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LYMPHOCYTE SUBPOPULATIONS AND SERUM IMMUNOGLOBULIN E (IgE) LEVELS IN ASCARIASIS*

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T and B lymphocytes are known to interact and act in cooperation with each other. In vitro studies support the fact that T-T and T-B cell interaction occurs by means of some soluble factors[1].

During antibody production, B lymphocytes are under control of the T cell subpopulations. Contrary to the facilitating effect of helper T lymphocytes on antibody production, suppressor T lymphocytes tend to decrease production[1-3]. A failure in this coordinating function of T lymphocytes will result in disorders in antibody production.

IgE is often found in high levels in atopic diseases, in most of the parasitic diseases and in some of the immune deficiency syndromes concurrent with depressed cellular immune response[2,3]. High serum levels of IgE in conditions such as the Wiskott-Aldrich syndrome, selective IgA deficiency, hyper-IgE syndrome and atopic diseases are explained by lack of the suppressing effect on antibody producing B cells because of insufficiency of suppressor T lymphocytes[2-8]. It is claimed that this insufficiency of T lymphocytes in atopic diseases is genetic[9,10]. In reviewing the literature, we were not able to find a study that examines the relationship between high IgE levels in ascaris infections and T cells.

This study aims at investigating the relationship between peripheral T and B lymphocytes, total eosinophil counts and serum IgE levels in cases of ascariasis.

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Material and Methods

The subjects of this study were chosen from among the patients visiting the out-patient clinic of Hacettepe University Children's Hospital. The study group consisted of 21 cases with ascariasis confirmed by stool examination. The control group consisted of 17 cases with negative stool examinations and with no history of parasitic disease. All of the subjects had negative personal and family histories of atopic diseases and their physical examinations were normal. In the ascariasis group there were 15 males and six females whose ages ranged between three and 17 years with a mean age of eight years. In the control group there were eight males and nine females whose ages ranged between two and 23 years with a mean age of 13.8 years.

During the study, none of the patients suffered from malnutrition nor any other disease which could effect the immune system. They were not receiving drugs. Leukocyte and total eosinophil counts were determined, and the absolute lymphocyte counts were calculated by the examination of blood smears. On the same day, venous blood with and without heparin was drawn. From the blood samples without heparin, sera were separated and stored in the refrigerator for IgE determination. Heparinized blood was used for quantitative determination of T and B cells. Total eosinophil counts were obtained by means of the Dacie and Lewis method[11]. Total serum IgE levels were determined by the PRIST method, using the Pharmacia Diagnostic kits.

Peripheral T and B lymphocyte counts were determined by the methods we had described in detail in a former study[8]. These methods for T and B lymphocyte determination may be summarized as follows: Peripheral lymphocytes were separated by the Boyum method[12]. E and HEAC rosettes were prepared[13], and the cells forming rosettes were counted to obtain the counts of T and B lymphocytes carrying Fc (IgG) and complement receptors, respectively. The monocyte/macrophage series and a minor subpopulation of T cells have receptors for Fc of IgG and C3b as well[14]; this fact was taken into consideration while evaluating the results.

Statistical evaluations were made by the Mann-Whitney U test and Student's *t* test.

Results

Our findings regarding the patients and control subjects are shown in Tables I and II. Cumulative results of both groups and statistical evaluations are indicated in Table III.

Serum IgE levels were significantly higher in the group with ascariasis than in the control group ($p<0.01$).

TABLE I: Ascariasis Group

Case No.	Age/sex (yrs)	Total eosinophils (per mm^3)	IgE (U/ml)	Total leukocytes (per mm^3)	Total lymphocytes (per mm^3)	E rosettes Absolute (per mm^3)	HEA rosettes Absolute (per mm^3)	HEAC rosettes Absolute (per mm^3)
1	5/M	550	550	8500	1530	826 (54%)	275 (18%)	245 (16%)
2	7/M	650	1000	6500	2210	840 (38%)	199 (9%)	111 (5%)
3	5/M	1750	2240	6000	2760	994 (36%)		
4	15/F	750	1000	11400	4332	1906 (44%)	520 (12%)	433 (10%)
5	7/M	750	2800	5200	1664	666 (40%)	283 (17%)	233 (14%)
6	11/F	500	260	7000	3220	1352 (42%)	515 (16%)	386 (12%)
7	17/M	600	430	4000	2000	700 (35%)		400 (20%)
8	12/M	450		6200	3720	1339 (36%)	409 (11%)	744 (20%)
9	7/M	500	800	8000	4000	1960 (49%)	560 (14%)	560 (14%)
10	11/M	700	290	7600	2288	1444 (50%)	520 (18%)	404 (14%)
11	7/M	500	170	8400	5040	1966 (39%)	504 (10%)	605 (12%)
12	3/M	625	240	6400	3200	1184 (37%)	224 (7%)	384 (12%)
13	7/M	450		6800	2040	612 (30%)	204 (10%)	122 (6%)
14	6/M	850		8000	4800	1872 (39%)	816 (17%)	528 (11%)
15	6/F	2000		10400	4368	1179 (27%)	437 (10%)	349 (8%)
16	12/F	650	680	10400	3952	1304 (33%)	474 (12%)	514 (13%)
17	9/M	400		7200	3312	2451 (74%)		
18	3/M	2600		10000	5400	2484 (46%)		
19	6/M	1200		5800	2784	1865 (67%)		
20	7/F	400		4200	1932	908 (47%)		
21	5/F	750		5600	2800	1680 (60%)		

TABLE II: Control Group

Case No.	Age/sex (yrs)	Total eosinophils (per mm^3)	IgE (U/ml)	Total leukocytes (per mm^3)	Total lymphocytes (per mm^3)	E rosettes Absolute (per mm^3)	HEA rosettes Absolute (per mm^3)	HEAC rosettes Absolute (per mm^3)
1	22/M	150	60	5800	2204	1014 (46%)	242 (11%)	529 (24%)
2	13/F	250	5	6500	1170	667 (57%)	222 (19%)	234 (20%)
3	13/M	200	33	6000	1080	626 (58%)	162 (15%)	184 (17%)
4	23/M	150	90	5200	1768	1096 (62%)	283 (16%)	318 (18%)
5	8/F	250	81	8000	3360	1714 (51%)	403 (12%)	571 (17%)
6	11/F	175	210	7000	1960	921 (47%)	314 (16%)	214 (11%)
7	16/M	175	25	6000	3000	1410 (47%)	600 (20%)	450 (15%)
8	18/F	200	32	9000	2880	1984 (55%)	374 (13%)	374 (13%)
9	2/F	250		7500	3150	1544 (49%)	473 (15%)	914 (29%)
10	4/M	175		9000	5400	2916 (54%)	594 (11%)	1134 (21%)
11	18/F	150	20	8600	2398	1727 (72%)	312 (13%)	337 (16%)
12	22/M	600	43	5000	2400	1344 (56%)	360 (15%)	456 (19%)
13	12/F	350	72	6500	2080	1123 (54%)	354 (17%)	374 (18%)
14	15/F	350	57	4600	1380	552 (40%)	193 (14%)	290 (21%)
15	21/M	225		5000	2000	740 (37%)	280 (14%)	480 (24%)
16	7/F	425		6000	2640	1320 (50%)	264 (10%)	471 (22%)
17	9/M	300		8000	4000	1960 (49%)	560 (14%)	560 (14%)

TABLE III: Comparison of the Total Findings of the Ascariasis Group with Those of the Control Group

	Ascariasis Group		Control Group		
	n	mean	n	mean	p
Total leukocytes (per mm^3)	21	7314.29 ± 2022.20	17	6688.24 ± 1438.29	$p > 0.05$
Absolute lymphocytes (per mm^3)	21	3207.24 ± 1157.57	17	2521.76 ± 1079.05	$p > 0.05$
Absolute E rosettes (per mm^3)	21	1405.86 + 570.08	17	1274.59 ± 493.97	$p > 0.05$
Absolute HEA rosettes (per mm^3)	14	423.64 ± 173.08	17	352.35 ± 135.15	$p > 0.05$
Absolute HEAC rosettes (per mm^3)	15	401.20 ± 175.70	17	487.65 ± 254.10	$p > 0.05$
Relative E-rosettes (per mm^3)	21	43.95 ± 11.89	17	52.00 ± 8.19	$p < 0.05$
Relative HEA-rosettes (per mm^3)	14	13.57 ± 3.92	17	14.41 ± 2.72	$p > 0.05$
Relative HEAC-rosettes (per mm^3)	15	12.47 ± 4.31	17	19.06 ± 4.13	$p < 0.05$

	n	Median	n	Median	p
IgE (U/ml)	12	490	12	50	$p < 0.01$
Total eosinophils (per mm^3)	21	650	17	200	$p < 0.01$

There was no difference observed in the absolute lymphocyte counts of the study group and the control group ($p>0.05$), but relative T lymphocyte counts were significantly lower in the patients with ascariasis ($p<0.05$).

Absolute and relative HEA rosette counts had shown a similar distribution in both groups ($p>0.05$). While there was no difference in absolute HEAC rosette counts of the patients and controls, the relative HEAC rosette counts were found to be lower in the patient group than in the control group ($p<0.001$).

Although total leukocyte and lymphocyte counts showed a similar distribution in patients and the control subjects, eosinophil counts of the patient group were significantly higher than those of the control group ($p<0.01$).

In the study group, the relation between serum IgE levels, total eosinophil and absolute T cell counts was studied and it was found that there was a slight negative correlation ($r = -0.36$) between IgE levels and T lymphocyte counts and a slight positive correlation ($r = +0.25$) between T lymphocytes and eosinophils, both having no statistical significance.

Discussion

Ascaris causes high levels of IgE production in the host[3]. Accordingly, we found IgE levels of the patients with ascariasis to be considerably higher than those of the control subjects (Table III). The cause of high IgE levels that appear in nematode infections is not definitely clear. According to current concepts, T-helper cells cooperate in the production of specific antibody (including IgE) during parasitic infections[14,15]. In addition to the rise in specific antibodies, many parasitic infections provoke a non-specific hypergammaglobulinemia. While T cells contribute to some of the generalized increase in immunoglobulin production, it is probable that much of the increase is due to antigens released from the parasite acting as a polyclonal mitogen for B cells (in a T-independent fashion)[14,15]. On the other hand, we have considered that an insufficiency of suppressor T lymphocytes may also lead to an increase in antibody production in ascaris infections.

Recently, it has become possible to identify T lymphocyte subsets by using monoclonal antibodies and theophyline modulation of E rosette formation[16]. Because of limited facilities and financial resources we were not able to determine lymphocyte subpopulations by using these new techniques in this study. Although we could not detect suppressor T cell counts directly, we calculated T and B lymphocyte counts indirectly by way of rosette formation. In the patients with ascariasis, although we found an important decrease in relative T lymphocytes and in relative B lymphocytes with complement receptors, we found absolute lymphocytes to be normal. However, we found a tending-to-a-negative correlation between absolute T lymphocytes and IgE levels. In a previous study,

we found this negative correlation to be statistically significant in patients with atopic dermatitis and a prominent suppression of T cells[8]. In this study, in addition to this slightly tending-to-negative correlation between absolute T lymphocytes and IgE, the important decrease in relative T lymphocytes may imply a possible T cell suppression in ascariasis which cannot be reflected in absolute T lymphocyte counts. However, this suppression is not as prominent as in atopic diseases. Non-specific immunosuppression has been demonstrated for both antibody and cell-mediated responses during parasitic infections[14]. Many immune responses are depressed in schistosomiasis[17]. The activity of T-helper cells is suppressed, as shown by decreased antibody responses to sheep red blood cells or to tetanus toxoid. Lymphocyte proliferation in response to mitogens is depressed. A factor produced by the worm inhibits lymphocyte proliferation directly; the existence of suppressor T cells and suppressive macrophages has been demonstrated[17].

The decrease we found in complement receptor carrying cells was an unpredictable finding which has not been seen in atopic diseases. It is known that the complement system plays a role in controlling parasitic infections[14]. Direct damage caused by antibody activating the classical pathway of complement causes damage to the parasite membrane and increased susceptibility to other mediators; complement C3b deposited on the parasite membrane opsonizes it for phagocytosis by cells with C3b receptors e.g., macrophages[14]. Perhaps the decrease we found in cells with complement receptor may result from the consumption of these cells in phagocytosis.

Eosinophils often accompany parasitic diseases. Studies involving mice having schistosomiasis show that eosinophils play an important role in the immunity of the host against parasites[18-19]. Predictably, the eosinophil counts of our patients were high. However there was no important statistical relationship between IgE levels and eosinophil counts.

In a study on patients with bronchial asthma, Gupta et al[20] reported that an increase in eosinophil counts correlated with a decrease in absolute T lymphocytes. On the other hand, Ghanzanshahi et al[21], stated that there was no relationship between these two. Similarly, we were not able to find a statistically significant correlation between absolute T lymphocytes and eosinophil counts.

Our limited number of patients and our failure to determine IgE levels in some of them may have had an effect on our results. More detailed studies on larger groups investigating the functions of the cellular immunity system as well, may prove to be helpful.

Summary

An increasing number of papers published recently suggest that high levels of serum IgE present in association with certain immune deficiency conditions and atopic disorders may be due to suppressor T lymphocyte subpopulation insufficiency. This led us to study the cause of high serum IgE levels in cases with ascariasis.

Serum IgE levels, the total number of eosinophils and peripheral T and B lymphocytes were studied in 21 children with ascariasis and in 17 controls. The peripheral T and B lymphocytes were enumerated by the rosette-formation test.

The number of relative T lymphocytes was found to be lower in cases with ascariasis, but the number of absolute T lymphocytes in patients did not differ from those of the control subjects. No statistically significant correlation was found between the serum IgE levels and absolute T lymphocyte counts in the group with ascariasis.

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