

Mite-specific induction of interleukin-2 receptor on T lymphocytes from children with mite-sensitive asthma: Modified immune response with immunotherapy

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Background: The efficacy of immunotherapy is still controversial. To elucidate the mechanisms of immunotherapy, we studied mite-specific induction of IL-2 receptor (IL-2R) expression on T lymphocytes from children with mite-sensitive asthma.

Methods: Peripheral blood mononuclear cells were obtained from 28 children with mite-sensitive asthma: 13 had never received house dust immunotherapy (nonimmunotherapy group), 15 had been receiving house dust immunotherapy at the time of the study (immunotherapy group). After a 6-day culture with or without *Dermatophagoides farinae* (Df) antigen, the expression of IL-2R α (CD25) and p75 on CD4⁺ or CD8⁺ T lymphocytes was measured by flow cytometry.

Results: The nonimmunotherapy group showed significant Df-specific CD25 induction on CD4⁺ T lymphocytes (Δ CD4⁺ CD25⁺) but little induction on CD8⁺ T lymphocytes (Δ CD8⁺ CD25⁺). Δ CD4⁺ CD25⁺ was correlated with the severity of the disease. In the immunotherapy group Δ CD8⁺ CD25⁺ was significantly higher than in the nonimmunotherapy group or in normal subjects and correlated with Df-specific IgG₄ and cumulative doses of house dust extract, whereas Δ CD4⁺ CD25⁺ was similar in the nonimmunotherapy and the immunotherapy groups. IL-2R β was not induced either on CD4⁺ or CD8⁺ T lymphocytes.

Conclusions: Our data suggest that house dust immunotherapy may have induced Df-specific CD8⁺ T lymphocytes in patients with mite-sensitive asthma and that the efficacy of immunotherapy may be attributed to the generation of Df-specific CD8⁺ T lymphocytes. (J ALLERGY CLIN IMMUNOL 1996;97:680-8.)

Key words: IL-2 receptor, CD25, Mik- β 1, asthma, immunotherapy, children, *Dermatophagoides farinae*, CD8⁺ T lymphocyte, IgG₄

Although the pathogenesis of bronchial asthma is still in controversy, important roles of antigen-specific T lymphocytes in exacerbation of bronchial asthma have been demonstrated by in vitro studies, such as antigen-specific proliferative response,¹

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Abbreviations used

Df:	<i>Dermatophagoides farinae</i>
FITC:	Fluorescein isothiocyanate
HD:	House dust
IL-2R:	Interleukin-2 receptor
MFI:	Mean fluorescent intensity
PBMCs:	Peripheral blood mononuclear cells
PBS:	Phosphate-buffered saline
PE:	Phycoerythrin

IL-2 production² and responsiveness,^{3,4} and production of various cytokines by antigen-specific T-cell clones⁵ from patients with atopic asthma. Also, recent in vivo studies have shown a signifi-

cant increase of activated T lymphocytes in peripheral blood from patients with acute severe asthma⁶ and in the bronchial submucosa of patients with stable asthma.⁷ And further, allergen inhalation challenge induced activation of T lymphocytes in the bronchial mucosa with the expression of messenger RNAs for cytokines relevant to eosinophil recruitment and activation.⁸ These findings clearly indicate that the antigen-specific activation of T lymphocytes plays a key role in exacerbation of asthma.

On the other hand, the immunologic mechanisms of immunotherapy are not yet defined. Several lines of evidence, such as generation of antigen-specific suppressor T lymphocytes during antigen-specific immunotherapy,^{9,10} altered antigen-specific proliferative response,¹¹ and IL-2 production and responsiveness¹² of T lymphocytes after antigen-specific immunotherapy, indicate that antigen-specific immunotherapy may modify the immune responses of T lymphocytes to the antigen.

To elucidate the mechanism of immunotherapy, we studied *Dermatophagoides farinae* (Df)-specific IL-2 receptor (IL-2R) induction on T lymphocytes from patients with mite-sensitive asthma by two-color flow cytometry with anti-IL-2Rp55 (CD25) and p75 monoclonal antibodies. We found that CD25 was induced on CD8⁺ T lymphocytes only in asthmatic patients who received antigen-specific immunotherapy, whereas the levels of CD25 induction on CD4⁺ T lymphocytes were similar in the patients who had never received immunotherapy and in those who received immunotherapy.

METHODS

Subjects

Twenty-eight children with house dust (HD) mite-sensitive bronchial asthma were enrolled in this study. Diagnoses were based on criteria listed in an international consensus report on treatment of asthma.¹³ All patients had positive skin test responses to HD extract. Patients who had acute exacerbations at the time of examination or those who had steroid therapy within 1 month of the study were excluded. Severity of asthma was determined for each patient.¹³ Patients were divided into two groups, those who had never received HD immunotherapy (nonimmunotherapy group) and those who had been receiving HD immunotherapy at the time of this study (immunotherapy group). The nonimmunotherapy group was further divided into subgroups on the basis of current severity of asthma. The immunotherapy group was divided on the basis of response to immunotherapy as follows: (1) poor responders, patients whose asthma severity was the same or worse after immuno-

therapy was initiated; (2) good responders, patients whose asthma severity was improved after immunotherapy was initiated; (3) in remission, patients who had been free of acute exacerbations for more than 2 years without any medication since immunotherapy was initiated. Eight healthy nonatopic individuals were selected as normal subjects (Table I).

Informed consent was obtained from all subjects, and this study was approved by the Institutional Research Committee.

Immunotherapy protocol

Commercial HD extract was obtained from Torii Pharmaceuticals (Tokyo, Japan) and used for immunotherapy and intradermal skin tests. One milligram of the extract was demonstrated to contain 9.8 ng of major allergen of mite (Der 1 + Der 2), of which 5.4 ng was Der f1.¹⁴ Immunotherapy was started with 1:100,000 or 1:1,000,000 wt/vol extract, which was a 10-fold dilution of the minimum concentration that produces skin erythema. Once or twice a week injections were administered with increasing volume (0.03, 0.05, 0.07, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5 ml). Then, a 10-fold higher concentration was administered in the same dosing increments until maintenance was reached, which consisted of 0.5 ml of 1:100 wt/vol extract or the highest tolerated dose. Injection dose was maintained with 0.1 ml of 1:100 wt/vol extract or highest tolerated dose and administered weekly for 10 weeks, every other week for 2 months, every 3 weeks for 3 months, and monthly. Duration of immunotherapy ranged from 3 to 72 months (mean, 18.5 months), and total cumulative doses of HD extract ranged from 0.2 to 78.0 mg (mean, 27.5 mg).

Atopic status

The numbers of peripheral blood eosinophils were counted by staining cells with Hinkelman's solution (0.5% yellow eosin, 0.5% formalin, 0.5% phenol in distilled water). Total IgE in sera was measured by enzyme immunoassay.¹⁵ Serum Df-specific IgE was quantitated by Phadebas IgE RAST (Pharmacia Diagnostics, Uppsala, Sweden) and expressed in terms of RAST score.¹⁶ Serum Df-specific IgG₄ was measured by ELISA.¹⁷

Culture of mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were separated from fresh heparinized venous bloods by Ficoll-Paque (Pharmacia Diagnostics) density gradient centrifugation, washed three times in phosphate-buffered saline (PBS), and then suspended in RPMI-1640 (Gibco, Grand Island, N.Y.) at 1×10^6 cells/ml with 10% heat-inactivated human AB serum. One milliliter of cell suspension was cultured with 10 μ g/ml Df antigen (Torii) or with nothing for 6 days or with anti-CD3 antibody (1:5000 immune mouse ascites) for 48 hours. All cultures were carried out at 37°C in a humidified atmosphere containing 5% CO₂. After the cells were

TABLE I. Characteristics of subjects

Subject No.	Age (yr)	Sex	Severity			Eosinophil (/mm)	IgE (IU/ml)	Df-IgE	Df-IgG ₄ (U/ml)	Maintenance dose of IT (wt/vol)
			Current	Pre-treatment	Response to IT			RAST (RAST score)		
Normal subjects										
1	24	F				ND	ND	0	ND	
2	23	F				75	ND	0	ND	
3	28	F				ND	ND	0	ND	
4	11	M				113	729	0	ND	
5	13	M				100	24	0	0	
6	11	M				531	22	0	0.9	
7	11	F				106	95	0	0	
8	28	M				ND	50	0	ND	
Mean ± SD	19 ± 8					137 (63-297)	71 (17-298)		0.3 ± 0.5	
Non-IT group										
9	13	F	S	S		263	911	4	0	
10	9	M	S	S		263	1735	4	0.7	
11	11	M	S	S		413	1840	4	0.3	
12	22	M	S	S		281	2667	4	0	
13	11	F	S	S		468	167	4	ND	
14	17	M	Mo	Mo		350	480	4	ND	
15	13	F	Mo	S		625	570	4	0	
16	15	F	Mo	S		231	572	4	1.1	
17	10	M	Mo	S		844	282	4	0	
18	14	M	M	Mo		563	352	4	0	
19	12	M	M	S		213	601	4	0.3	
20	13	M	M	S		350	661	4	0.9	
21	14	M	M	S		988	117	4	ND	
Mean ± SD	13 ± 3					400 (244-655)*	585 (235-1457)*		0.2 ± 0.3	
IT group										
22	11	M	S	S	Poor	119	593	4	ND	1:100
23	15	F	S	S	Poor	181	2693	4	0	Build-up
24	13	F	Mo	Mo	Poor	644	1767	4	0.3	1:10,000
25	7	M	Mo	S	Good	1044	1062	4	ND	1:100
26	11	F	Mo	S	Good	600	509	4	0	1:1,000
27	8	F	M	S	Good	763	219	4	0.3	1:10,000
28	12	M	M	S	Good	269	876	4	ND	1:1,000
29	9	M	M	S	Good	575	946	4	1.6	1:100
30	12	M	M	S	Good	669	1578	4	0.9	1:100
31	16	M	M	Mo	Good	188	313	4	ND	Build-up
32	8	M	M	Mo	Good	331	6598	4	2.2	1:100
33	12	M	attack(-)	Mo	Remission	175	280	4	0.5	1:100
34	13	F	attack(-)	M	Remission	519	1269	4	3.2	1:100
35	16	M	attack(-)	Mo	Remission	238	291	4	1.4	1:100
36	11	F	attack(-)	Mo	Remission	294	809	4	0.7	1:100
Mean ± SD	12 ± 3					364 (190-701)*	842 (330-2148)*		1.0 ± 1.0†	

IT, Immunotherapy; S, severe; Mo, moderate; M, mild; ND, not done.

* $p < 0.01$ versus normal subjects.† $p < 0.05$ versus nonimmunotherapy group.

cultured for certain days, they were washed twice, resuspended in PBS, and used for further analyses. Some of the freshly purified PBMCs were suspended in PBS and used immediately.

To demonstrate the antigen specificity of CD25 induction, PBMCs from some of the normal subjects and the patients with mite-sensitive asthma were cultured for 6 days with 10 μ g/ml *Aspergillus* antigen (Torii).

Immunofluorescent staining of cell surface antigens

Monoclonal antibodies. Fluorescein isothiocyanate (FITC)-conjugated mouse anti-Leu3a (CD4) and anti-Leu2a (CD8), phycoerythrin (PE)-conjugated mouse anti-Tac (CD25) were obtained from Becton Dickinson Immunocytometry Systems (Mountain View, Calif.). Mik- β 1, which is mouse anti-human IL-2Rp75 monoclonal antibody, was kindly provided by Dr. Mitsuru Tsudo (Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan).

Approximately 5×10^5 cells of freshly purified or cultured cells were used for each staining. To detect the expression of CD25 on T lymphocytes, 10 μ l of PE-anti-CD25 was added to the cell suspension with 10 μ l of FITC-anti-CD4 or FITC-anti-CD8 and incubated for 15 minutes at 4° C. To detect the expression of IL-2Rp75, the cell suspension was incubated first with 5 μ l of Mik- β 1 for 15 minutes at 4° C. After washing once with PBS, cells were placed in contact with 5 μ l PE-conjugated goat F(ab')₂ anti-mouse IgG (1:10 dilution; TAGO Inc., Burlingame, Calif.) for 15 minutes at 4° C. Unoccupied sites were blocked with excess mouse IgG (0.3 mg/ml, 10 μ l; Sigma Chemical Co., St. Louis, Mo.) for 10 minutes at room temperature, then FITC-anti-CD4 or FITC-anti-CD8 was added without washing and incubated for 15 minutes at 4° C. Ten microliters of PE-conjugated preimmune goat F(ab')₂ (TAGO) or nothing was used as a negative control for PE-anti-CD25 or Mik- β 1, respectively. After the cells were stained with monoclonal antibodies, they were washed twice, resuspended in PBS, and stored at 4° C in the dark until use.

Flow cytometric analyses

The expression of the surface antigens on T lymphocytes was detected by using FACScan (Becton Dickinson) as described previously.¹⁸

The expression of CD25 was evaluated as the percentage of CD25⁺ cells in CD4⁺ or CD8⁺ T lymphocytes (CD25⁺ CD4⁺ or CD8⁺). *Df*-specific CD25 induction (Δ CD25⁺ CD4⁺ or CD8⁺) was evaluated as follows:

Δ CD25⁺ CD4⁺ or CD8⁺ (%) = Percentage of CD25⁺ CD4⁺ (or CD8⁺) T lymphocytes cultured with *Df* antigen – Percentage of CD25⁺ CD4⁺ (or CD8⁺) T lymphocytes cultured without *Df* antigen

Mean fluorescent intensity (MFI) was used to evaluate the expression of IL-2Rp75 because IL-2Rp75 is constitutionally expressed on T lymphocytes.¹⁸ *Df*-specific IL-2Rp75 induction (Δ IL-2Rp75) was evaluated as follows:

Δ IL-2Rp75 = {MFI of IL-2Rp75⁺ CD4⁺ (or CD8⁺) T lymphocytes cultured with *Df* antigen} – {MFI of IL-2Rp75⁺ CD4⁺ (or CD8⁺) T lymphocytes cultured without *Df* antigen}

Statistical analyses

Statistical analyses of the experimental data were performed by means of paired or unpaired *t* tests. Values for total IgE, numbers of eosinophils, and cumulative doses of HD extract were logarithmically transformed.

RESULTS

Atopic status

The numbers of eosinophils and the levels of serum total IgE and serum *Df*-specific IgG₄ are shown in Table I. The levels of *Df*-specific IgG₄ in the immunotherapy group were significantly higher ($p < 0.05$) than those in the nonimmunotherapy group, although the numbers of eosinophils and the levels of total IgE were similar in the nonimmunotherapy and the immunotherapy groups. IgE RAST scores for *Df* were 4 in all asthmatic patients and 0 in all normal subjects.

Immunotherapy and clinical responses

Cumulative doses of HD extract in the remission group (57 ± 14.4 mg) were significantly higher than those in the good responder (18.0 ± 20.8 mg, $p < 0.01$) and the poor responder (13.5 ± 21.1 mg, $p < 0.05$) groups.

Subpopulations and IL-2R expression of T lymphocytes in peripheral blood

There were no significant differences in the percentages of CD4⁺ and CD8⁺ T lymphocytes and the expression of CD25 and IL-2Rp75 on CD4⁺ and CD8⁺ T lymphocytes between any two groups before PBMCs were cultured (data not shown).

Df-specific CD25 induction on T lymphocytes

Table II shows the expression of CD25 after 6-day culture with *Df* antigen. When PBMCs were cultured with *Df* antigen, the expression of CD25 on CD4⁺ T lymphocytes was significantly increased in the nonimmunotherapy group ($p < 0.001$), the immunotherapy groups ($p < 0.001$), and even in normal subjects ($p < 0.01$), although the increase was subtle. On the other hand, the increase in the expression of CD25 on CD8⁺ T lymphocytes was significant only in the immunotherapy group ($p < 0.001$). Compared with *Df* antigen, the expression of CD25 was remarkably increased on CD4⁺ and CD8⁺ T lymphocytes in all groups when cells were cultured with anti-CD3 antibody.

The levels of *Df*-specific CD25 induction were compared between groups. The levels of Δ CD25⁺ CD4⁺ in the nonimmunotherapy and the immuno-

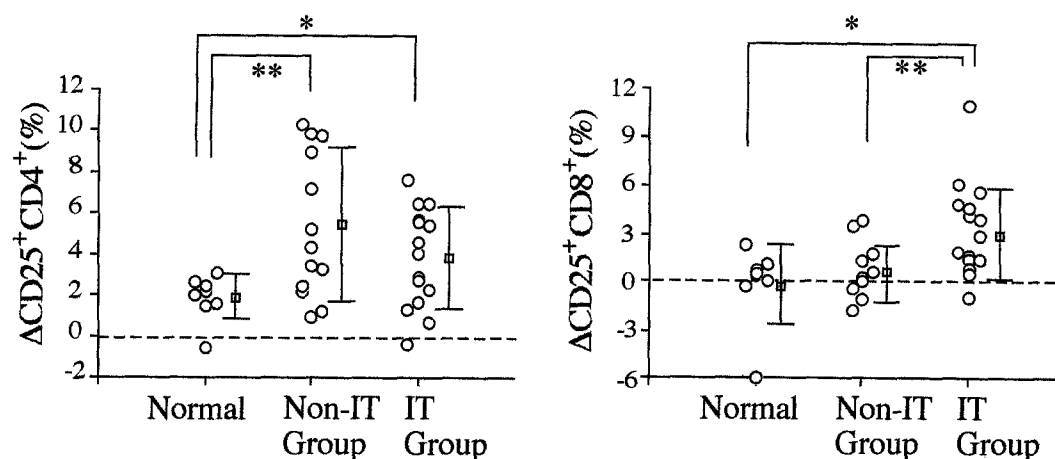


FIG. 1. *Df*-specific CD25 induction on T lymphocytes in normal subjects and patients with mite-sensitive asthma who had never received immunotherapy (nonimmunotherapy group) and those who had been receiving immunotherapy (immunotherapy group). Square and bar represent mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$. IT, Immunotherapy.

TABLE II. CD25 expression after 6-day culture with *Df* antigen

Antigen	CD25 ⁺ CD4 ⁺ (%)			CD25 ⁺ CD8 ⁺ (%)		
	Normal	Non-IT	IT	Normal	Non-IT	IT
None	10.1 $\pm$ 4.4	9.5 $\pm$ 2.8	10.5 $\pm$ 3.1	1.7 $\pm$ 2.6	0.7 $\pm$ 0.8	1.7 $\pm$ 1.6
<i>Df</i>	11.9 $\pm$ 4.9	14.9 $\pm$ 3.1	14.3 $\pm$ 3.5	1.5 $\pm$ 1.3	1.5 $\pm$ 1.9	5.0 $\pm$ 3.8
<i>p</i> Value	<0.01	<0.001	<0.001	NS	NS	<0.001
anti-CD3*	29.0 $\pm$ 8.2	30.5 $\pm$ 9.8	28.3 $\pm$ 10.8	26.4 $\pm$ 13.4	22.8 $\pm$ 13.1	26.2 $\pm$ 11.6

Values are expressed as means $\pm$ SD. To evaluate the effect of *Df* antigen on CD25 induction, paired *t* tests were used for statistical analyses.

IT, Immunotherapy; NS, not significant.

*PBMCs were cultured for 48 hours with anti-CD3 antibody.

therapy groups were significantly higher than those in normal subjects ($p < 0.01$ and $p < 0.05$, respectively), and there was no significant difference in the levels of Δ CD25⁺ CD4⁺ between the nonimmunotherapy and immunotherapy groups. The levels of Δ CD25⁺ CD8⁺ in the immunotherapy group were significantly higher than those in normal subjects ($p < 0.05$) and in the nonimmunotherapy group ($p < 0.01$) (Fig. 1).

In the nonimmunotherapy group patients with severe asthma showed significantly higher levels of Δ CD25⁺ CD4⁺ than those with moderate asthma ($p < 0.01$) and normal subjects ($p < 0.001$), and patients with moderate asthma also showed significantly higher levels of Δ CD25⁺ CD4⁺ than normal subjects ($p < 0.05$). Most patients with mild asthma showed low levels of Δ CD25⁺ CD4⁺; however, one patient showed a remarkably high level of Δ CD25⁺ CD4⁺. This patient had acute exacerbation of asthma soon after completing this

study. The levels of Δ CD25⁺ CD8⁺ were similar in three subgroups and in normal subjects (Fig. 2, A). In the immunotherapy group, in the good responder group, and even in the remission group subjects significantly higher levels of Δ CD25⁺ CD4⁺ than normal subjects ($p < 0.05$ and $p < 0.05$, respectively). The good responder and remission groups also showed significantly higher levels of Δ CD25⁺ CD8⁺ than normal subjects ($p < 0.05$ and $p < 0.01$, respectively) and the nonimmunotherapy group ($p < 0.05$ and $p < 0.01$, respectively). Compared with the good responder group, the poor responder group showed lower, but not significant, levels of Δ CD25⁺ CD8⁺, although there were no significant differences in the duration of immunotherapy and cumulative doses of HD extract between the poor responder and good responder groups (Fig. 2, B).

In comparison with *Df* antigen, *Aspergillus* antigen did not induce CD25 expression either on CD4⁺ or

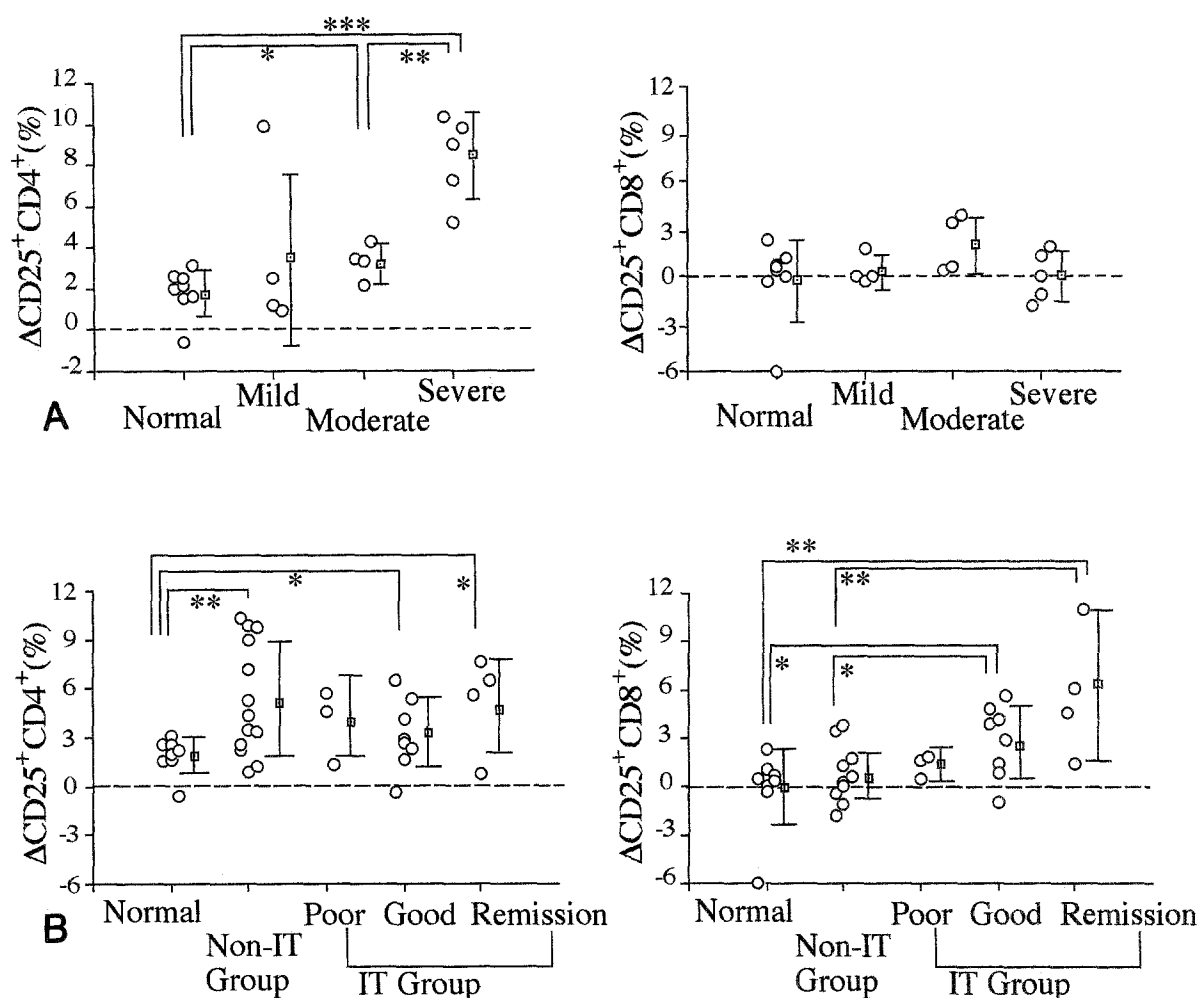


FIG. 2. *Df*-specific CD25 induction of T lymphocytes: Comparison of current severity and response to immunotherapy. **A**, Patients in the nonimmunotherapy group were grouped according to current asthma severity. **B**, Patients in the immunotherapy group were grouped according to responses to immunotherapy. Square and bar represent mean $\pm$ SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. IT, Immunotherapy.

CD8⁺ T lymphocytes from normal subjects and patients with mite-sensitive asthma (Table III).

CD25 induction and atopic status

The numbers of eosinophils and the levels of total IgE were not correlated with the levels of either $\Delta CD25^+ CD4^+$ or $\Delta CD25^+ CD8^+$ in any group. In the immunotherapy group the levels of *Df*-specific IgG₄ were significantly correlated with those of $\Delta CD25^+ CD8^+$ ($r = 0.64$, $p < 0.05$; Fig. 3, *A*) but not with those of $\Delta CD25^+ CD4^+$ ($r = 0.06$, $p > 0.8$).

Influence of the dose of immunotherapy on CD25 induction and *Df*-specific IgG₄

In the immunotherapy group cumulative doses of HD extract that the patients had received were

significantly correlated with the levels of serum *Df*-specific IgG₄ ($r = 0.62$, $p < 0.05$) and $\Delta CD25^+ CD8^+$ ($r = 0.53$, $p < 0.05$) but not with $\Delta CD25^+ CD4^+$ ($r = 0.02$, $p > 0.4$) (Fig. 3, *B*, *C*, and *D*, respectively).

Df-specific IL-2Rp75 induction on T lymphocytes

The expression of IL-2Rp75 on T lymphocytes cultured with *Df* antigen and $\Delta IL-2Rp75$ was negligible on either CD4⁺ or CD8⁺ T lymphocytes in normal subjects and in the asthmatic groups (data not shown).

DISCUSSION

In this study we investigated *Df*-specific IL-2R induction on CD4⁺ and CD8⁺ T lymphocytes from

TABLE III. Antigen specificity of CD25 induction

Antigen	CD25 ⁺ CD4 ⁺ (%)		CD25 ⁺ CD8 ⁺ (%)	
	Normal	Asthma	Normal	Asthma
None	10.0 ± 1.1	12.5 ± 2.4*	1.4 ± 1.9	1.4 ± 1.0
<i>Df</i>	12.0 ± 0.4	18.1 ± 1.8*†	2.2 ± 2.3	6.8 ± 6.2
<i>Asp</i>	11.1 ± 0.1	14.2 ± 0.1†	2.4 ± 1.1	2.1 ± 1.5
anti-CD3	31.7 ± 6.8	37.1 ± 10.7	25.7 ± 5.1	28.5 ± 15.1
ΔCD25‡				
<i>Df</i>	2.1 ± 0.6†	5.6 ± 1.0†	0.8 ± 2.4	5.4 ± 5.3
<i>Asp</i>	1.1 ± 1.1	1.7 ± 2.5	1.0 ± 0.7	0.7 ± 0.7

Two normal subjects and three mite-sensitive (not sensitive to *Aspergillus*) asthmatic patients in the immunotherapy group were enrolled in this experiment. Values are expressed as means ± SD.

Asp, *Aspergillus*.

* $p < 0.01$.

† $p < 0.05$.

‡ΔCD25⁺ CD4⁺ or CD8⁺ was determined as defined in Methods.

patients with atopic asthma by two-color flow cytometric analysis. It has been reported that *Df*-specific induction of IL-2 responsiveness was observed only on CD4⁺ T lymphocytes from patients with atopic asthma in vitro.^{3,4} We demonstrated that CD25, which is a well-established marker for activated T lymphocytes,¹⁹ was significantly induced on CD4⁺ T lymphocytes, but not on CD8⁺ T lymphocytes, in asthmatic patients who had never received HD immunotherapy, especially in patients with severe asthma. These findings are consistent with the results of previous reports, and remarkably high levels of CD25 induction in patients with severe asthma may indicate that the numbers of *Df*-specific helper T lymphocytes are increased in the peripheral blood, probably because of frequent allergen exposure.

On the other hand, in patients who had been receiving immunotherapy with good clinical response, significant *Df*-specific CD25 induction was observed on CD8⁺ T lymphocytes, as well as on CD4⁺ T lymphocytes. And further, the levels of *Df*-specific CD25 induction on CD8⁺ T lymphocytes were correlated with the cumulative doses of HD extract that the patients had received. These findings suggest that asthmatic patients who are responding well to immunotherapy have *Df*-specific CD8⁺ T lymphocytes in the peripheral blood, as well as *Df*-specific CD4⁺ T lymphocytes, and that HD immunotherapy might generate *Df*-specific CD8⁺ T lymphocytes.

Several clinical studies have demonstrated that immunotherapy inhibits the development of the allergen-induced late asthmatic reaction^{20,21} and the late cutaneous reaction.^{22,23} Despite these clinical effects, IL-2 responsiveness of antigen-

stimulated T lymphocytes from asthmatic patients who have received immunotherapy was reported to be higher than that of T lymphocytes from normal subjects and patients with a new diagnosis of asthma.¹² And further, it was reported that the numbers of infiltrated CD25⁺ or HLA-DR⁺ T lymphocytes in skin biopsy specimens of the late cutaneous reactions are significantly increased after antigen-specific immunotherapy, even though the late cutaneous reactions themselves are inhibited.²⁴ These two previous findings, showing discrepancy between the clinical improvement and the paradoxically enhanced reactivity of T lymphocytes to the antigen, can be reconciled by our findings demonstrating that both CD4⁺ T lymphocytes and CD8⁺ T lymphocytes were activated in patients who had been receiving immunotherapy, whereas only CD4⁺ T lymphocytes were activated in those who had never received immunotherapy. Taken together, these findings suggest that immunotherapy may promote the generation of an antigen-specific, but probably functionally different, type of T lymphocyte rather than suppress the entire immunologic responses to the antigen.

Although the importance of CD8⁺ T lymphocytes in atopic asthma is not well known, increases in the numbers and percentages of CD8⁺ T lymphocytes were observed in bronchoalveolar lavage from asthmatic patients who had only the immediate asthmatic reaction after allergen inhalation challenge but not from those who had both the immediate and late asthmatic reactions.²⁵ And further, it has been demonstrated that transfer of allergen-sensitized interferon-γ-producing CD8⁺ T lymphocytes into sensitized recipients results in selective suppression of IgE production^{26,27} and

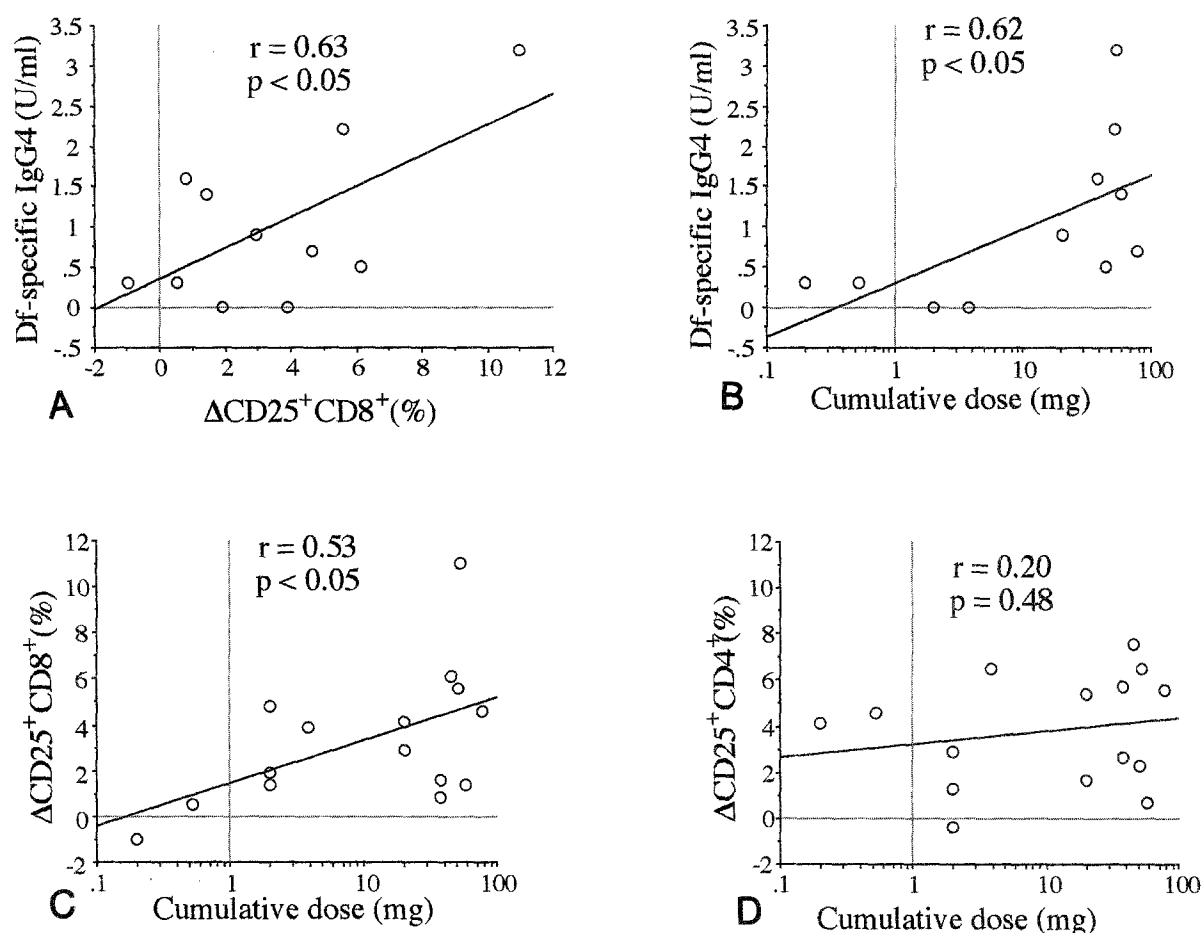


FIG. 3. Relationship between cumulative doses of HD extract and *Df*-specific CD25 induction on CD8⁺ T lymphocytes and serum *Df*-specific IgG₄ in the immunotherapy group. **A**, ΔCD25⁺ CD8⁺ versus *Df*-specific IgG₄ ($r = 0.63$, $p < 0.05$). **B**, Cumulative dose versus *Df*-specific IgG₄ ($r = 0.62$, $p < 0.05$). **C**, Cumulative dose versus ΔCD25⁺ CD8⁺ ($r = 0.53$, $p < 0.05$). **D**, Cumulative dose versus ΔCD25⁺ CD4⁺ ($r = 0.20$, $p = 0.48$).

normalization of airway responsiveness²⁷ in animal models. These findings suggest that antigen-specific CD8⁺ T lymphocytes are responsible for downregulation of the hypersensitive response by producing regulatory cytokines, such as interferon- γ , which is known to inhibit the proliferation of T_{H2} cells²⁸ and IgE synthesis.²⁹ As shown in this study, the levels of *Df*-specific CD25 induction on CD8⁺ T lymphocytes were significantly correlated with those of serum *Df*-specific IgG₄ and cumulative doses of HD extract. The significance of *Df*-specific IgG₄ on the beneficial effect of immunotherapy is still unclear³⁰; however, our results indicate the possibility that antigen-specific CD8⁺ T lymphocytes may regulate antigen-specific IgG₄ production.

To avoid inaccuracy of flow cytometric analysis caused by background noise, at least 5,000 lympho-

cytes were gated and analyzed for each staining. Further, as shown in Table II, the expression of CD25 was analyzed by paired *t* tests, which measured how *Df* antigen affected the expression of CD25 on T lymphocytes in each subject. These analyses revealed the significant effects of *Df* antigen on the increased expression of CD25 on T lymphocytes, and the background noise of flow cytometer did not seem to affect our results.

In conclusion, we demonstrated that asthmatic patients who have been receiving HD immunotherapy successfully have *Df*-specific CD8⁺ T lymphocytes in the peripheral blood. The function of *Df*-specific CD8⁺ T lymphocytes and the mechanism of their generation remain to be studied; however, CD25 induction on CD8⁺ T lymphocytes will be one of the promising markers for evaluating the efficacy of antigen-specific immunotherapy.

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