Peripheral blood lymphocyte subsets in children with frequent upper respiratory tract infections

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SUMMARY: Kendirli T, İkincioğulları A, Doğu F, Babacan E. Peripheral blood lymphocyte subsets in children with frequent upper respiratory tract infections. Turk J Pediatr 2008; 50: 63-66.

It is a common and well-known fact that infants and preschool children undergo frequent episodes of upper respiratory tract infections. The majority of these children do not have a recognized immunodeficiency. The aim of the present study was to evaluate the effects of frequent upper respiratory tract infections on cellular immunity, using peripheral blood lymphocyte subsets and activation markers as defining parameters. The study group consisted of 16 children (aged 2-6 years) with frequent upper respiratory tract infections; 30 age-matched healthy children served as controls. Peripheral blood T, B, NK cells; T lymphocyte subsets; naive and memory cells; and activation markers were analyzed by using monoclonal antibodies and flow cytometry. White blood cell count (WBC) was found to be markedly increased in the study group compared to controls ($p<0.05$). The absolute number of lymphocytes was also higher than that of the healthy children. The relative size of the CD3+CD8+ T lymphocytes and the relative and absolute numbers of CD3-CD16+56+ NK cells were found to be higher in patients than the controls. All the remaining percentages and numbers of the T cell subgroups including naive and memory cells and B lymphocytes did not show any difference, while CD3+CD25+ cell numbers were markedly increased ($p<0.05$).

In conclusion, the examination of peripheral blood lymphocyte subsets in children with frequent upper respiratory tract infections is important in evaluating cellular immune alterations due to antigenic stimulation; however, it is neither essential nor cost-effective in the management of the disease. This study has shown that both the percentage and absolute numbers of peripheral blood lymphocyte subsets maintain their normal status in children with frequent upper respiratory tract infections.

***Key words:** frequent upper respiratory tract infections, lymphocyte subsets, children, immune deficiency.*

Frequent upper respiratory tract infection (URTI) is a common problem in children, particularly during wintertime. A minimum of eight episodes of URTI experienced by school age is considered as "frequent"[1]. Although the majority of these children do not have diagnosed immunodeficiencies, some of them may show low levels of certain laboratory parameters, usually of immunoglobulin isotypes[2,3-5]. Many of the observed immunological alterations are of questionable significance, without any convincing evidence related to an increased susceptibility to RTIs. On the other hand, frequent URTI may cause severe medical, educational, and social problems in children with apparent normal immune systems. It has been reported that a number of drugs such as levamisole, thymic hormones, bacterial extracts, and isoprinosine have been used to prevent frequent RTIs in children, with various rates of success[2-8]. The aim of the present study was to evaluate the effects of frequent URTI on cellular immunity using peripheral blood (PB) lymphocyte subsets and activation markers as defining parameters.

Material and Methods

The study group consisted of 16 children 2-6 years of age referred to our clinic with URTI such as rhinopharyngitis, tonsillitis, otitis and

sinusitis. Symptoms related to the structures of the respiratory tract above the larynx were accepted as URTI[6]. Children with infections involving the upper respiratory tract with a history of more than eight episodes per year of nasal discharge, nasal blockage, cough and/or fever were accepted as "frequent"[1]. Information gathered through questionnaires determined that all subjects had normal growth patterns, with no family history of immune deficiency or atopic disease. Again, no patient with a recognized severe immunodeficiency was included in the study. They were evaluated by the Ear, Nose and Throat (ENT) Department as free of chronic tonsillitis or adenoid hypertrophy and none had adenoidectomy or tonsillectomy. The results were then compared with the reference values of 30 healthy age-matched Turkish children [9]. We obtained informed consent from the parents of every child. Demographic characteristics of the study group and the controls are shown in Table I.

Table I. Demographic Characteristics of the Patients

Group	Frequent URTI (n=16)	Controls (n=30)
Age range (years)	2-6	2-6
Median age (years)	3.5	3
Boys (n)	9	16
%	56.2	53.3
Girls (n)	7	14
%	43.8	46.7

URTI: Upper respiratory tract infection.

Immunological studies were carried out in the Pediatric Immunology-Allergy Research Laboratory of Ankara University. Venous blood (2 ml) was collected from each subject into tubes containing ethylenediamine tetra-acetic acid (EDTA). Complete blood count (CBC), including an automated differential, was performed with Coulter JT. Lymphocyte counts were calculated according to following formula: Lymphocyte % x WBC/100[9].

Peripheral blood lymphocyte subgroups were examined using a panel of monoclonal antibodies (MoAb) (Table II) with flow cytometry (Coulter EPICS XL-MCL) through lysed whole blood technique[10]. The following combination of mouse antihuman moAbs was used for two-color staining: CD45-FITC/CD14-PE, CD3-FITC/CD4-PE, CD3-FITC/CD16+56-PE, CD3-FITC/CD8-PE, CD2-FITC/CD19-PE, CD45RA-FITC/CD4-PE, CD45RA-FITC/CD8-PE, CD3-FITC/CD25-PE, HLA-DR-FITC/CD20-PE, CD4-FITC/CD45RO-PE, and CD8-FITC/CD45RO-PE (Immunotech, Marseille, France). Phycoerythrin (PE) or fluoroisothiocyanate (FITC)-labeled Mouse IgG of the appropriate isotypes was used as negative control. Data were analyzed using Coulter System IITM software.

Table II. MoAbs and Corresponding Cells

CD Designation	Main Cellular Expression
CD45	Common leukocyte antigen
CD14	Monocyte
CD3	Total T lymphocyte
CD4	Helper T lymphocyte
CD8	Cytotoxic T lymphocyte
CD3-CD16+56+	Natural Killer cells
CD19	Total B lymphocyte
CD20	Total B lymphocyte
CD45RA	Naive lymphocyte
CD45RO	Memory lymphocyte
CD25	IL-2 receptor p55 antigen, activated cells
HLA-DR	MHC Class II antigen, activated cells

MoAb: Mononuclear antibodies. IL: Interleukin.
MHC: Major histocompatibility complex.

Statistical Analysis

For statistical analysis, the mean and standard deviations (mean±SD) were obtained. Mann-Whitney U test was used for the comparison of the study and control groups. p-values <0.05 were considered as significant for all parameters. Median values of lymphocyte subsets for the study group and 5^{th}-95^{th} percentiles (median and 5-95%) of lymphocyte subsets for the control group were also determined.

Results

The relative size, absolute numbers (mean±SD), median and median (5-95%) values of lymphocyte subsets of 16 children with frequent URTI and of 30 healthy children are given in Table III.

White blood cell (WBC) counts of the study group were distinctly higher than of the control group ($p<0.05$). The absolute number of the CD3+CD4+ T lymphocytes, the relative size of the CD3+CD8+ T lymphocytes, and the relative and absolute numbers of CD3-CD16+56+ NK cells were found to be higher in patients compared to controls. All the remaining percentages and numbers of the T cell subgroups including naive and memory

Table III. Relative Size (%) and Absolute Numbers (x10/L) of Peripheral Blood Lymphocyte Subsets of Patients and Controls (Mean±SD) and [Median (5-95%)]

	URTI (n:16)	Healthy children (n:30)	URTI (n:16)	Healthy children (n:30)
	Mean±SD		Median	Median (5-95%)
†WBC ($x10^9$/L)	8.5±2.6	6.9±1.7	7.9	6.8 (4.0-10.4)
Lymphocyte (%)	46±11	51±11	45	51 (27-69)
($x10^9$/L)	3.9±1.5	3.5±1.0	3.8	3.5 (1.5-5.2)
CD2+(%)	72±5	74±6	74	74 (65-83)
($x10^9$/L)	2.6±1.1	2.6±0.7	2.6	2.6 (1.1-3.9)
CD3+CD16+56-(%)	65±4	68±7	66	67 (55-79)
($x10^9$/L)	2.5±0.9	2.4±0.6	2.2	2.4 (1.9-3.6)
CD3+CD4+(%)	32±9	39±6	33	38 (26-49)
†($x10^9$/L)	1.4±0.6	1.3±0.4	1.3	1.5 (0.6-2.0)
†CD3+CD8+(%)	25±4	22±7	26	22 (9-35)
($x10^9$/L)	1.0±0.3	0.8±0.3	0.9	0.7 (0.3-1.3)
CD4+CD45RA+(%)	36±8	31±6	28	29 (20-41)
($x10^9$/L)	1.4±0.9	1.4±2.1	1.2	1.1 (0.5-2.5)
CD8+CD45RA+(%)	22±4	21±5	22	21 (13-31)
($x10^9$/L)	0.8±0.3	0.7±0.3	0.8	0.7 (0.3-1.3)
CD4+CD45RO+(%)	14±4	17±8	13	13 (8-42)
($x10^9$/L)	0.5±0.2	0.5±0.2	0.5	0.5 (0.2-0.8)
CD8+CD45RO+(%)	6±2	6±2	6	6 (2-10)
($x10^9$/L)	0.3±0.3	0.2±0.1	0.2	0.2(0.06-0.5)
CD25+(%)	7±4	4±1	4	4 (2-6)
($x10^9$/L)	0.2±0.1	0.1±0.05	0.1	0.1 (0.08-0.2)
CD3+CD25+(%)	2±1	3±1	2	2 (1-4)
†($x10^9$/L)	0.2±0.1	0.08±0.03	0.1	0.1 (0.04-0.2)
HLA-DR+(%)	26±6	36±16	26	26 (18-38)
($x10^9$/L)	0.9±0.3	0.9±0.3	1.0	0.9 (0.4-1.5)
CD19+(%)	21±5	21±6	21	20 (11-31)
($x10^9$/L)	0.8±0.3	0.7±0.3	0.8	0.7 (0.3-1.2)
CD20+(%)	18±8	20±5	19	20 (11-29)
($x10^9$/L)	0.7±0.4	0.7±0.3	0.6	0.6 (0.3-1.1)
CD20+HLA-DR+(%)	22±7	19±5	21	19 (10-28)
($x10^9$/L)	0.7±0.5	0.7±0.3	0.6	0.7 (0.3-1.1)
†CD3-CD16+56+(%)	16±7	11±6	12	10 (5-28)
†($x10^9$/L)	0.5±0.2	0.4±0.3	0.5	0.3 (0.2-1.2)

†: $p<0.05$.
URTI: Upper respiratory tract infection.

cells and B lymphocytes did not show any difference, while CD3+CD25+ cell numbers were markedly increased ($p<0.05$).

Discussion

Upper respiratory tract infections are the most common complaint for hospital admission in childhood. It has been suggested that frequent infections during childhood lead to a non-specific stimulation of the immune system. Allergy, hypertrophy and inflammation of the adenoids and the tonsils, socioeconomic and environmental conditions, attendance at day care centers, and passive smoking are the major risk factors causing frequent URTI[1-3,11-14]. The type or severity of the infection, family history and physical examination provide clues that the immune system is not functioning properly in these children, revealing some serological abnormalities. According to our previous data, primary humoral immunodeficiencies (such as IgA or IgG subclass) are determined as the second most common cause of frequent complaints related to the respiratory tract[12].

In fact, in our study among 16 patients, two had been diagnosed as partial IgA and two others as IgG subclass deficiencies [Subjects with IgA serum levels that fell more than 2 SD below the mean serum levels for their age were defined as partial IgA deficiency patients][7]. IgG subclass deficiency was defined as serum IgG subclass levels more than 2 SD below the normal mean value for age[8]. Therefore, it is once more obvious that the humoral immune evaluation is crucial in explaining the frequency of URTI in childhood.

Peripheral blood T and B lymphocyte enumeration is frequently used for the diagnosis of immune and malignant discrepancies, especially in primary immune deficiencies such as SCID (severe combined immunodeficiency). Age-specific reference ranges of lymphocyte subsets should be used for appropriate clinical evaluation of pediatric patients[9]. In our study, peripheral lymphocyte subsets were studied in 16 children with frequent URTI and 30 age-matched healthy subjects. A number of significant changes were noticed in certain parameters of the two groups. Significant lymphocytosis, increased relative size and absolute numbers of CD3+CD8+T and CD3-CD16+56+ NK cells and elevated absolute numbers of CD3+CD25+ activated T lymphocytes in children with frequent URTI point to viral infections causing antigenic stimulation leading in turn to in vivo immune activation in these patients. However, all these results fell within the normal range and no cellular immunodeficiency was detected in patients with frequent URTI.

In conclusion, the examination of peripheral blood lymphocyte subsets in children with frequent URTI is only important in evaluating the cellular immune alterations due to antigenic stimulation. Otherwise, it is obvious that the evaluation of cellular immunity in children with recurrent URTI is not an essential or cost-effective management method. This study has shown that both the percentage and absolute numbers of peripheral blood lymphocyte subsets maintain their normal status in children with frequent URTI.

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