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Correlations Among Age, Cytokines, Lymphocyte Subtypes, and Platelet Counts in Autoimmune Thrombocytopenic Purpura

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While autoimmune thrombocytopenic purpura is mediated by autoantibodies, accumulating evidence suggests that T helper cells and the cytokines they produce also play a key role. We determined correlations among age, serum cytokine concentrations, circulating lymphocyte, and platelet counts in adult (n = 19) and children (n = 29) with autoimmune thrombocytopenic purpura. Correlations between age and cytokine levels were also assessed in healthy controls (n = 50). Significant positive correlations between age and serum levels of interferon-gamma, age and CD4+ lymphocytes, age and natural killer cell count were observed in these patients. Absolute lymphocyte and CD8+ cell count was significantly inversely correlated with age. In adult patients, a significant inverse correlation between platelet and absolute lymphocyte count was observed. In pediatric patients, an inverse correlation of platelet count with serum concentration of interleukin-3 was recorded. In 50 healthy volunteers there were significant positive correlations between age and interleukin-3, -4, -6, and interferon-gamma, and significantly negative correlations with interleukin-2, tumor necrosis factor-alpha, and interferon-alpha. Additional evaluations are necessary to identify the impact of age-related changes in immune function on the clinical course of autoimmune thrombocytopenic purpura. Pediatr Blood Cancer 2006;47:671–674.

Key words: adult; age; autoimmune thrombocytopenic purpura; cytokine; lymphocyte; pediatric

INTRODUCTION

Autoimmune thrombocytopenia (AITP) is a common immune-mediated platelet disorder. The incidence is 0,46/10(5) children per year [1]. The incidence in UK is around 4 per 100,000 children per year [2]. In adult patients, the incidence of AITP appears to increase with age, being 1.94/million/year [3]. The majority of children have a favorable outcome, with resolution within 6 months. In contrast, AITP in adults is generally chronic and relapsing. It is still impossible to predict which patients will develop chronic disease.

Although both acute and chronic forms of AITP are immune-mediated, different pathogenetic mechanisms may be responsible. Characterization of these may permit identification of acute AITP patients who are likely to develop the chronic form of the disorder. Semple and colleagues [4] revealed elevated levels of serum interleukin-2 (IL-2), interferon-gamma (IFN-γ), and IL-10 in 53% of patients with chronic, but only 9% of patients with acute AITP.

Changes in the immune system with advancing age may contribute to the abnormalities observed in chronic AITP. Aging is associated with a reduction in B cell turnover, a decline in numbers of naïve T cells and an increase in memory T cells [5,6]. Epidemiologic data show a rise in the incidence of autoimmunity with increased age [7]. Altered T cell homeostasis has been postulated to invoke an immune-risk phenotype, at higher risk of developing autoimmune disease [8,9]. In aged mice, activation of natural killer T (NKT) cells is correlated with an increase in IL-10 and contributes to suppression of effector T cell immunity [10].

Constitutive cytokine secretion is generally regarded as elevated in the elderly [11]. Tumor necrosis factor-alpha (TNF-α) and IL-6 have been associated with morbidity and mortality in the elderly. TNF-α plays a direct role in the pathogeneses of several chronic diseases and increasing serum levels seem to be the best predictor of mortality in elderly population. T cells from aged mice respond to antigen with decreased IL-2 production [12].

Improved understanding of changes in the immune system during life may help to elucidate the pathogenesis of AITP in different age groups and enable prediction of disease course. Our study was designed to determine levels of cytokines, lymphocytes, and platelets in pediatric and adult patients with AITP and examine their correlations. Levels of cytokines were also measured in healthy controls.

MATERIALS AND METHODS

Patients

Forty-eight patients with AITP (age range, 8 months to 86 years), comprising 29 pediatric patients (age range, 8 months to 15 years) and 19 adult patients (age range, 15 years to 86 years), and 50 healthy volunteers (age range, 2 years to 50 years) were enrolled between January 1999 and

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May 2003. At diagnosis, all AITP patients met standard diagnostic criteria: platelet count less than 80×10^9/L; normal or increased number of megakaryocytes in normal bone marrow; and no other cause for thrombocytopenia. All patients received written information about the study and gave informed consent before study entry.

Assays

Absolute lymphocyte count (ALC) and percentage of circulating T, B, CD4+, and CD8+ lymphocytes and NK cells were measured at diagnosis in all patients by flow cytometric analysis (FACSCalibur, Becton Dickinson Immunocytometry Systems, San Jose, CA (#E3906), Software: MultiSET V1.1.2 or SimulSET v. 3.1.) [13]. Serum levels of IL-1α, IL-2, IL-3, IL-4, IL-6, IL-10, TNF-α, IFN-α, and IFN-γ were determined at diagnosis in all patients and healthy volunteers according to the quantitative sandwich enzyme immunoassay (ELISA) technique (Quantikine Immunoassay, R&D Systems, Inc., Minneapolis, MN).

Statistical Methods

Data were analyzed by SPSS 11.0 software. Pearson's correlation coefficients analysis was performed. Probability values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Cytokine Levels and Age

Serum concentrations of IL-2, TNF-α, and IFN-α in the healthy controls were significantly inversely correlated with age. There were significant positive correlations between age and serum concentrations of IL-3, IL-4, IL-6, and IFN-γ, meaning that their levels increase with age. There were no significant correlations between age and serum concentrations of IL-1α or IL-10. Analysis in patients revealed a significant positive correlation between age and levels of IFN-γ. No significant correlations between age and other cytokines were observed (Table I).

Lymphocyte Counts and Age

ALC ($P = 0{,}039$) and CD8+ lymphocyte ($P = 0.066$) count at diagnosis in AITP patients was significantly inversely correlated, while percentage of NK cells ($P = 0.074$) and CD4+ lymphocyte ($P = 0.005$) was significantly positively correlated with age. There were no significant correlations of age with either T or B lymphocyte count.

Lymphocyte Counts

In children with AITP, significant inverse correlations between lymphocyte subsets were observed as follows: NK cells and CD4+ lymphocytes; CD8+ and B lymphocytes, and B and T lymphocytes. CD8+ and T lymphocyte counts were significantly positively correlated. No significant correlation between ALC and lymphocyte subsets was noted. In the adult subgroup, significant positive correlations were noted as follows: CD4+ and T lymphocytes; ALC and B lymphocytes. A series of significant negative correlations were observed: NK cells and B lymphocytes; NK cells and ALC; CD8+ and B lymphocytes; CD4+ and B lymphocytes, and B and T lymphocytes (Table II).

TABLE I. Correlations Between Age and Cytokine Levels (pg/ml) in Healthy Controls and Patients With AITP

	Cytokine	Mean (SD)	*P*	R
Healthy controls				
Age (years); mean (SD);	IL-1α	2.5 (1.3)	0.495	0.1
19.4 (15.9); n = 50	IL-2	27.9 (3.6)	<0.01	−0.51
	IL-3	59.1 (24.3)	<0.01	0.52
	IL-4	9.4 (1.8)	<0.01	0.55
	IL-6	5.9 (1.9)	<0.01	0.77
	IL-10	18.2 (2.9)	0.340	−0.14
	IFN-γ	50.7 (8.3)	<0.01	0.52
	TNF-α	21.8 (3.5)	0.014	−0.35
	IFN-α	36.0 (25.4)	0.004	−0.40
AITP patients				
Age (years); mean (SD);	IL-1α	3.6 (2.7)	0.926	0.01
13.8 (17.4); n = 48	IL-2	26.3 (5.5)	0.800	0.04
	IL-3	56.9 (21.5)	0.747	0.05
	IL-4	13.6 (9.5)	0.128	0.22
	IL-6	6.9 (4.3)	0.108	0.24
	IL-10	17.5 (5.4)	0.211	−0.18
	IFN-γ	51.9 (11.6)	0.015	0.35
	TNF-α	20.2 (5.4)	0.954	0.01
	IFN-α	44.4 (32.8)	0.136	−0.22

AITP, autoimmune thrombocytopenic purpura; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Platelet Counts and Cytokine Levels/Lymphocyte Counts

There was no significant correlation between platelet counts and ALC at diagnosis in pediatric patients. However, in adult patients, platelet counts at diagnosis were significantly inversely correlated with ALC ($P = 0.003$). In pediatric patients, there were a significant inverse correlation between platelet count and levels of IL-3 at diagnosis ($P = 0.021$), but no relationships between platelet count and concentrations of any other cytokines were observed. In adult patients, there were no significant correlations between platelet count and any of the cytokines measured.

DISCUSSION

Our data suggest that in healthy controls, serum concentrations of the cytokines IL-3, IL-4, IL-6, and IFN-γ significantly increase with age, while concentrations of IL-2, TNF-α, and IFN-α significantly decrease. In our patients with AITP, we observed a significant positive correlation between age and serum concentration of IFN-γ at diagnosis, but no significant relationship between age and other cytokines was

TABLE II. Correlations Between Lymphocyte Subsets in Pediatric and Adult Patients With AITP

Lymphocyte subset	Pediatric AITP (n = 29); r (*P*)					Adult AITP (n = 19); r (*P*)				
	ALC	B cells	CD4+ cells	CD8+ cells	NK cells	ALC	B cells	CD4+ cells	CD8+ cells	NK cells
ALC			0.05 (0.863)	0.11 (0.698)	−0.20 (0.462)			−0.38 (0.199)	−0.43 (0.143)	−0.60 (0.029)
T cells	0.21 (0.437)	−0.82 (<0.01)	0.32 (0.192)	0.72 (0.001)	−0.36 (0.154)	−0.54 (0.055)	−0.79 (<0.01)	0.67 (0.007)	0.42 (0.117)	−0.03 (0.911)
B cells	−0.01 (0.981)		−0.11 (0.680)	−0.68 (0.002)	−0.19 (0.462)	0.78 (0.002)		−0.52 (0.049)	−0.53 (0.043)	−0.57 (0.028)
CD4+ cells				−0.29 (0.229)	−0.55 (0.021)				−0.29 (0.229)	−0.09 (0.749)
CD8+ cells					−0.01 (0.970)					−0.40 (0.137)

AITP, autoimmune thrombocytopenic purpura; ALC, absolute lymphocyte count.

noted. We also found an inverse correlation between age and ALC, age and CD8+ lymphocyte count, and positive correlations between age and CD4+ lymphocyte and NK cell count. In pediatric patients, CD4+ lymphocyte and NK cell count were inversely correlated. We also noted an inverse correlation between platelet count and IL-3 level in pediatric, but found no significant relationships between platelet count and cytokine levels in adult patients. A significant negative correlation between ALC and platelet count was observed at diagnosis in adult, but not in pediatric patients.

A number of other groups have studied T cell reactivities and cytokine secretion in AITP. Semple and colleagues first postulated that AITP was associated with a defect in CD4+ lymphocytes leading to autoantibody production [14]. Other studies, recently reviewed by Semple and Friedman, produced similar findings. As discussed above, increased levels of IL-2, IFN-γ, and IL-10, together with an absence of IL-4, were found in patients with chronic AITP [4]. Several subsequent studies confirmed an abnormal distribution of Th1-associated cytokines in this disorder [15]. Garcia-Suarez et al. showed that CD2+ lymphocytes from chronic ITP patients secreted elevated levels of IFN-γ and TNF-α [16]. These findings are consistent with our observation of positive correlations between age and IFN-γ level and NK cell count in adult AITP; a higher incidence of chronic AITP would be expected in older individuals.

Our findings add to the literature describing T cell and cytokine abnormalities in patients with AITP. For adult patients with AITP, ALC at diagnosis is strongly inversely correlated with platelet count. A high ALC may be predictive of a poor prognosis and close monitoring of patients with this laboratory finding should be considered. Increased levels of IFN-γ, CD4+, and NK cells at diagnosis may suggest a chronic disease course. Additional evaluation in human studies will be useful in order to clearly identify the impact of altered cytokine production on age-related changes in immune function and AITP.

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