Acta Dermatovenerol Croat 2006;14(1):8-20 ORIGINAL SCIENTIFIC ARTICLE

Immunological Parameters in the Sera of Patients with Atopic Dermatitis and Airborne Allergy Treated with Allergy Vaccines

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SUMMARY Patients with atopic disorders present an increased production of IgE, which is usually limited to specific antibodies against various environmental allergens. It has also been suggested that the production of these antibodies may be influenced by effective specific immunotherapy (SIT). Of course, a decline of serum antigen specific IgE in the course of such a treatment cannot explain the clinical efficacy of SIT and is probably not a key mechanism. However, SIT may at least participate in the final clinical result.

In this study, 37 patients with atopic dermatitis were treated with allergy vaccines (Novo-Helisen Depot) for a time period of 48 months. The control group consisted of 29 patients with atopic dermatitis who were treated with classical methods. The clinical score (W-AZS), total IgE and antigen specific IgE (asIgE) in the sera of patients were assessed before treatment and after 24 and 48 months of therapy (FEIA CAP System, Pharmacia). There was a significant difference between the two investigated groups from both the clinical and immunological standpoints after 2 and 4 years of observation. There was a significant decrease of serum total IgE and asIgE (directed against airborne allergens) in the course of specific immunotherapy. In the control group, the total IgE level tended to increase, and this tendency was also recorded in case of asIgE measurements. We also evaluated the influence of specific immunotherapy on the serum level of IFN-γ, sIL-2R, IL-4, IL-5 and IL-10 before treatment and after 4 years of therapy with the quantitative 2-step colorimetric sandwich ELISA method (R&D Systems).

In the group of patients treated with allergy vaccines, a significant decrease of the serum sIL-2R level was observed after 48 months of therapy ($p<0,01$). In the control group, a significant increase of serum IL-4 ($p<0,01$) as well as IL-5 ($p<0,05$) was registered at the end of the observation period. There was no significant correlation between the clinical score and serum level of any of the investigated cytokines in either group of patients before the treatment or after 48 months of therapy.

KEY WORDS: atopic dermatitis, specific immunotherapy, total IgE, antigen specific IgE, cytokines

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INTRODUCTION

Although allergen-specific immunotherapy (SIT) has been widely used for the treatment of allergic diseases for more than 90 years, the exact mechanism of its action remains to be determined. A variety of immunological changes have been described during and after clinical courses of SIT, and several mechanisms have been proposed for SIT's beneficial clinical effect. However, it remains rather unclear which, if any, of these immunological changes are involved in the mechanisms by which this therapy improves the clinical status of patients.

Recently, considerable attention has been directed to the mechanisms of SIT. One reason for this increased interest is that clarification of the immunological mechanisms underlying the clinical efficacy of SIT may provide a new understanding of the basic mechanisms of human allergic diseases. Of the many different hypotheses, the clinical role of serum immunoglobulins and alternations in T-cell response are of special interest. Patients with allergic disorders present an increased production of IgE, which is usually limited to specific antibodies against environmental allergens. IgE molecules bind specifically to receptors located on the surface of tissue mast cells and circulating basophils, which then produce and release several potent mediators involved in the clinical manifestations of allergic diseases. Therefore, a decrease of antigen specific IgE (asIgE) could theoretically lead to a good clinical course. It may then be suggested that SIT may influence the production of asIgE. In the 1970s, it was postulated that a reduction of asIgE in the sera of patients treated with SIT was a major mechanism of this therapeutic method. Later, it was demonstrated that the IgE response shows an initial increase, followed by a gradual decrease, and after several years of SIT or asIgE, the serum level may be not affected (1-7). It seems that although a decline of serum asIgE in the course of SIT cannot explain the clinical efficacy of SIT and is not always a key mechanism in clinically successful SIT, it may at least participate in the final result of this treatment.

Atopic dermatitis (AD) represents an inflammatory skin disorder which has been well known since the beginning of the 20th century, but its pathogenesis is not yet clearly understood (8). In addition to a multifactorially determined genetic predisposition, AD is characterized by various changes in humoral and cell-mediated immunity (8-13). Profound immunological alternations in AD may be associated with imbalances within the cytokine network (14), and the altered production of immuno-modulating cytokines may be the cause of changes in the immune response, finally contributing to the clinical picture of the disease. It seems that the development of AD skin lesions results from the sequential activation of Th2 and then Th1 type cells. This process may be at least partially controlled by cells in the microenvironment of the skin of AD patients. It is therefore important to stress that from an immunological standpoint, Th2 and Th1-type immune responses are not mutually exclusive, and human inflammatory diseases like AD are based on interactions between different Th-cell subsets, which may account for the clinical characteristics of the disease (15). A considerable number of studies published over the last several years demonstrated that SIT may influence the deviated immune response of allergic patients in a specific manner and finally redirect the immune system towards normal immunity (16-18). The potential underlying mechanism of the repolarization of T-cell activity towards a Th1 pattern in the course of SIT might either be anergy or immune deviation. IL-10 seems to be involved in case of T-cell anergy. Perhaps the combination of anergy and a reactivation step by microenvironmental cytokines occurs, and anergic cells induced by IL-10 may recover through the influence of IL-2 and IL-15 to produce Th0/Th1 cytokines (16).

In this study, serum total IgE and asIgE directed against common airborne allergens were evaluated in the course of the 4-year SIT in patients with AD and airborne allergy. During the course of SIT, we also monitored serum levels of IFN-γ, sIL-2R, IL-4, IL-5 and IL-10 in order to analyse the possible influence of this type of therapy on the cytokine release in AD.

MATERIAL AND METHODS

Two groups of AD patients were investigated in this study. The SIT group consisted of 37 patients (25 females, 12 males, age range 5-44 years, mean age 18 years) with moderate to severe disease activity evaluated by the W-AZS index (19,20) 94.5 $\pm$ 39.7pts. All patients had excoriated skin lesions with intensive pruritus. In all cases, the IgE-mediated airborne allergy was confirmed (clinical evaluation, skin prick tests with aeroallergens, total IgE, and asIgE against aeroallergens). seventeen patients were allergic to grass pollen allergens, and 6 patients were allergic to grass and mugwort pollen allergens. In 14 cases, allergy

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to house dust mite allergens (*Dermatophagoides pteronyssinus – Dp and Dermatophagoides farinae – Df)* was diagnosed. In this group of patients, SIT with Novo-Helisen Depot vaccines was performed for the time period of 4 years.

The control group consisted of 29 patients with AD (19 females, 10 males, age range 5-41 years, mean age 17 years) with moderate to severe disease activity assessed by the W-AZS index – 86.9 ± 24.0 pts. No significant difference in the clinical activity of AD between these two investigated groups was detected before treatment. Patients selected for the control group filled all required criteria for SIT but did not decide to participate in allergy vaccinations. Eleven patients were allergic to grass pollen allergens, and 4 were allergic to grass and mugwort pollen allergens. In 14 cases, allergy to house dust mite allergens (*Dp* and *Df*) was diagnosed. In this group of patients, conventional methods of treatment were applied for the time period of 4 years.

- *Specific immunotherapy*

Novo-Helisen Depot allergy vaccines were used in the treatment of the SIT group. The composition of vaccines was individually selected according to the clinical and allergological characteristics of patients. The following allergy vaccines were used:

1. grass pollen allergens 100% in 17 cases
2. grass pollen allergens 80% mugwort pollen allergens 20% in 6 patients
3. house dust mite allergens *Dp* 50% and *Df* 50% in 14 cases

- *Conventional treatment* consisted of antihistaminic drugs (I and II generation), antipruritic agents, antiinflammatory agents, topical steroids, moisturizing and greasing agents.

- *Clinical assessment* in both investigated groups was performed before treatment and after 12, 24, 36 and 48 months of treatment. The W-AZS index was used for the clinical evaluation of patients (Tables 1 and 2).

- *Measurement of serum total IgE and asIgE* by the FEIA-CAP System technique (Pharmacia) was performed before treatment and after 24 and 48 months of treatment in both investigated groups of patients.

W-AZS INDEX

Table 1.

I. EVALUATION OF PRURITUS AND LOSS OF SLEEP IN PATIENTS WITH ATOPIC DERMATITIS

A. PRURITUS EVALUATION:

	POINTS
No pruritus	0
Pruritus is present:	
Extensiveness:	
1. Single or multiple localization of pruritus	2
2. Extensive pruritus involving the whole body surface	6
Frequency:	
1. Short episodes of pruritus - less than 30 minutes	2
2. Long-lasting pruritic episodes	4
3. Constant pruritus	8
Severity:	
1. Scratching is not necessary	2
2. Scratching is necessary	4
3. Anxiety and irritation caused by pruritus	8

B. LOSS OF SLEEP EVALUATION:

1. No loss of sleep	0
2. Problems in falling asleep	3
3. Night awakening caused by pruritus	6
4. Sleeplessness	12

I = A + B

W-AZS INDEX

Table 2.

II. EVALUATION OF EXTENSIVENESS AND SEVERITY OF SKIN INFLAMMATION IN PATIENTS WITH

Extensiveness of skin	lesions A	Severity of skin inflammation: erythema oedema	vesicles erosions	crusts scaling	lichenisation pigmentation B	A x B / 10
1. Face and neck	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
2. Scalp and nucha	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
3. Trunk (anterial surface)	( )x4=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
4. Trunk (posterial surface)	( )x4=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
5. Right arm	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
6. Right forearm and hand	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
7. Left arm	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
8. Left forearm and hand	( )x1=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
9. Right thigh	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
10. Right shank and foot	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
11. Left thigh	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
12. Left shank and foot	( )x2=	( )x3 +	( )x3 +	( )x2 +	( ) = ...	.....
					TOTAL	II

- Score extensiveness of skin lesions from 0 to 3:
  - 0 = not present
  - 1 = 1-10% of skin surface involved
  - 2 = 11-30% of skin surface involved
  - 3 = 31-100% of skin surface involved
- Score severity of skin inflammation from 0 to 3:
  - 0 = not present
  - 1 = mild
  - 2 = moderate
  - 3 = severe

TOTAL W-AZS : I + II

- *Measurement of IFN-γ, sIL-2R, IL-4, IL-5 and IL-10 serum levels*

In both investigated groups of AD patients, serum levels of selected cytokines were measured before the treatment and after 4 years of therapy by a colorimetric ELISA-Quantikine technique using a quantitative 2-step colorimetric sandwich ELISA (R&D System).

Statistical analysis

Statistical evaluations were performed by using the Student's t-test, ANOVA/MANOVA polydimensional analysis, Wilcoxon test and Mann-Whitney's U test. For an evaluation of correlations, Spearman's rank correlation coefficient was calculated for statistical significance.

RESULTS

- *Clinical assessment*

The results of the clinical evaluation of AD patients are presented in Table 3. From a clinical standpoint, there was no significant difference between the investigated groups before treatment. After 48 months of treatment, the mean value of W-AZS in the SIT group declined significantly ($p<0.001$). The clinical picture of patients in the control group also improved significantly ($p<0.001$). However, statistical analysis performed in order to compare the clinical efficacy of SIT and conventional methods showed a significant difference after 12, 24, 36 and 48 months of treatment, indicating a better efficacy of SIT ($p<0.01$; $p<0.001$; $p<0.001$; $p<0.001$ respectively).

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Table 3. Mean value of W-AZS index ± SD before treatment and after 12, 24, 36 and 48 months of therapy in both investigated groups

		Mean value of W-AZS index (pts) ± SD	
		SIT Group (n-37)	Control Group (n-29)
Before treatment	a	94.5 ± 39.7	86.9 ± 24.0
After 12 months of treatment	b	37.5 ± 30.4	56.0 ± 22.8
After 24 months of treatment	c	18.4 ± 17.6	53.3 ± 27.4
After 36 months of treatment	d	12.8 ± 17.1	45.4 ± 22.0
After 48 months of treatment	e	11.1 ± 19.1	47.2 ± 25.3

SIT Group
a/b – p<0.01
a/c; a/d; a/e – p<0.001

Control Group
a/b; a/c; a/d; a/e – p<0.001

- *Serum total IgE level*

The mean serum total IgE decreased significantly after 48 months of treatment with allergy vaccines (p<0.001). It also decreased in the control group, but the difference was not statistically significant (Table 4). Statistical analysis performed in order to compare this parameter in both investigated groups showed that before treatment there was no significant difference between the SIT and control groups. On the other hand, after 24 and 48 months of treatment, there was a significant difference between the two investigated groups (p<0.001) to the advantage of the SIT patients.

- *asIgE serum level*

Fourteen patients in the SIT group were allergic to d1(*Dp*) and d2 (*Df*). The mean serum level of asIgE against d1 and d2 decreased significantly after 48 months of treatment (p<0.001) (Table 5). In the control group, the mean serum level of asIgE against d1 and d2 also decreased during 48 months of conventional therapy, but the difference was not significant (Table 5). Comparative statistical analysis showed no significant differences between the SIT and control groups before treatment. After 24 months of therapy, however, a significant difference was detected between the asIgE measurement results of the investigated groups, d1 – p<0.01 and d2 – p<0.01 to the advantage of the SIT group. In addition, after 48 months of therapy, a significant difference was found between the groups: d1 – p<0.001 and d2 – p< 0.001.

Seventeen patients in the SIT group and 11 patients in the control group were allergic to grass pollen allergens: g3 - cocksfoot, g4 - meadow fescue, g5 – rye grass, g6 – timothy grass, g12 – cultivated rye. The mean serum levels of asIgE against grass pollen allergens are presented in Table 6. In the SIT group, the mean serum level of asIgE against selected grass pollen allergens was significantly lower after 48 months of treatment than before treatment (g3: p<0.001, g4: p<0.05, g5: p<0.001, g6: p<0.001, g12: p<0.001). In the control group, the mean serum level of asIgE

Table 4. Mean serum IgE level (KU/l) ± SD in the SIT group (n-37) and in the control group (n-29) in the course of therapy

		Mean value of total IgE (KU/l) ± SD	
		SIT group	Control Group
Before treatment	a	2691.9 ± 3984.1	1839.6 ± 962.7
After 24 months of treatment	b	805.9 ± 732.2	2013.6 ± 128.8
After 48 months of treatment	c	71.,8 ± 669.2	2586.1 ± 1223.4

SIT Group
a/b - p<0.001
a/c - p<0.001

Control Group
a/b; a/c; b/c – N.S.

Table 5. Mean serum level of serum asIgE against house dust mite allergens in the SIT group (n-14) and in the control group (n-14).

Mean value of antigen specific IgE (KU/l) ± SD					
		SIT Group		Control Group	
		d1	d2	d1	d2
Before treatment	a	244.7 ± 320.4	255.2 ± 268.7	222.3 ± 269.2	251.0 ± 241.8
After 24 months of treatment	b	101.4 ± 203.9	87.9 ± 150.2	217.9 ± 268.5	255.0 ± 235.2
After 48 months of treatment	c	32.2± 41.4	27.5 ± 33.4	207.7 ± 246.4	229.2 ± 209.9

d1 – *Dermatophagoides pteronyssinus*
d2 – *Dermatophagoides farinae*

SIT Group
d1: a/b – p<0.01
a/c – p<0.001
d2: a/b – p<0.05
a/c – p<0.001

Control Group
d1: a/b; a/c - N.S.
d2: a/b; a/c - N.S.

against selected grass pollen allergens decreased significantly after 48 months of therapy only in case of g3 and g4 (p<0.01 and p<0.05 respectively). Statistical comparative analysis of the results of asIgE measurements performed before treatment between the SIT and control groups showed no significant difference. On the other hand, after 24 and 48 months of therapy there was a significant difference between these groups to the advantage of the SIT group. After 24 months of therapy: g3 – p<0.0001; g4, g5, g6, g12 – p<0.001, and after 48 months of therapy: g3, g4, g5, g6, g12 – p<0.001.

Table 6. Mean serum level of as IgE against grass pollen allergens in the SIT group (n-17) and in the control group (n-11)

		Mean value of antigen specific IgE (KU/l) ± SD				
		SIT Group				
		g3	g4	g5	g6	g12
Before treatment	a	121.3 ± 95.3	143.1 ± 158.6	128.0 ± 152.7	139.9 ± 177.7	143.9 ± 185.0
After 24 months of treatment	b	40.9 ± 34.3	44.4 ± 37.9	35.6 ± 27.7	36.4 ± 32.6	37.7 ± 34.4
After 48 months of treatment	c	20.4 ± 22.0	20.0 ± 22.0	20.7 ± 19.4	19.0 ± 20.6	17.4 ± 17.3
		Control Group				
		g3	g4	g5	g6	g12
Before treatment	a	143.7 ± 50.7	149.1 ± 46.6	138.0 ± 51.4	140.0 ± 70.9	110.4 ± 40.4
After 24 months of treatment	b	186.0 ± 66.9	182.4 ± 73.9	142.5 ± 49.9	176.8 ± 79.6	133.6 ± 54.6
After 48 months of treatment	c	196.2 ± 85.7	194.7 ± 65.3	156.3 ± 54. 2	170.8 ± 70.1	128.6 ± 50.7

SIT Group
g3: a/b – p<0.05
a/c – p<0.001
g4: a/b – N.S.
a/c – p<0.001
g5: a/b – p<0.05
a/c – p<0.001
g6: a/b – p<0.05
a/c – p<0.001
g12: a/b – p<0.05
a/c – < 0.001

Control Group
g3: a/b; a/c – p<0.01
g4: a/b; a/c – p<0.05
g5: a/b; a/c – N.S.
g6: a/b; a/c – N.S.
g12: a/b; a/c – N.S.

Table 7. Mean serum level of as IgE against grass and mugwort pollen allergens in the SIT group (n-6) and control group (n-4)

	Mean value of antigen specific IgE (KU/l) ± SD					
	SIT Group					
	g3	g4	g5	g6	g12	w6
Before treatment a	35.5 ± 28.3	39.0 ± 34.3	53.9 ± 52.4	56.7 ± 80.2	38.9 ± 39.5	37.3± 33.8
After 24 months of treatment b	66.7± 107.8	47.7 ± 47.5	47.5 ± 63.3	64.4 ± 104.6	36.9 ± 42.2	28.2± 57.3
After 48 months of treatment c	29.3 ± 45.2	26.1 ± 31.5	24.6± 31.2	26.4 ± 42.9	23.3± 35.8	14.0± 30.3
	Control Group					
	g3	g4	g5	g6	g12	w6
Before treatment a	48.8 ± 39.9	40.0 ± 24.1	44.0 ± 29.7	38.3 ± 27.1	45.0 ± 14.2	43.0± 19.0
After 24 months of treatment b	82.3 ± 50.0	57.9 ± 32.3	62.2 ± 10.3	54.5 ± 18.7	82.6 ± 11.7	37.8± 20.6
After 48 months of treatment c	103.6± 36.9	77.0 ± 16.4	80.4 ± 36.1	68.9 ± 22.2	102.4± 39.7	36.1± 21.4

SIT Group
g3: a/b; a/c - N.S.
g4: a/b; a/c - N.S.
g5: a/c - $p < 0.05$
a/b - N.S.
g6: a/b; a/c - N.S.
g12: a/b; a/c - N.S.
w6: a/c - $p < 0.01$
a/b - N.S.

Control Group
g3: a/b; a/c - $p < 0.001$
g4: a/b; a/c – N.S.
g5: a/b; a/c – N.S.
g6: a/b – N.S.
a/c - $p < 0.05$
g12: a/b; a/c – $p < 0.05$
w6: a/b; a/c – N.S.

six patients in the SIT group and 4 patients in the control group were allergic to grass pollens and mugwort pollen allergens (w6). The mean serum level of asIgE against these allergens is presented in Table 7. After 48 months of treatment with allergy vaccines, we recorded a significant decrease of asIgE directed against g5 and w6 ($p<0.05$ and $p<0.01$ respectively). In the control group, a significant decline was observed in case of g3, g6 and g12 after 48 months of therapy ($p<0.001$, $p<0.05$ and $p<0.05$ respectively). Comparative statistical analysis of measurements of serum as IgE performed between the SIT and control groups indicated that there was no significant difference before treatment. After 48 months of therapy, however, significant differences were found in case of g3 ($p<0.05$), g4 ($p<0.01$), g5 ($p<0.05$) and g12 ($p<0.05$) to the advantage of the SIT group. In case of g6 and w6, there was no significant difference after both 24 and 48 months of therapy.

• *Correlational analysis between the clinical score and the results of total IgE and asIgE measurements*

In the global population of AD patients investigated in this study (the SIT and control groups), there was a significant correlation before treatment between the clinical score assessment and total serum IgE measurements ($p<0.001$) (Fig. 1). After 48 months of treatment, a significant correlation between the above-mentioned parameters was found in the SIT group ($p<0.01$) (Fig. 2). There was no significant correlation between the clinical score and serum asIgE measurements in either investigated group before treatment or after 48 months of therapy.

Serum levels of selected cytokines (Table 8)

• *IFN-γ*

In the SIT and control groups, the mean serum level of IFN-γ increased during 48 months of treatment but with no statistical significance. Also, there was no significant difference between the two investigated groups either before treatment or after 4 years of therapy.

• *sIL-2R*

The mean serum level of sIL-2R in the SIT group decreased significantly after 4 years of therapy ($p<0.01$). It also decreased in the control group but with no significant difference. There was

Table 8. Mean serum levels of selected cytokines (pg/ml) ± SD in atopic dermatitis patients before treatment and after 48 months of therapy in the SIT group (n-37) and in the control group (n-29)

	Mean serum level of selected cytokines (pg/ml) ± SD				
	SIT Group				
	IFN-γ	sIL-2R	IL-4	IL-5	IL-10
Before treatment	9.3 ± 16.7	2090 ± 897.9	25.1 ± 37.7	0.5 ± 1.4	5.2 ± 4.8
After 48 months of treatment	27.1 ± 62.4	1798.5± 624.6	20.6 ± 29.0	0.8 ± 2.4	5.6 ± 7.0
	Control Group				
	IFN-γ	sIL-2R	IL-4	IL-5	IL-10
Before treatment	8.9 ± 18.4	1929.6± 703.8	22.5 ± 30.7	0.3 ± 1.1	3.1 ± 3.7
After 48 months of treatment	19.8 ± 46.0	1617.5± 446.0	28.7 ± 31.9	0.9 ± 2.6	3.7 ± 3.3

no significant difference between the two investigated groups of AD either before or after 4 years of therapy.

• *IL-4*

In the group of SIT patients, the mean serum level of IL-4 after 4 years of therapy slightly decreased, while it increased significantly in the control group ($p<0.01$). There was no significant difference between the SIT and control groups either before treatment or after 4 years of therapy.

• *IL-5*

The mean serum level of IL-5 in the SIT group slightly increased after 4 years, and in the control group it increased significantly ($p<0.05$). There was no significant difference between patients treated with SIT and patients in the control group either before or after 48 months of therapy.

• *IL-10*

Patients treated with SIT and conventional therapy presented a slight increase in their IL-10 level after 4 years of treatment. There was also no significant difference between the two investigated groups either before or after 4 years of therapy (Table 7).

• *Correlation between serum IFN-γ, sIL-2R, IL-4, IL-5 and IL-10 levels and the clinical score*

There was no significant correlation among the serum levels of IFN-γ, sIL-2R, IL-4, IL-5 and IL-10 in the two investigated groups of AD patients either before treatment or after 48 months of therapy. No significant correlation between the clinical score (W-AZS) and measurements of serum cytokines levels was found in either the SIT or control group before treatment or after 48 months of therapy.

DISCUSSION

AD patients selected for the study presented high serum levels of total IgE and asIgE against aeroallergens. Therefore, it was indicated that

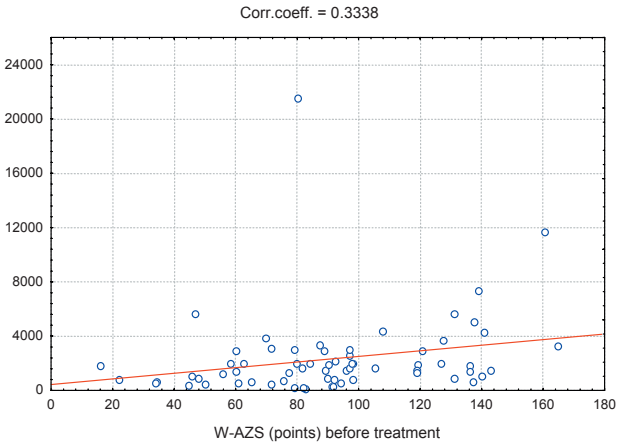

Figure 1. Correlation between total IgE measurements and the clinical score (W-AZS) in the global population of AD patients (n-66) before treatment ($p<0.01$)

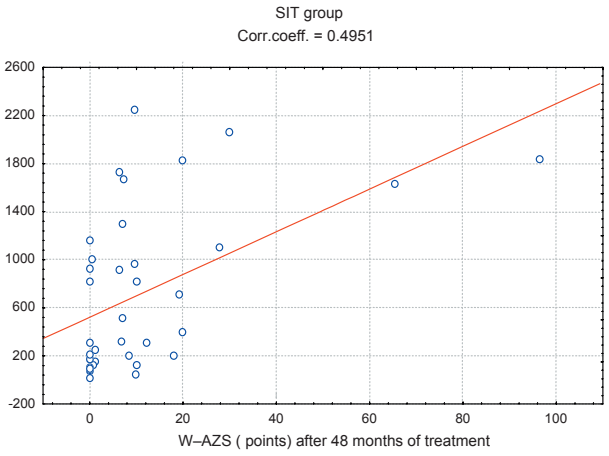

Figure 2. Correlation between total IgE measurements and the clinical score (W-AZS) in the SIT group of AD patients (n-37) after 48 months of therapy ($p<0.01$)

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IgE-mediated allergy was involved in the development of their skin lesions. Patients in the SIT group and in the control group were highly comparable from both the clinical (W-AZS index score) and allergological standpoints (total IgE, asIgE serum levels). Thus, the impact of different methods of treatment on the mentioned parameters could be assessed. In the group of SIT patients, a gradual decline in the mean total IgE level was recorded in the course of the 4 year treatment, which was especially noticeable after the first year of SIT. After both 2 and 4 years of treatment with allergy vaccines, the serum total IgE measurements were significantly lower for SIT patients than in the control group. Patients treated with conventional methods presented a noticeable increase tendency in the mean total IgE level. Therefore, the opposite influence of treatment applied to total serum IgE was registered in the two investigated groups. This immunological parameter is still considered to be very important in about 80% of AD patients with an allergic type of the disease, and the decreasing influence of SIT on total IgE should therefore be highlighted.

In the case of the whole population of AD patients (the SIT group and the control group), there was a significant correlation between the clinical status of patients and IgE measurements before treatment. After 48 months of treatment, a significant correlation was recorded only in the SIT group It could therefore be concluded that SIT modulates the immunological status of AD patients with noticeable implications on the severity of the inflammatory process. Various data in the literature concerning the correlation between the clinical picture of AD patients and the serum total IgE level are rather contradictory, although many authors have observed that these parameters are strongly correlated (21-25). Perhaps these contradictions are related to a different selection of patients for investigations and various methods of clinical and statistical assessment.

The results of asIgE measurements demonstrated a clear tendency of decrease in the SIT group. This was rather expected, as SIT is known to result in an initial increase and further decrease of serum asIgE levels in atopic patients (26-28). However, all available information about the mentioned phenomenon are based on studies performed in other than AD atopic diseases. This study therefore demonstrated that in AD patients, SIT has an effect on asIgE levels similar to the effect observed in other investigated atopic diseases.

In the group of patients allergic to house dust mites, a significant difference between asIgE measurements before and after 24 and 48 months of SIT was observed in case of both d1 and d2 allergens. There was also a decrease of mean serum asIgE (d1,d2) in the control group, but no significant difference was detected. After both 24 and 48 months of therapy, asIgE levels against d1 and d2 in the control group were significantly higher than in the SIT group. SIT has been previously reported by Pacor *et al.* (29) to decrease the level of asIgE against *Dp* in patients with AD in the course of a 3-year therapy. On the other hand, Zachariae *et al. (37)* have not recorded any influence of SIT on a group of 12 patients with severe AD during 6 months of treatment. Perhaps the 6 month period of SIT was not sufficient to modulate the level of asIgE in a distinct manner, and a prolonged and systematic treatment would be necessary for this phenomenon to occur. Likewise, Glover and Atherton (30) have not registered a decreasing impact of SIT on asIgE levels in AD patients allergic to house dust mite allergens. But again, only 12 weeks of therapy seems to be rather short to expect such changes to take place. In the cited investigation, no significant influence on the results of skin prick tests and total IgE serum levels was observed, which is contradictory to our results. (Skin prick tests in our group of investigated patients showed a clear tendency for negativization – results are not presented in this paper.)

In case of monoallergy (*Dp*), we observe a very good clinical efficacy of SIT, and noticeable variations of clinical score and immunological parameters are expected. As mentioned above, however, a longer period of SIT is required for these events to occur. In the group of AD patients allergic to grass pollen allergens and treated with Novo-Helisen Depot allergy vaccines, a noticeable decrease of mean serum asIgE against g3, g4, g5, g6 and g12 was observed. On the other hand, there was a clear tendency of the asIgE mean level in the control group to increase. After both 24 and 48 months of therapy, there was a significant difference of asIgE levels against all investigated grass pollen allergens between the SIT and control groups to the advantage of patients treated with allergy vaccines.

Patients allergic to grass and mugwort pollen allergens reacted to the treatment somewhat differently. In fact, it was the smallest group–only 10 patients (6 in the SIT group and 4 in the control group)-and therefore a proper analysis of the results is rather impossible. At any rate, it is inter-

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esting that in case of g3, g4 and g6, there was an increase in the mean serum asIgE level after 24 months of SIT, which then decreased twofold after 48 months of therapy in comparision with the mean level of asIgE before treatment. In case of asIgE against g5 and g12, a decrease in the mean serum levels during the course of treatment was observed. Only in case of g12 and w6 a significant difference was detected after 24 months of SIT in comparison with results obtained before the treatment.

In the control group, there was a clear tendency of the asIgE level against grass pollen allergens to increase and the asIgE level against w6 to decrease. After 4 years of treatment, there was a significant difference in asIgE measurements for all grass pollen allergens investigated between the SIT and control groups to the advantage of the SIT group. Such a difference was detected for w6 after both 24 and 48 months of treatment. These results indicated that in patients allergic to grass pollen allergens, an additional hypersensivity to w6 created a noticeable difference in the course of SIT from both the clinical and immunological standpoints. It might be related to a polyvalency of allergy, a wider exposition to allergens and the complexity of allergy vaccines' composition. Thus, it seems that AD patients allergic to grass pollen allergens and those who are additionally allergic to w6 should be regarded as two different subpopulations. Unfortunately, there are no data in the literature which consider this problem, as all investigations on the influence of SIT on asIgE serum levels in AD patients were performed on populations allergic to house dust mites.

It is also of allergological and clinical interest to note whether there are any correlations between the clinical severity of the disease and asIgE levels in the sera of patients. No such correlations have been detected in either group of AD patients in this study, either before treatment or after 24 and 48 months of treatment.

Very little is known about cytokine production and release in AD patients in the course of SIT. We evaluated Th1 and Th2 related cytokines before treatment and after 4 years of SIT or conventional therapy. No significant differences were found between the two investigated groups of patients either before treatment or after 4 years of therapy. In the SIT group, there was a significant decrease in the serum sIL-2R level after 4 years of therapy, clearly indicating an immunomodulatory influence of SIT in AD. The mean serum levels of IL-4 and IL-5 increased significantly in the control group after 4 years of treatment. These results provided clear evidence that patients with AD react in a different manner to SIT than to conventional methods of treatment.

It is known that AD patients with moderate to severe disease activity are characterized by an impaired capacity of their T-cells to release IL-2 in vitro (31). This deficiency may be relatively specific, since no significant changes could be detected in patients with severe plaque-type psoriasis. Decreased IL-2 production may significantly correlate with the eczematous involvement of patients' skin. It is also documented that T lymphocyte activation represents a very complex process, which involves a variety of cytokines, including IL-1 and IL-2 (31). IL-1 is released by monocytes and thought to represent one of the accessory cell-derived signals required for efficient T-cell activation. In contrast, IL-2 is produced by activated T-cells and known to upregulate the expression of its own receptor (31). Functional alternations of T-cells such as decreased lymphoproliferative responses (32-34), could be the result of a diminished capacity of mononuclear cells to release IL-1 and IL-2 upon appropriate stimulation. It has been shown, however, that the sera of AD patients as well as psoriasis contained significantly increased levels of sIL-2R, as compared with healthy controls (35). Thus, the finding of increased sIL-2R levels is in apparent conflict with decreased lymphoproliferative responses *in vitro*. A supposed T-cell defect which could be shown by functional assays in the peripheral blood is therefore in direct contrast to signs of hyperactivation.

One possible explanation for the contradictory findings would be that the inflammatory process in AD is focused on the skin. Therefore, perhaps the decreased production of cytokines by blood mononuclear cells is due to a down-regulation of the immune response in the peripheral blood induced by cytokines released from hyperactivated immune cells in the inflammed skin. Alternatively, the hyporesponsiveness of mononuclear cells in vitro could be a sign of "exhaustion" following excessive stimulation *in vivo*. These suggestions are supported by the finding that alternations of district T-cell functions normalized with clinical remissions (33,34,36).

It seems that a decrease of sIL-2R serum in the course of treatment indicates the clinical improvement of AD patients (37). This phenomenon occurred in both groups of investigated patients, but

it was significant only in the SIT group. It should also be mentioned that a clinical improvement was noticed in both the SIT and control groups, but it was more distinct on patients treated with allergy vaccines. Therefore, it is not surprising to us that the sIL-2R serum level decreased more significantly in the SIT group.

Data in the literature on IL-4 production in AD are controversial. Kasamatsu *et al*. (38) detected higher levels of IL-4 in AD patients than in healthy controls, while other authors have not confirmed this observation (39). In our study, mean levels of IL-4 and IL-5 were not higher than the reference values for healthy controls. In the course of treatment in the SIT group, the mean level of IL-4 decreased slightly and the mean level of IL-5 increased slightly. On the other hand, conventional therapy resulted in a significant increase of IL-4 and IL-5 serum levels. IL-10 serum levels were characterised by a slight tendency to increase in both investigated groups after 4 years of therapy.

In conclusion, SIT appeared to be an effective method of treatment in case of selected patients with AD and airborne allergy. The possible application of this method in selected cases of AD has recently been one of our major interests, and our previous clinical results were satisfactory (40-44). In the course of SIT, a significant decrease of total IgE and asIgE against aeroallergens in the sera of patients was observed. These observations differentiated the SIT group from the control group of AD patients treated with conventional methods. Although it is not clear what the real role of IgE is in the mechanism of successful SIT, it seems that IgE definitely participates in this event not only in case of venom allergy or allergic rhinitis but also in AD patients treated with allergy vaccines. At the moment, it is difficult to properly evaluate the possible effect of SIT on the release of investigated cytokines, but these preliminary results indicate a complexity of events occurring in the course of allergy vaccinations in AD. We hope to investigate this problem further in the future.

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