

Variations in serum levels of interleukin (IL)-1 β , IL-2, IL-6, and tumor necrosis factor- α during specific immunotherapy

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Background: Cytokine production by T helper cells is essential for the induction and maintenance of allergic inflammation in the bronchial mucosa. According to recent views, specific immunotherapy (SIT) favors the differentiation of T lymphocytes into cells of the Th1 rather than those of the Th2 subset.

Objective: To determine whether or not SIT induces a decrease in the inflammatory reaction by studying eventual variations in the serum levels of IL-1 β , IL-2, IL-6, and TNF- α in allergic subjects during SIT.

Methods: Serum levels of IL-1 β , IL-2, IL-6, and TNF- α were determined before and after 3, 6, and 9 months of SIT by an immunoradiometric assay (IRMA) in 11 adults with perennial allergic asthma and/or rhinitis caused by house dust mites and in 6 nonatopic healthy volunteers.

Results: Median serum IL-1 β and TNF- α levels of the patients were significantly higher at baseline than those of the controls and decreased during SIT to values similar to or lower ($P < .01$) after 6 months of SIT for TNF- α than those of the controls. Median serum IL-2, significantly lower at baseline than in the controls, increased during SIT to a level similar to that of the controls. Although the median values of IL-1 β and TNF- α in the patients tended to decrease and those of IL-2 to increase during SIT, the differences were not significant; the correlation coefficients (r) of the serum levels of IL-1 β , IL-6, and TNF- α versus duration of SIT were negative, while that of IL-2 was positive.

Conclusions: Decreases in median serum IL-1 β and TNF- α levels during SIT, together with the increases in serum IL-2 and IL-6, compared with those of the controls furnish evidence supporting a reduction in the inflammatory response in the course of SIT.

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INTRODUCTION

Specific IgE production to environmental allergens, a characteristic of the atopic population, is attributable to bypass of the mechanism of immune tolerance, which blocks activation of Th2 cells. What distinguishes atopic from nonatopic subjects is the subpopulation of T helper (Th) cells that is activated; in atopics it consists predominantly of Th2 cells, while in nonatopics it consists predominantly of Th1 cells.¹

These two clones of lymphocytes play a fundamental role in the pathogenesis of the allergic reaction. IgE synthesis does not occur in vitro or in vivo in the absence of IL-4, which together with IL-3, IL-6, IL-10, IL-13, and TNF- α , is produced by Th2 lymphocytes, whereas IL-2, INF- γ , and TNF- α are produced by Th1 lymphocytes. Indeed, it has been shown that cytokine production by Th cells is essential for the induction and maintenance of allergic inflammation in the bronchial mucosa.^{2–6}

Various other cytokines enhance IgE production. IL-2 induces expansion of T cell clones. IL-5 stimulates the proliferation and differentiation of cells of the eosinophil lineage, while both IL-5 and TNF- α activate eosino-

phils. IL-6 stimulates the proliferation and differentiation of B cells and IL-9 promotes mastocyte proliferation. IL-10 inhibits cytokine synthesis by Th1 cells and also potentiates the effects of IL-4 by inducing the expansion of T cell clones.^{7–9}

IL-1, a ubiquitous pleiotropic cytokine composed of α and β subunits, is produced mainly by monocytes and macrophages. IL-1 is the principal mediator of the acute phase of the inflammatory reaction. In addition to inducing the proliferation and differentiation of T and B cells, IL-1 activates eosinophils and neutrophils and stimulates the production of chemotactic cytokines (ie, IL-8).^{10,11}

In this study, serum levels of IL-1 β , IL-2, IL-6, and TNF- α were determined on peripheral blood samples of 6 nonatopic controls and 11 allergic patients with respiratory symptoms (asthma and/or rhinitis) before and after 3, 6, and 9 months of specific immunotherapy (SIT). The decreases in serum IL-1 β and TNF- α during SIT at the same time as the increases in serum IL-2 and IL-6 to levels similar to those of the controls suggest a reduction of the allergic inflammatory response in the allergic patients.

SUBJECTS

Eleven adults with perennial allergic asthma and/or rhinitis caused by house dust mites were recruited from new outpatients attending the Allergy Clinic of the Institute of Respiratory Diseases of the University of Pavia. All patients tested positive on skin testing and RAST for *Dermatophagoides pteronyssinus* (DPP). Six healthy volunteers with a negative history of atopic disease and negative skin test results were included in the study as controls.

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Table 1. Median Values of Sera IL-6, IL-1 β , IL-2 and TNF- α in the Controls and in the Patients at Baseline (Time 0) and after 3,6, and 9 Months of SIT

	CONTROLS n = 6	PATIENTS n = 11			
		T(0)	T(3)	T(6)	T(9)
IL-6	7.98	88.2	198.6	67.71	32.16
pg / mL		$P < 0.001$	$P < 0.01$	$P = 0.0007$	
IL-1 β	4.96	114.32	1.73	13.91	15.58
pg / mL		$P = 0.000007$	ns	ns	ns
IL-2	0.79	0.28	0.74	1.76	0.72
U / mL		$P = 0.02$	ns	ns	ns
TNF- α	10.99	30.48	10.65	3.53	9.59
pg / mL		$P = 0.001$	ns	$P = 0.01$	ns

ns = not significant (Mann Whitney *U* test)

After obtaining informed consent, all subjects underwent skin testing (prick) with a battery of common aeroallergens (NeoAbello, Madrid, Spain) and a histamine control to ensure normal skin responsiveness. Oral antihistamines and antidepressants with antihistaminic properties were withheld for at least 48 hours before skin testing, and patients on astemizole were skin tested only after suspension of the drug for at least 6 weeks. Skin test responses were read within 15 to 20 minutes after testing; the largest wheal and flare diameters were recorded for each allergen tested. A response was considered positive in the presence of a wheal diameter of at least 5 mm with a flare diameter of 8 mm or more.

All study patients were treated with SIT using commercial extracts of DPP (Neo Abello). The extracts were administered subcutaneously according to a standard dose schedule at weekly intervals for about 3 months with increasing doses of DPP extract up to a maintenance dose of 0.8 mL (1000 STU), which was continued once a month for the next 6 months.

Peripheral blood samples were drawn from all patients and controls for determination of IL-1 β , IL-2, IL-6, and TNF- α under baseline conditions over a period of 2 months, in the patients 15 to 30 days before the first dose of SIT, and again after 3, 6, and 9 months of SIT.

METHODS

IL-1, IL-2, IL-6, and TNF- α were determined by an immunoradiometric as-

say (IRMA, Medgenix) based on coated-tube separation and an oligoclonal system which employs several monoclonal antibodies (MAbs) directed against distinct epitopes of IL-2.

MAbs1, the captured antibodies, are coated to the lower inner surface of the plastic tube. Standards or samples added to the tubes first show low-affinity antibodies. The signal antibodies labeled with ^{125}I complete the oligoclonal system and trigger the immunologic reaction. After washing, the radioactivity bound to the tube reflects antigen concentration. The use of several distinct MAbs avoids hyperspecificity common to two-site IRMA.

Statistical Analysis

The median serum cytokine levels in the allergic patients at baseline and after 3, 6, and 9 months of SIT were compared with those of the controls using the Mann-Whitney *U* test. The relationship between the median serum

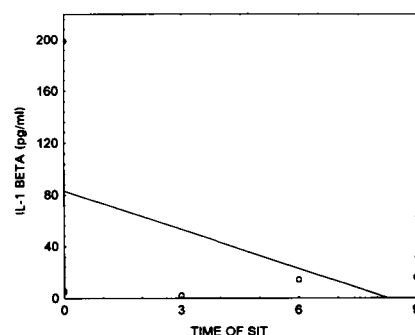


Figure 1. Correlation coefficient ($r = -0.2$) between the median values of IL-1 β and the duration of SIT in months.

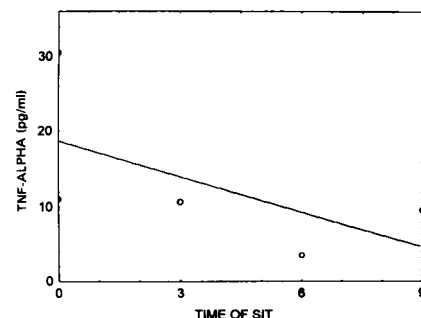


Figure 2. Correlation coefficient ($r = -0.8$) between the median values of TNF- α and the duration of SIT in months.

values of each cytokine and time after SIT was analyzed by the Spearman rank test (r). These nonparametric tests were used because the sample size was small and the distribution of the data was not normal.

RESULTS

The median values of the four cytokines in the controls as well as in the patients at baseline (time 0) and after 3, 6, and 9 months of SIT are reported in Table 1. The results of the statistical analyses comparing the medians of the control group with those of the patients at each of the four sampling times are also shown there. The baseline values of IL-1 β , IL-2, IL-6, and TNF- α were significantly different in the two groups; in the patients, baseline values of IL-1 β , IL-6, and TNF- α were higher and IL-2 was lower than in the controls. Comparison of cytokine concentrations during SIT in the patients with those of the controls revealed similar

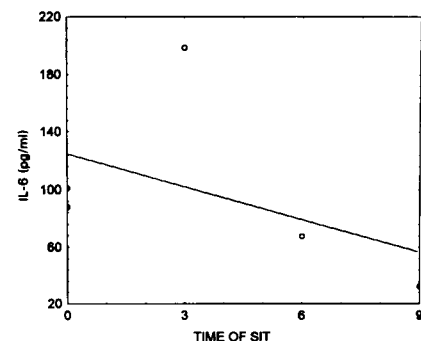


Figure 3. Correlation coefficient ($r = -0.8$) between the median values of IL-6 and the duration of SIT in months.

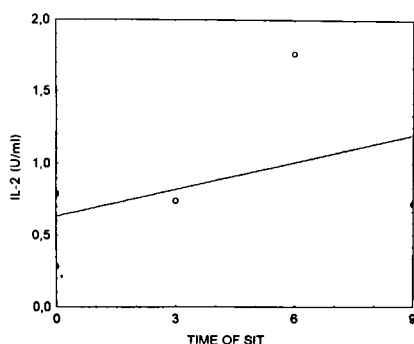


Figure 4. Correlation coefficient ($r = 0.4$) between the median values of IL-2 and the duration of SIT in months.

levels of IL-1 β and TNF- α in the two groups at all times, except after 6 months of SIT when TNF- α was significantly lower ($P < .01$). Serum IL-6 remained significantly higher in the patients after 3 and 6 months of SIT than in the controls but returned to similar levels after 9 months of SIT.

Serum levels of IL-1 β and IL-6 during SIT tended to decrease with respect to baseline, whereas IL-2 increased in the patients; however, the differences did not reach statistical significance. The correlation coefficients (r) between the median cytokine values and the sampling times (Figs 1 to 4) are negative for IL-1 β ($r = -0.2$, $P = \text{ns}$), TNF- α ($r = -0.8$, $P = \text{ns}$), and IL-6 ($r = -0.8$, $P = \text{ns}$), but positive for IL-2 ($r = 0.4$, $P = \text{ns}$).

CONCLUSIONS

The variations observed in the serum levels of the cytokines evaluated in this study suggest a marked reduction in the inflammatory reaction as well as in the phenomena responsible for the bypass of immune tolerance during SIT. The increases of serum IL-2 and IL-6 levels, in light of their enhancement of cell-mediated immune responses, could represent a mechanism for overcoming the bypass of immune tolerance. In addition, the most com-

mon inflammatory mediators, TNF- α and IL-1 β , decreased during specific immunotherapy; IL-1 β decreased to levels similar to the control values and TNF- α to levels below the control values.

As hypothesized by others,^{12,13} SIT probably induces a switch from a predominantly Th2 to a predominantly Th1 response which results in increased IL-2 synthesis, decreased IgE production, and the recruitment of eosinophils and mastocytes. The presence of elevated numbers of eosinophils in the bronchial mucosa and secretions is associated with alterations of the mucosa, which are considered responsible for the symptoms of asthma. Thus, as some authors propose,^{14,15} it is possible that specific immunotherapy modulates the response of T cells to subsequent antigen exposure.

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