 1213–19.
25. SCHMID I, SCHMID P, GIORGI JV. Conversion of logarithmic channel numbers into relative linear fluorescence intensity. *Cytometry* 1988; 9: 533–8.
26. SPRINGER TA. Adhesion receptors of the immune system. *Nature* 1990; 346: 425–34.
27. STEVENS WJ, VERHELST JA, VAN DEN BOGAERT W, BRIDTS CH. Clinical and biological evaluation of semi-rush and ordinary immunotherapy schemes in Type I allergic respiratory diseases. *Allergy* 1985; 40: 447–52.
28. SUGAI T, SAKIYAMA Y, MATUMOTO S. Eosinophil cationic protein in peripheral blood of pediatric patients with allergic diseases. *Clin Exp Allergy* 1992; 22: 275–81.
29. TAMIR R, CASTRACANE JM, ROCKLIN RE. Generation of suppressor cells in atopic patients during immunotherapy that modulate IgE synthesis. *J Allergy Clin Immunol* 1987; 79: 591–8.
30. TSAI LC, TANG RB, HUNG MW, CHEN HM, TSAI SJ. Expression of serum IL-2, IL-2R and CD8 levels during hyposensitization in house-dust-sensitive asthmatics. *J Asthma* 1990; 27: 307–13.
31. VAN BEVER HP, BOSMANS J, DE CLERCK LS, STEVENS WJ. Modification of the late asthmatic reaction by hyposensitization in asthmatic children allergic to house dust mite (*Dermatophagoides pteronyssinus*) or grass pollen. *Allergy* 1988; 43: 378–85.
32. VENGE P, ROXIN LE, OLSSON I. Radio-immunoassay of human eosinophil cationic protein. *Br J Haematol* 1977; 37: 331–5.
33. VENGE P, DAHL R. Are blood eosinophil number and activity important for the development of the late asthmatic reaction after allergen challenge? *Eur Respir J* 1989; 2: 430s–4s.
34. WALKER C, KAEGI MK, BRAUN P, BLASER K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991; 88: 935–42.
35. WARNER JO. Significance of late reactions after bronchial challenge with house dust mite. *Arch Dis Child* 1976; 51: 905–11.
36. WARNER JO, PRICE JF, SOOTHILL JF, HEY EN. Controlled trial of hyposensitization to *Dermatophagoides pteronyssinus* in children with asthma. *Lancet* 1978; ii: 912–15.

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