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Anti-inflammatory plasma cytokines in children and adolescents with Down syndrome

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Abstract: Cytokines participate in many physiological processes including the regulation of immune and inflammatory responses. Production of some important cytokines in children with Down syndrome (DS) is depressed or increased . In this study we analysed the selected anti- inflammatory cytokines: interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13) in plasma of children and adolescents with DS. The study group consisted of 20 patients with Down syndrome and 33 healthy subjects at the age of 5-17 years. Levels of: IL-4, IL-10 and IL-13 in plasma samples were determined by specific enzyme- linked immunosorbent assay (ELISA) techniques according to manufacturer's instructions. IL-4 was detectable in 25% subjects with Down syndrome and in 28.6% healthy subjects. IL-13 was detectable in 15% patients with Down syndrome and in 15.2% healthy subjects, respectively. IL-10 was detectable in 1 of 20 patients with Down syndrome and in 2 of 33 healthy subjects only. No significant correlations between measurable cytokine levels and age and gender were found. No significant increased concentration of selected anti- inflammatory cytokines were detected.

Key words: Down syndrome, children, cytokines, interleukin-4, interleukin-10, interleukin-13

Introduction

Down's syndrome, the most frequent chromosomal disorder with mental retardation, results from triplicated chromosome 21, or from a triplication of its restricted regions. The immune function in individuals with DS has been shown to be defective [1-3]. Dysregulation of the immune system is one characteristic pathological feature of the syndrome, and leads to increased susceptibility to viral or bacterial infections and leukemia. These observations, together with the demonstration of a frequent occurrence of HBsAg carrier state and of autoantibodies, have prompted investigations of the immune function in DS patients [4].

Thymic morphological and functional abnormalities have been also demonstrated [5].

The trisomic chromosome 21 carries genes for receptors and ligands of the interferon family. In DS patients, abnormalities in thymus anatomy depend on interferon-γ (IF-γ) and tumour necrosis factor-α (TNF-α) overexpression was found [6].

Interferon-γ can also cause neurodegeneration and β-amyloid production in Down syndrome and in its animal model, the trisomy 16 mouse [7], and accounts for cognitive impairment. Apolipoprotein E (ApoE) is a polymorphic protein that plays a central role in plasma lipoprotein metabolism. Its production and accumulation are increased in central nervous system disorders like a Alzheimer´s disease and in DS [8]. DS is associated with high frequency of celiac disease, a chronic inflammatory disease of the small intestinal mucosa.

Cytokines participate in many physiological processes including the regulation of immune and inflammatory responses. These effector molecules are produced transiently and locally controlling the amplitude and duration of the response.

IL-4, is a cytokine that induces differentiation of naive helper T cells (Th0 cells) to Th2 cells. It is a key regulator in humoral and adaptive immunity [9].

IL-10 is produced primarily by monocytes and to a lesser extent by lymphocytes. This cytokine has pleiotropic effects in immunoregulation and inflam-

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mation. It also enhances B cell proliferation, and antibody production [10]. It is capable of inhibiting synthesis of pro-inflammatory cytokines like interferon-γ, interleukin-2, interleukin-3 [10].

IL-13 is a cytokine secreted by many cell types, but especially T helper type 2 (Th2) cells. In addition to effects on immune cells that are similar to those of the closely related cytokine IL-4, IL-13 is more importantly implicated as a central mediator of the physiologic changes induced by allergic inflammation in many tissues[11].

Our previous studies suggest an important role of changes of serum levels of cytokines and lipid peroxidation factors in the pathogenesis of acute and chronic diseases of the central nervous system [12-15].

In the literature there a lot of studies on pro-inflammatory interleukins (interleukin-1, interleukin-2, interleukin-6 and interleukin-8) in subjects with DS [6,16-18] but there are only few data on anti-inflammatory cytokines in those subjects [19,20]. The aim of this study was to evaluate interleukin concentrations (IL-4, IL-10 and IL-13) in plasma in children with DS.

Materials and methods

Patients. The study involved 53 children and adolescents, including 20 with DS (10 males and 10 females; mean age 8.57±6.19 years; range 5-17). All of them were patients of the Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok. All Down syndrome subjects were assessed by clinical examination and karyotype analysis, they showed a mild and variable degree of mental retardation, were free of other pathological conditions at the moment of the study and were in good health status. We recruited thirty three healthy subjects as controls. The healthy children were in the age 8-17 years (mean age 12.11±3.46). There were 22 females and 11 males in this group. Patients with significantly abnormal basic laboratory findings, indicating liver dysfunction and with lipid metabolism disorders, were excluded from the study.

ELISA testing. The patients affected by allergic, inflammatory, infectious or immune disorders which could interfere with the study were also excluded. Plasma for analysis of cytokines was obtained simultaneously with routine laboratory tests. Blood samples were obtained under a fasting and rest condition, in the morning. Blood was drawn from the antecubital vein, centrifuged, frozen and stored until -20°C until the assay. Immunoassay kits of BioSource (Bio Source Internationallnc. 542 Flynn Road, Camarillo, California 93012, USA) were used. Levels of: 1. IL-4, 2. IL-10 and 3. IL-13 in plasma samples were determined by specific enzyme- linked immunosorbent assay (ELISA) techniques according to manufacturer's instructions (Catalogue numbers: KAP1281, KAP1321, KPMS113). The minimum detectable concentration of assay was 1.2 pg/mL for IL-4, 0.2 pg/mL for IL-10 and 0.73 pg/mL for IL-13. We tested both groups in each assay. Laboratory staff was blind to clinical data.

Ethical issues. The protocol was approved by the Ethics Committee at the Medical University of Białystok.

Statistical analysis. Statistical evaluation was carried out by means ±SD with software STATISTICA 6.0 PL.

Table 1. Selected data of plasma cytokines in children and adolescents with Down syndrome and in control group presented as mean±SD.

Studied interleukins	Number of measurable IL cases	
	Down syndrome (n=20)	Control group (n=33)
IL-4 pg/mL	5 (25%) 3.16±1.43 pg/mL	10 (28.6%) 3.39±1.44 pg/mL
IL-13 pg/mL	3 (15%) 1.043±0.116 pg/mL	5 (15.2%) 1.78±0.79 pg/mL
IL-10 pg/mL	1 (5%) 7.321 pg/mL	2 (6.1%) 24.24 pg/mL and 37.68 pg/mL

Results

IL-4 was detectable in 5 out of 20 (25%) subjects with DS (3.16±1.43 pg/mL) and 10 out of 33 (28.6%) healthy subjects (3.39±1.44 pg/mL). IL-13 was detectable in 3 out of 20 (15%) patients with DS (1.043±0.116 pg/mL) and 5 out of 33 (15.2%) healthy subjects (1.78±0.79 pg/mL). IL-10 was detectable in one subject with DS only (7.321 pg/mL) and two healthy children (24.24 pg/mL and 37.68 pg/mL). Cytokine plasma levels are presented in Tables 1 and 2.

The number of measurable samples was too small for statistical analysis. Moreover, the data were not normally distributed. Therefore, we did not find any significant differences or trends between both studied groups.

No significant correlations between measurable cytokine levels and age or gender were found.

Discussion

We examined the interleukins plasma concentrations from aged individuals with Down syndrome (age 5-17 years old, 10 female, 10 female) in order to correlate with age and gender. In the present study, we did not find significant increase of plasma IL-4, IL-10 and IL-13 concentrations in children with DS compared with healthy children. We also did not note a correlation between anti-inflammatory interleukins plasma levels and age or gender. Weakness of our study was a small study group and determination of the interleukins in plasma. In contrast, Guuzzarotti *et al* [20] found increase of serum IL-10 concentration in adolescents with DS. They assessed cytokine production, immune activation, T lymphocytes maturation, and serum interleukin-7 concentration in 24 adolescents with DS and 42 age- and gender-matched controls. The IF-γ, IL-10 production, as well as serum IL-7 concentrations and activation markers-bearing T lymphocytes were significantly increased. The discrepancy in these studies

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Table 2. Clinical characteristic and cytokine levels in patients with Down syndrome, in all of the studied subjects the were no symptoms of systemic inflammation in clinical examination.

Subject (n=20)	Age	Gender	C-reactive protein level	IL-4	Il-10	Il-13
WM	5	M	Normal	ND	ND	1.01
WB	6	F	Normal	ND	ND	ND
PŁ	14	M	Normal	5.179	ND	ND
MS	12	F	Normal	2.342	ND	0.92
KU	10	M	Normal	ND	ND	ND
LM	5	F	Normal	ND	ND	ND
RK	14	M	Normal	ND	ND	ND
PP	14	M	Normal	1.036	ND	ND
BJ	6	M	Normal	4.143	ND	ND
ŁK	12	F	Normal	ND	ND	ND
KK	5	M	Normal	3.107	ND	ND
EW	5	F	Normal	ND	ND	ND
DF	10	M	Normal	ND	ND	ND
EF	14	M	Normal	ND	ND	ND
FK	5	M	Normal	ND	ND	ND
AK	6	F	Normal	ND	ND	ND
NS	5	F	Normal	ND	7.321	ND
JB	5	F	Normal	ND	ND	ND
NP	6	F	Normal	ND	ND	1.20
ON	5	F	Norma	ND	ND	ND

ND – not detectable

may be due to the differences in the ages of the individuals studied.

In another study, Park *et al*. [21] found that cellular proliferation and IL-2 production in inactivated cells were not different in adult with DS and controls.

It was previously reported that serum IL-6 levels from the sporadic type of Alzheimer disease and that of a similar stage of demented persons with DS were increased compared with normal age-matched healthy controls [22].

Griffin *et al*. [23] demonstrated that brain IL-1 immunoactivity increased in individuals with DS and Alzheimer disease.

IL-4 was believed to be solely responsible for the expression of immunoglobulin E the mouse [24,25].

The discovery of interleukin-13 and the demonstration that this cytokine could also induce immunoglobulin E production by human B cells established that a further layer of complexity existed in the regulation of immunoglobulin E [26].

Smooth muscle cells in the tunica media of many blood vessels also produce IL-6 as a pro-inflammatory cytokine. IL-6 role as an anti-inflammatory cytokine is mediated through its inhibitory effects on TNF-alpha and IL-1, and activation of IL-1ra and IL-10 [27].

Intracranial production of cytokines has been demonstrated after acute disseminated encephalomyelitis [28] and encephalopathy [29]. The cerebrospinal fluid IL-6, IL-10, TNF-α, and sTNFR1 concentration were elevated in the patients with acute disseminated encephalomyelitis. Myelin basic protein levels in cerebrospinal fluid of the patients with elevated cerebrospinal fluid sTNFR1 levels were significantly higher than those in cerebrospinal fluid of the patients with normal cerebrospinal fluid sTNFR1 levels. It was suggested that IL-6 and TNF-α were mediate inflammation in the central nervous system in acute disseminated encephalomyelitis [28].

Franciosi *et al*. [18] demonstrated that IL-8 potentiates the effect of amyloid beta peptide in inducing secretion of inflammatory cytokines from cultured human microglia, suggesting a possible role in the early development of Alzheimer neuropathology in DS.

Inflammation might play a role in the curtailed growth in DS brains, as it is postulated to do in the precocious development of Alzheimer pathology in DS [30].

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In conclusion, present study does not confirm any changes anti-inflammatory interleukins in plasma among children with DS. However, our study focused on few plasma cytokines only. So we could not exclude immune dysfunction and cytokine levels disturbances in DS. Further studies seems to be necessary.

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