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A PRELIMINARY STUDY ON IL4 LEVELS IN EXTRINSIC ATOPIC ASTHMATIC CHILDREN*

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Interleukin 4, (IL4) known as a lymphokine secreted by type II helper T-cells, is thought to regulate IgG and IgE secretions. Therefore, elevated IL4 levels are expected in atopic allergic disesase and parasitoses.

The purpose of this study was to determine IL 4 levels in allergic asthmatic children. In 50 extrinsic atopic children (19 females, 31 males, mean age 8±4), IgE and IL 4 were found to be 469±296 U/L and 0.318±0.09 ng/ml, respectively. In seven control cases, IgE and IL4 levels were 62±25 and 0.106±0.017, respectively.

Comparison of the two groups disclosed statistically significant differences in IL4 and IgE levels, suggesting the ability of IL4 to augment IgE production. *Key words: helper T cell, lymphokine, interleukin 4, atopic asthma.*

T-lymphocytes regulate immunoglobulin production via lymphokines, soluble immunomodulator substances. These T-cell-derived, multifunctional lymphokines adjust the activation, growth and differentiation of multiple cell types[1,2].

A subgroup of the T-lymphocytes, helper T-cells are classified according to the lymphokines they secrete. Type I helper T-cells (THI) secrete interleukin 2, interferon, granulocyte and macrophage colony stimulating factors and interleukin 3; type II helper T-cells release interleukin 3, interleukin 4, mast cell and T-cell growth factors[2,3].

The subject of this study, interleukin 4, (IL4) also named B-cell stimulating factor 1 (BSFI), was first described in 1982 as a cofactor. In vivo and in vitro studies have since shown that IL4 is a potent stimulator of IgG and IgE production[3,4].

The human condition most commonly associated with increased IgE antibody production is atopy, the common familial allergic disorder of immediate-type

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hypersensitivity to environmental allergens. In vivo studies have demonstrated that IgE production is critically dependent on IL4.

The present study was designed to determine IL4 levels and the association between IgE and IL4 in children with allergic asthma and a healthy control group.

Material and Methods

This study involved 50 extrinsic atopic asthmatic children attending Cerrahpaşa Faculty of Medicine-Immunology and Allergy out-patient clinics. Seven similar-aged healthy children chosen from the outpatient clinic as healthy children without a history of atopy comprised the control group.

The diagnosis of atopic asthma fit the related criteria[5]. Blood samples for IgE and IL4 determinations were collected at the time of first enrollment in the study. Nineteen of the patients were girls and 31 were boys each of them having a serum IgE level of 50 IU or more. IgE and IL4 levels were measured with RIA technique (Pharmacia), and ELISA assay (Genzym, human IL4 Elisa test strips), respectively.

The IL4 Elisa test kit is a solid phase enzyme immunoassay employing the multiple antibody sandwich principle. A murine monoclonal antibody specific for human IL4 was attached to microtiter wells in a 96 well plate. IL4 present in standard samples and unknown specimens was captured by the solid phase monoclonal. A rabbit polyclonal anti-human IL4 antibody was added to bind to multiple epitopes on the IL4 contained on solid phase. A third antibody, biotin labelled goat anti-rabbit Ig, was then applied to bind to the rabbit polyclonal antibody already attached to IL4.

Also added was a horseradish peroxidase conjugated streptavidin reagent, which binds to biotinylated goat anti-rabbit Ig. This enzyme acts with a peroxide and OPD containing substrate buffer to produce increased absorbance, indicating the presence of IL4. This reaction was halted at the appropriate point by the addition of a stop reagent.

Immunoreactive IL4 could then be quantitated by an ELISA reader. The measured absorbance was proportional to the concentration of IL4 that was present in the sample. A reference curve was obtained by plotting the IL4 concentrations of several dilutions of standard versus absorbance. Since the IL4 concentrations used to produce the standard curve were known, the amount of IL4 in the experimental samples could be determined by comparing their absorbance to that produced by the standards. Values between 0.025-2 ng/ml were the range that could be read by this system.

Student's t test was used for statistical analysis and a level of $p<0.05$ was considered significant.

Table I: Age, Sex Differences, IgE and IL4 Levels of the Patients

No	Name	Age (yrs)	Sex	IgE (IU/100 ml)	IL4 (ng/ ml)
1	B.O.	6	M	226	0.25
2	E.A.	8	F	462	0.30
3	B.T.	3	F	605	0.4
4	E.T.	3	M	319	0.3
5	S.M.	3	F	374	0.3
6	B.G.	7	F	231	0.3
7	S.K.	4	M	814	0.4
8	H.Ö.	6	M	550	0.35
9	O.G.	3	M	352	0.3
10	U.A.	7	M	154	0.2
11	A.Ç.	3	M	616	0.3
12	O.E.	4.5	M	200	0.3
13	Ö.Ç.	6	F	148	0.5
14	E.T.	6	M	330	0.3
15	S.B.	7	M	286	0.2
16	İ.Ş.	6	M	308	0.3
17	G.K.	3.5	F	429	0.3
18	S.K.	7	M	440	0.2
19	M.A.	3	M	550	0.3
20	Ö.A.	12	M	218	0.3
21	Ç.K.	9	M	134	0.3
22	İ.A.	12	M	978	0.45
23	A.K.	9	M	253	0.3
24	H.S.	13	M	550	0.2
25	N.O.	10	F	286	0.45
26	K.K.	7	M	297	0.5
27	N.B.	8	F	506	0.5
28	C.A.	14	M	566	0.3
29	F.Ç.	7	M	143	0.5
30	M.K.	10	F	385	0.2
31	T.K.	15	M	695	0.3
32	M.T.	11	M	1650	0.45
33	Ş.O.	10	M	660	0.3
34	Y.P.	14	F	664	0.3
35	Ü.Ç.	11	F	616	0.3
36	E.Ö.	6	F	1144	0.5
37	B.F.	11	M	814	0.35
38	M.P.	4	M	148	0.2
39	E.B.	8	F	462	0.3
40	E.B.	6	M	616	0.3
41	M.I.	4	F	145	0.2
42	B.G.	5	M	396	0.2
43	M.Ş.	12	M	682	0.2
44	Y.Y.	10	M	127	0.2
45	F.K.	13	F	264	0.3
46	İ.G.	13	F	527	0.35
47	G.H.	9	F	374	0.3
48	F.B.	14	F	1034	0.45
49	G.G.	7	F	248	0.3
50	F.E.	7	M	374	0.3
Mean		8±4	19 Female 31 Male	469±296	0.318±0.09

Results

Age, sex distribution, IgE and IL4 levels of the patients and the control group are illustrated in Tables I and II. The mean age of the 50 asthmatic children was 8±4 years. Mean concentrations of IL4 and IgE of the patient population were found to be 0.318±0.09 ng/ml and 469±296 U/L successively (Table I). For the control group, the mean age was 4.2±2 years and the mean concentration of IL4 was 0.106±0.017 and of IgE was 6.2±25 U/L (Table II).

Table II: Age, Sex Distribution and IgE and IL4 Levels of the Control Group

No	Name	Age (yrs)	Sex	IgE (IU/100 ml)	IL4 (ng/ml)
1	A.Y.	6	M	16	0.09
2	T.Y.	7	F	46	0.12
3	P.A.	6	F	82	0.085
4	Z.D.	2	M	70	0.095
5	E.E.	2	F	72	0.12
6	H.E.	4	F	57	0.105
7	T.Y.	3	M	90	0.13
Mean		4.2±2	4F 3M	62±25	0.106±0.017

The presence of limited IL4 test samples restricted the number of control cases. None of the atopic patients revealed an IgE level considered to be normal in our laboratory (0-50 IU/L).

The assessment of IgE and IL4 levels between the asthmatic and control cases disclosed statistically significant differences ($p<0.001$) (Table III).

Table III: Comparison of the Study Group with the Control Group

	n	Mean Age (yrs)	IgE (IU/100 ml)	IL4 (ng/ml)
Asthmatic patients	50	8±4	469±296	0.318±0.09
Control group	7	4.2±2	62±25	0.106±0.017
			t: 3.6	t: 5.34

Discussion

During the past several years, it has become clear that regulation of the growth of both T-cells and B-cells involves signals not only from antigen-specific cell surface receptors but also from lymphokines. The understanding of the mechanism by which lymphokines affect the growth of lymphocytes is crucial in evaluating the regulation of immune responses.

The subject of this study, IL4, was first described in 1982 as a co-factor in the proliferation of resting B-cells stimulated through the cross-linkage of their membrane Ig by anti-Ig antibodies[6]. IL4, a 20,000 dalton glycoprotein produced by some activated T-cells, has gained popularity especially after Mosmann et al's work[7-9]. These investigators indicated the existence of two broad classes of mouse T-lymphocytes, designated as T_{H1} and T_{H2}, and stated that IL4 is produced by the T_{H2} subgroup.

It has now become clear that IL4 acts on resting B-cells as well as late G_1 B-cells, T-cells, and multiple hematopoietic cell lineages[10-12].

IL4 is a potent stimulant of IgG_1 and IgE secretion by B-cells[13,14]. Experimental studies show that in vivo production of IgE is highly dependent on IL4. Mice infected with the larvae of the nematode Nippostrongylus brasiliensis display a striking increase in serum IgE. Administration of monoclonal anti-IL4 antibodies to these animals markedly inhibits this increase[15].

Likewise, injection of anti-IgD antibodies into mice causes a strong polyclonal activation of B-cells, which results in a marked increase in both serum IgG_1 and serum IgE levels. Purified monoclonal anti-IL4 antibody inhibits the increase in IgE completely, suggesting that anti-IgD induced IgE production is modulated at least in part by T-cell mediated IL4 production[16]. In our clinical study carried out on atopic asthmatic children, this fact is confirmed, implying that a correlation exists between IL4 secretion and IgE production.

Recently it has been shown that IL4 has the capacity to regulate the so-called VCAM-1 expression on endothelial cells, which is a very important clue in the early events after an allergen challenge in asthmatics[17]. It has again been shown in biopsy specimens from patients with asthma that pre-formed IL4 can be localized to mast cells[18]. These events provide an important link between mast cell activation, regulation of adhesion molecules and chemotaxis priming.

In certain diseases, B-cells could be induced to switch to IgE-producing cells in response to IL4. In common variable immunodeficiency, it is demonstrated that peripheral blood mononuclear cells could be induced to produce IgE[19].

Obviously, on the basis of this study, we do not presume to draw conclusions about the pathogenesis of atopy and of other diseases characterized by the hyperproduction of IgE. Future studies on the mechanisms underlying defective IgE synthesis, as in patients with immunodeficiencies, might contribute to the understanding of IgE production in both normal and allergic individuals.

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