

Proinflammatory plasma cytokines in patients with Down syndrome

Cytokiny prozapalne w surowicy pacjentów z zespołem Downa

Joanna Śmigiełska-Kuzia¹, Krzysztof Sendrowski¹, Anna Jakubiuk-Tomaszuk¹, Leszek Boćkowski¹, Beata Olchowik¹, Magdalena Cholewa¹, Wojciech Sobaniec¹, Beata Żelazowska-Rutkowska², Anna Stasiak-Barmuta³, Milena Żochowska²

¹ Klinika Neurologii i Rehabilitacji Dziecięcej, Uniwersytet Medyczny w Białymstoku

² Zakład Laboratoryjnej Diagnostyki Pediatricznej, Uniwersytet Medyczny w Białymstoku

³ Zakład Immunologii Klinicznej, Uniwersytet Medyczny w Białymstoku

STRESZCZENIE

Wstęp. Pomimo rosnącego zainteresowania udziałem układu immunologicznego w zespole Downa (ZD), rola cytokin w tym zespole jest wciąż niejasna. Większość dotychczasowych doniesień dotyczyła osób dorosłych. **Cel pracy.** Celem tego badania było oznaczenie stężeń wybranych cytokin prozapalnych: ludzkiej interleukiny 1 α (IL-1 α), ludzkiej interleukiny 2 (IL-2), ludzkiej interleukiny 6 (IL-6), rozpuszczalnego receptora dla czynnika martwicy nowotworu (sTNFR1) oraz czynnika martwicy nowotworu (TNF- α) w surowicy dzieci i dorosłych z ZD. **Materiał i metodyka.** Badaniami objęto 84 dzieci i młodzieży, w tym 45 z ZD (20 chłopców i 25 dziewcząt, średnia wieku 10,44 \pm 4,66). Grupę kontrolną stanowiło 39 dzieci bez ZD (21 chłopców i 18 dziewcząt, średnia wieku 11,03 \pm 4,72). Do badania włączono również 81 osób dorosłych, w tym 41 z ZD (19 mężczyzn i 22 kobiety, średnia wieku 41,34 \pm 8,52). Grupę kontrolną stanowiło 40 dorosłych bez ZD (20 mężczyzn i 20 kobiet, średnia wieku 40,4 \pm 10,29). Do oznaczeń użyto zestawów testów immunologicznych z eBioscience (eBioscience Campus Wien Biocenter 2, 1030 Wiedeń, Austria). **Wyniki.** Uzyskano istotnie statystycznie wyższe stężenia IL-1, IL-2, IL-6, sTNFR1 w surowicy krwi dzieci i dorosłych z ZD w porównaniu z grupami kontrolnymi. Stężenie TNF- α w surowicy krwi dzieci z ZD było wyższe w porównaniu z grupą kontrolną, ale różnica nie była istotna statystycznie. W grupie pacjentów z ZD wykazano wzrost stężenia IL-2 i sTNFR1 wraz z wiekiem. W przypadku IL-2 różnice te były statystycznie istotne.

Słowa kluczowe: ludzka interleukina 1 α , ludzka interleukina 2, ludzka interleukina 6, rozpuszczalny receptor dla czynnika martwicy nowotworu, czynnik martwicy nowotworu, zespół Downa

ABSTRACT

Introduction. Despite a growing interest in the contribution of the immune system in Down syndrome (DS), the role of cytokines in DS is still unclear. Most studies were conducted in adults with DS. **Aim of the study.** In this study we determined the levels of the selected proinflammatory cytokines: interleukin-1 α (IL-1 α), interleukin-2 (IL-2), interleukin-6 (IL-6), human soluble tumor necrosis factor receptor1 (sTNFR1) and human tumor necrosis factor alpha (TNF- α) in plasma of children and adults with DS. **Material and methods.** The study included 84 children and adolescents, including 45 with DS (20 boys and 25 girls; mean age 10.44 \pm 4.66 years). The control group consisted of 39 children without DS (21 boys and 18 girls; mean age 11.03 \pm 4.72). We recruited 81 adults, including 41 with DS (19 males and 22 females; mean age 41.34 \pm 8.52). The control group consisted of 40 adults without DS (20 males and 20 females; mean age 40.4 \pm 10.29). Immunoassay kits of eBioscience (eBioscience Campus Vienna Biocenter 2, 1030 Vienna, Austria) were used. **Results.** Compared with the control group, the DS group (children and adults) had statistically significant increases in mean serum IL-1 α , IL-2, IL-6 and sTNFR1 levels. The TNF- α levels were also increased in the DS children, but the difference was not statistically significant. A positive correlation was found between IL-2 levels and age and sTNFR1 levels and age in the DS group but only in the case of IL-2 these differences were statistically significant.

Key words: human interleukin-1 α , human interleukin-2, human interleukin-6, human soluble tumor necrosis factor receptor 1, human tumor necrosis factor α , Down syndrome

INTRODUCTION

Down syndrome (DS) is the most common chromosomal abnormality in humans that occurs in 1 out of every 800–1,000 births. It is an autosomal disorder resulting from triplication of chromosome 21 [1, 2].

The current report is a continuation of our earlier research on DS. We focused on some aspects of oxidative stress in the pathogenesis of DS [3, 4]. Our results showed that disturbances of lipid peroxidation processes increased with age.

Children with DS are at increased risk for epilepsy and attention-deficit/hyperactivity disorder [5, 6]. Our previous results showed that 6% patients with DS had epileptic seizures [7].

Down syndrome is the most frequent chromosomal disorder with long lasting infections caused by deficiency of the original immune system. Up-to-date investigations on immunological conditions did not show any coherent results. The aim of our previous study was the assessment

of cell immunity in children with DS using CD3+, CD4+ and CD8+ lymphocyte in peripheral blood T ratio in comparison with a reference group. Based on the obtained results, it can be concluded that cell-mediated immunity dysfunction in children with DS might lead to an increased susceptibility to infections [8].

Down syndrome is well established to be associated with multiple immune aberrations. Compared with healthy subjects, DS patients are significantly more sensitive to infections and have a higher frequency of coexistent hepatitis B and malignant disease (especially leukemia) in addition to the enhanced predisposition to autoimmune disease [9, 10]. There is a large number of autoimmune diseases in DS. Autoimmune thyroid disease is the most frequent autoimmune disorder coexisting with DS [11]. DS is associated with a high frequency of celiac disease, an autoimmune gastrointestinal disorder characterized by mucosal atrophy of the jejunum on exposure to gluten and related proteins from certain grains. There has been a number of studies concerning diabetes and autoimmune diseases (hypoparathyroidism, alopecia) which might be more prevalent in people with DS [12–15].

The interaction between immune cells is finely regulated by several mechanisms, among which cytokines play a crucial role in modulating the quality and the intensity of immune responses. Cytokines are small proteins produced by most cells in the body, which through multiple biological activities promote cell-cell interaction. Cytokines participate in many physiological processes including regulation of immune and inflammatory responses [16].

People with DS frequently show early dementia with compromised short-term memory, behavioural and anxiety disorders, deteriorating self-care and social skills. Post-mortem studies in subjects with DS have demonstrated the almost invariable presence of senile plaque after the 30-year build-up of deposits of amyloid- β peptide similar to those observed in Alzheimer's disease, suggesting a common pathogenesis for the two conditions [17]. In both Down syndrome and Alzheimer's disease an overproduction of amyloid has been described. The amyloid precursor protein is coded in chromosome 21 (gene locus 21q21.2-21q22.1). The presence of a supernumerary chromosome 21 in DS could be related to amyloid overproduction. Accordingly, the finding of increased amyloid deposition in Alzheimer's disease should be otherwise explained [17, 18].

Although the exact pathogenic mechanism at the basis of plaque formation has not yet been defined, some observations suggest that proinflammatory cytokine could be directly involved in Alzheimer's disease and in the degenerative and reactive processes of the central nervous system [19]. A great deal of evidence indicates a hypothetical, although still unclear, generic role of the entire proinflammatory cytokine class in Alzheimer's disease and in dementia in DS. Heyser et al. reported a decline in learning capacity in transgenic mice expressing elevated IL-6 production at the astrocytic level [20]. Kalman et al. pointed out a direct correlation between serum IL-6 levels and the dementia stage in subjects affected by Alzheimer's disease or DS [21].

Carta et al. showed higher levels of cytokines and chemokines in DS patients [17]. A correlation between the degree of mental retardation and IL-6 was found in patients with DS, but not in controls [17]. The authors suggested a possible involvement of chemokines in the inflammatory and degenerative processes similar to Alzheimer's disease in DS.

In other study, Park et al. reported no changes in plasma levels of interleukin-6 in adults with DS [22]. Some studies reported increased levels of interleukin-8, tumor necrosis factor alpha and soluble tumor necrosis factor receptor1, whereas others reported no changes in their plasma levels [23–26].

Despite a growing interest in the contribution of the immune system in DS, the role of cytokines in DS is still unclear. Most studies were conducted in adults with DS. Toward better understanding of the role of cytokines in DS, this study was focused on proinflammatory cytokines in plasma of children and adults with DS: human interleukin-1 α (IL-1 α), human interleukin-2 (IL-2), human interleukin-6 (IL-6), human tumor necrosis factor α (TNF- α) and human soluble tumor necrosis factor receptor1 (sTNFR1). Our hypothesis is that IL-1 α , IL-2, IL-6, TNF- α , sTNFR1 concentrations in the serum of children and adults with DS are elevated when compared with age-adjusted controls.

MATERIALS AND METHODS

The study included 84 children and adolescents, including 45 with DS (20 boys and 25 girls; mean age 10.44 ± 4.66 years; range 5–17). The control group consisted of 39 children without DS (21 boys and 18 girls; mean age 11.03 ± 4.72). All subjects with DS were assessed by clinical examination and karyotype analysis; they were free of other pathological conditions at the moment of the study and were in good health. All of them were patients at the Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok. We recruited 81 adults, including 41 with DS (19 males and 22 females; mean age 41.34 ± 8.52). The control group consisted of 40 adults without DS (20 males and 20 females; mean age 40.4 ± 10.29).

All patients had detailed internal and neurologic examinations, and all had basic laboratory tests: morphology of venous blood, platelet count, transaminases, bilirubin, ammonium, glucose, electrolytes (sodium, potassium, calcium, phosphorus, chlorine, magnesium), urea, creatinine, uric acid, cholesterol and urinalysis. Patients with significantly abnormal basic laboratory findings, indicating liver dysfunction or lipid metabolism disorders were excluded from the study. All patients affected by allergic, inflammatory, infectious, or immune disorders that could interfere with the study were excluded on the basis of a detailed history and laboratory investigation. Other exclusion criteria included thyroid hormone supplementation. Therefore, all subjects were generally healthy.

Blood samples were obtained in fasting and resting condition, in the morning.

Blood was drawn from the antecubital vein, centrifuged, frozen and stored at -20°C until the assay.

Immunoassay kits of eBioscience (eBioscience Campus Vienna Biocenter 2, 1030 Vienna, Austria) were used. For all patients in both groups (children, adults), the levels of IL-1, IL-2, IL-6, sTNFR1, and TNF- α in plasma samples were determined using enzyme – linked immunosorbent assay techniques according to the manufacturer’s instructions. The minimum detectable concentration of the assay was 1.1 pg/mL for IL-1 α , 0.4 pg/mL for IL-2, and 0.03 pg/mL for IL-6, 0.05 ng/mL for sTNFR1, and 0.106 pg/mL for TNF- α . We tested both groups in each assay. Laboratory staffs were blinded to clinical data.

Statistical evaluation was performed with Statistica software, version 6.0 PL. For data with normal distribution, *t*-test for independent samples was conducted; otherwise, the non- parametric Mann-Whitney *U*-test was used. Values are reported as means \pm 1 standard deviation. Significance was set at *P* < 0.05.

The study protocol was approved by the ethics committee of the Medical University of Białystok.

RESULTS

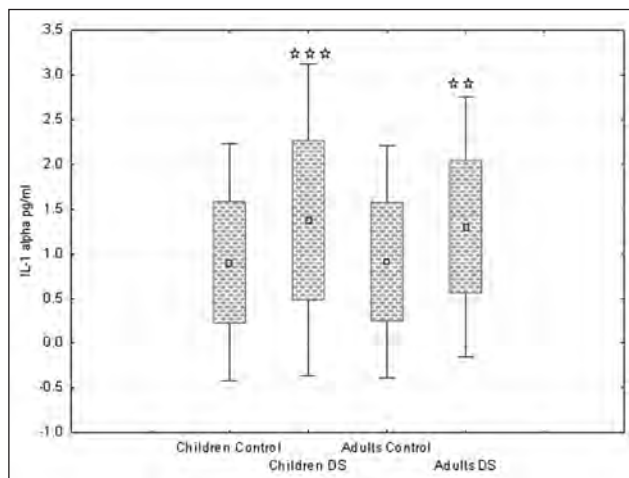
Compared with the control group, the DS group (children and adults) had statistically significant increases in mean serum IL-1 α , IL-2, IL-6 and sTNFR1 levels.

The IL-1 α mean concentration was significantly higher in children with DS (1.40 ± 0.88 pg/ml) compared with children control (0.90 ± 0.67 pg/ml) (*p* = 0.004). In adults with DS, the IL-1 α mean concentration was 1.30 ± 0.73 and in adults control 0.91 ± 0.65 (*p* = 0.01) (fig. 1). Levels of IL-2 were significantly higher in children with DS (1.43

± 0.55 pg/ml) compared with children control (1.10 ± 0.65 pg/ml) (*p* = 0.01) and in adults with DS (2.23 ± 1.14 pg/ml) compared with adults control (1.21 ± 0.52) (*p* < 0.001) (fig. 2). The IL-6 mean concentration was significantly higher in children with DS (2.54 ± 1.29 pg/ml) compared with children control (1.27 ± 0.71 pg/ml) (*p* < 0.001). In adults with DS, the IL-6 mean concentration was 2.69 ± 1.46 pg/mL and in adults control 1.09 ± 0.65 (*p* < 0.001) (fig. 3). We found a significant increase of plasma sTNFR1 concentrations in children with DS (0.31 ± 0.12 ng/ml) compared with children control (0.25 ± 0.09 ng/ml) (*p* = 0.01). There was also a significant increase of plasma sTNFR1 concentrations in adults with DS (0.50 ± 0.37 ng/mL) compared with adults control (0.29 ± 0.10 ng/mL) (*p* = 0.006) (fig. 4). The TNF- α levels were also increased in the DS children compared with children control, but the difference was not statistically significant. In the present study, we did not find a significant increase of plasma TNF- α concentrations in adults with DS compared with adults control (fig. 5).

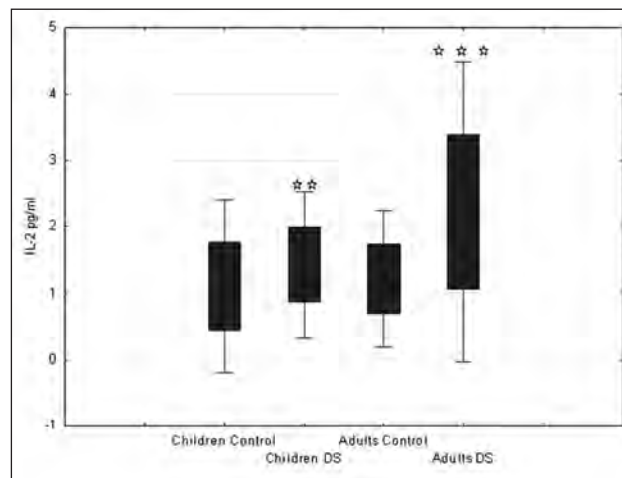
In adults with DS, the IL-2 mean concentration was significantly higher compared with children with DS (2.23 ± 1.14 pg/ml versus 1.43 ± 0.55 pg/ml) (fig. 2), similarly the sTNFR1 concentrations in adults with DS (0.50 ± 0.37 ng/mL) compared with children with DS (0.31 ± 0.12 ng/ml) (fig. 4).

A positive correlation was found between IL-2 levels and age (*R* = 0,2376, *P* = 0,0275) (fig. 6) and sTNFR1 levels and age (*R* = 0,1863, *P* = 0,0858 (fig. 7) in the Down syndrome group but only in the case of IL-2 these differences were statistically significant.



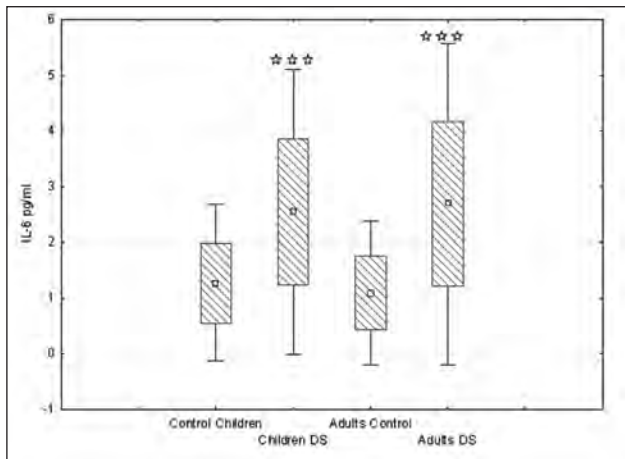
DS – Down syndrome; IL-1alpha – human interleukin-1 α .
 *** *p* = 0.004 versus children control; ** *p* = 0.01 versus adults control.

Figure 1. Comparison of human interleukin-1 α levels between Down syndrome and control subjects.



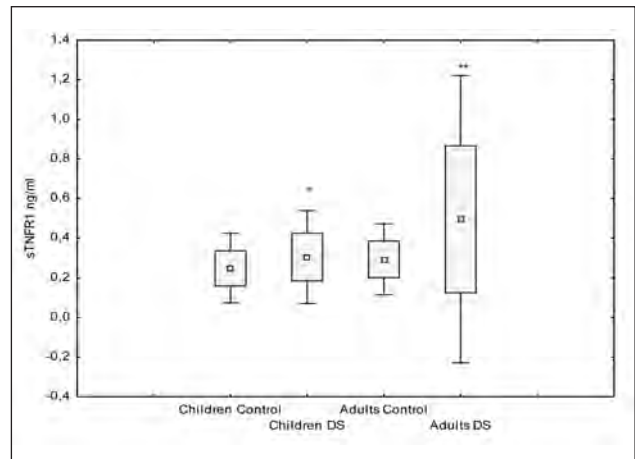
DS – Down syndrome; IL-2 – human interleukin-2.
 ** *p* = 0.01 versus children control; ****p* < 0.001 versus adults control.

Figure 2. Comparison of human interleukin-2 levels between Down syndrome and control subjects.



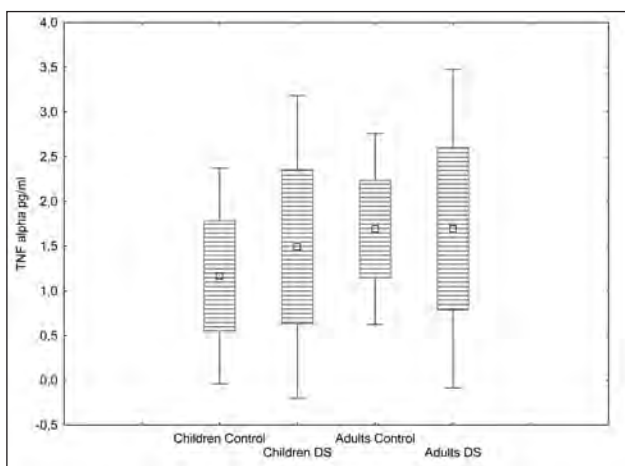
DS – Down syndrome; IL-6 – human interleukin-6.
 *** $p < 0.001$ versus children control; *** $p < 0.001$ versus adults control.

Figure 3. Comparison of human interleukin-6 levels between Down syndrome and control subjects.



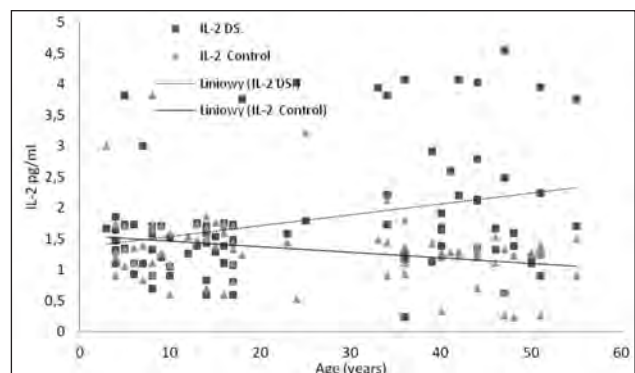
DS – Down syndrome; sTNFR1 – human soluble tumor necrosis factor receptor 1.
 ** $p = 0.01$ versus children control; *** $p = 0.006$ versus adults control.

Figure 4. Comparison of human soluble tumor necrosis factor receptor1 levels between Down syndrome and control subjects.



DS – Down syndrome; NS – not significant; TNF alpha – human tumor necrosis factor α .
 NS – Children DS versus children control ; NS – Adults DS versus adults control.

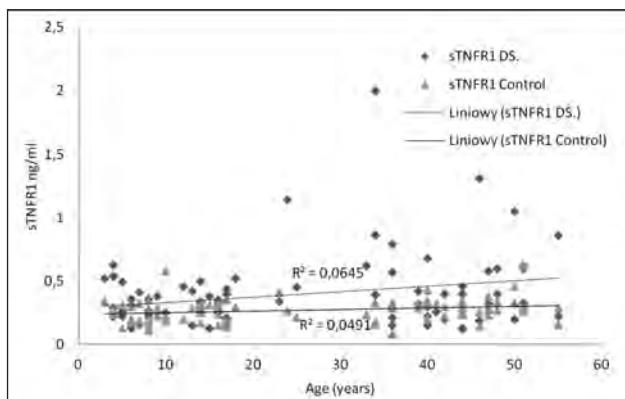
Figure 5. Comparison of human tumor necrosis factor alpha levels between Down syndrome and control subjects.



DS	R = 0,2376	T = 2,2421	P = 0,0275
Control	R = -0,2131	T = -1,91439	P = 0,0592

DS – Down syndrome; IL-2 – human interleukin-2.

Figure 6. The regression straight line and coefficient of correlation between the human interleukin- 2 levels and the age of Down syndrome group and control subjects.



DS	R = 0,1863	T = 1,7380	P = 0,0858
Control	R = 0,2370	T = 2,1414	P = 0,0353

DS – Down syndrome; sTNFR1 – human soluble tumor necrosis factor receptor 1.

Figure 6. The regression straight line and coefficient of correlation between the human interleukin- 2 levels and the age of Down syndrome group and control subjects.

DISCUSSION

To the best of our knowledge, this study is the first to report on plasma pro-inflammatory interleukin concentrations in children and adults with DS using enzyme-linked immunosorbent assay for quantitative detection of human interleukins. We found statistically significant differences in mean serum IL-1 α , IL-2, IL-6 and sTNFR1 concentrations between the DS group and controls. In the present study, children and adults with DS have increased TNF- α levels, compared with the control subjects, but the difference was not statistically significant. The topic has been rarely investigated in children and in adults with DS in the same study.

The present results are generally in agreement with previous data. Despite a growing interest in the contribution of the immune system in DS, the role of cytokines in DS is still unclear.

Interleukin 1, an immune response-generated cytokine that stimulates astrocyte proliferation and reactivity (astrogliosis), was present in up to 30 times as many glial cells in tissue sections of the brain from patients with DS and Alzheimer's disease compared with age-matched control subjects [27]. Most interleukin 1-immunoreactive glia in DS and Alzheimer disease were classified as microglia. The number of IL-1 immunoreactive neurons did not appear to differ in DS and Alzheimer's disease compared with a control brain. Numerous temporal lobe astrocytes in Alzheimer's disease and postnatal DS were intensely IL-1, S-100, and glial fibrillary acidic protein-immunoreactive and had a reactive structure. IL-1 levels in Alzheimer's disease temporal lobe homogenates were elevated, as were the levels of S-100 and glial fibrillary acidic protein, two proteins reportedly elevated in reactive astrocytes. These data suggest that increased expression of S-100 in DS, resulting from duplication of the gene on chromosome 21 that encodes the beta subunit of S-100, may be augmented by elevation of interleukin-1. As a corollary, the astrogliosis in Alzheimer's disease may be promoted by elevation of IL-1 [27].

Human IL-1 is a key mediator of the host response to various infectious, inflammatory, and immunologic challenges. Two distinct peptides, IL-1 α and IL-1 β , mediate the biological activities and bind to the same cell-surface receptors [28]. IL-1 α affects several unrelated tissues and it is a main mediator of acute-phase inflammatory responses characterized by alterations in metabolic, endocrine, and immunologic functions [28]. IL-1 β is the major form secreted by monocytes and macrophages, which are thought to be the main source of circulating IL-1. The IL-1 α form is constitutively produced by various epithelial cells, keratinocytes of the skin, and in the brain. In these locations, IL-1 α may contribute to cell growth and repair functions [28].

There are no previous reports addressing IL-1 α levels in patients with DS. Previous studies were focused on the form IL-1 β with controversial results [17, 22, 23]. Some reports confirm a significant increase of IL-1 β in adult DS patients [17], but others confirm a significant decrease of IL-1 β in adults with DS [22]. In another study, Meguid et al. reported that IL-1 β levels in children with DS showed

no significant difference compared with healthy subjects [23]. There are no previous reports addressing the levels of the other interleukin form, IL-1 α , in DS subjects. In the present study, the plasma IL-1 α levels were significantly higher in children with DS compared with healthy children and in adults with DS compared with healthy adults.

The response to IL-2 has been extensively studied as one potential mechanism underlying the age-related defect in cellular immunity [29–32]. Several laboratories have demonstrated decreased production of IL-2 after mitogen stimulation, decreased density of IL-2 receptor expression, decreased expression of IL-2 mRNA, and decreased proliferation of T cells in response to IL-2. IL-1 and IL-2 play a primary role in the activation, recruitment, and proliferation of T lymphocytes. Evidence has been accumulating that there are age-related declines in lymphocyte production and response to other cytokines, such as IL-1 and TNF- α . Meguid et al. indicate that serum IL-2 levels were significantly decreased in children with DS compared with the control group, and its production was correlated inversely with age [23]. IL-2 production was measured in DS patients in a study by Park et al. [22]. IL-2 production was significantly decreased in aged individuals with DS. Interestingly, IL-2 was significantly greater in older males than in older females with DS [22].

The results of our study are different from the data in the literature. In the present study, the plasma IL-2 levels were significantly higher in children and in adults with DS compared with subjects without DS.

IL-6 is secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation [21]. IL-6 is one of the most important mediators of the acute phase response. IL-6 levels were examined in the sera and CSF of patients with mild-moderate and severe stage of late onset sporadic type of Alzheimer's disease and in the sera of demented DS probands with similar stages of Alzheimer's disease and compared with data of age-matched healthy controls [21]. Normal serum IL-6 levels were found in the mild-moderate stage, but significantly increased levels were found in the severe stage of both dementia groups. CSF concentrations remained within the normal range in all groups. Positive correlations between the serum IL-6 levels and age and the severity of the disease were observed. The authors suggested a disease stage-dependent general activation of the immune system both in sporadic Alzheimer's disease and in DS with Alzheimer's disease [21].

In another study, Licastro et al. reported that DS subjects were at high risk of developing Alzheimer's disease [33]. Patients with Alzheimer's disease often show altered levels of some immune molecules in their peripheral blood, which correlate with cognitive impairment. The authors studied immune molecules in the blood of non-demented children with DS to investigate whether altered peripheral immune phenotype could be present in these subjects without dementia, many years before the presentation of clinical signs of cognitive deterioration [33]. Plasma levels of IL-6, and soluble interleukin-6 receptor were significantly higher in DS than in control children. Plasma levels of

soluble intercellular adhesion molecule-3, soluble vascular cell adhesion molecule-1 and C-reactive protein were also increased in DS. The increase of IL-6 and C-reactive protein in children with Down syndrome was similar to that found in elderly patients with clinical Alzheimer's disease. Peripheral altered immune phenotype in healthy young subjects with DS might be an early sign of CNS alterations leading many years later to cognitive deterioration and dementia [33].

In another study by Licastro et al. plasma levels of IL-6 were higher in children with DS than in controls [19]. Carta et al. [17] and Park et al. [22] reported that production of IL-6 in older patients with DS was not significantly different from controls. Our results are generally in agreement with the data of Licastro et al. [19]. In the present study, the plasma IL-6 levels were significantly higher in children and in adults with DS compared with subjects without DS.

Tumor necrosis factor receptor1 is one of two receptors of tumor necrosis factors present at the surface of many cells, able to bind both human tumor necrosis factor alpha and human tumor necrosis factor beta. Soluble forms of the receptors are shed from the cell membrane and are present in plasma, urine, and culture supernatants [34]. Different processes modulate their presence. Human sTNFR1 correlates, for example, with disease progression in human immunodeficiency virus infection, parasitemia, and disease severity in human malaria [35]. In the present study, serum concentrations of sTNFR1 in DS subjects was significantly increased compared with controls. In the literature, there is no data on sTNFR1 in those subjects. Most studies indicate

that human tumor necrosis factor alpha levels in children with DS showed no significant difference compared with healthy subjects [17, 22, 23]. The present results for TNF- α levels in children and adults with DS are the same.

In our previous study, we examined selected anti-inflammatory interleukins plasma concentrations (human IL-4, human IL-10 and human IL-13) from individuals with DS in order to correlate with age and sex [36]. No significant correlations between measurable cytokine levels and age and sex were found. No significant increased concentrations of selected anti-inflammatory cytokines were detected [36].

CONCLUSIONS

Based on obtained results the following conclusions can be drawn:

- The present results point to increased levels of the proinflammatory cytokines IL-1 α , IL-2, IL-6 and sTNFR1 in children and adults with DS compared with control patients.
- We observed an age-related increased concentration of IL-2 and sTNFR1 and in the case of IL-2 these differences were statistically significant.
- Taking into account the present results and the results of our previous study indicating no changes in activity anti-inflammatory cytokines, this imbalance between the anti- and pro-inflammatory processes may play a role in the development of humoral immunity dysfunction in DS.

REFERENCES

- [1] Mark R.E., Griffin W.S.: Trisomy 21 and the brain. *J Neuropathol Exp Neurol* 2004; 63: 679–685.
- [2] Marder E., Dennis J.: Medical management of children with Down's syndrome. *Current Pediatr* 1997; 7: 1–7.
- [3] Śmigielska-Kuzia J., Sobaniec W., Kulak W., et al.: Antioxidant enzymes and lipid peroxides in children with Down syndrome. *J Pediatr Neurol* 2007; 5: 117–120.
- [4] Cholewa M., Śmigielska-Kuzia J., Sendrowski K., et al.: Evaluation of antioxidant activity in the erythrocytes in patients with Down syndrome. *Neurol. Dziec.* 2011; 20: 43–47.
- [5] Stafstrom C.E., Patxot D.F., Gilmore H.E., et al.: Seizures in children with Down syndrome: etiology, characteristics and outcome. *Dev Med Child Neurol* 1991; 33: 191–200.
- [6] Ekstein S., Glick B., Weill M., et al.: Down Syndrome and Attention-Deficit/Hyperactivity Disorder (ADHD). *J Child Neurol* 2011; 26: 1290–1295.
- [7] Śmigielska-Kuzia J., Sobaniec W., Kulak W., et al.: Clinical and EEG Features of Epilepsy in children and adolescents in Down syndrome. *J Child Neurol* 2009; 24: 416–420.
- [8] Jakubiuk-Tomaszuk A., Śmigielska-Kuzia J., Wysocka J., et al.: Assessment of cellular immunity in children with Down syndrome by determining the ratio of subpopulations of T lymphocytes CD3+, CD4+ and CD8+ in peripheral blood using flow cytometric method. *Neurol. Dziec.* 2011; 20: 49–53.
- [9] Nespoli L., Burgio G.R., Ugazio A.G., et al.: Immunological features of Down syndrome: a review. *J Intellect Disabil Res* 1993; 37: 543–551.
- [10] Chistiakov D.: Down syndrome and coexistent autoimmune diseases. *J Appl Biomed* 2007; 5: 71–76.
- [11] Kinik S.T., Ozcay F., Varan B.: Type I diabetes mellitus, Hashimoto's thyroiditis and celiac disease in an adolescent with Down syndrome. *Pediatr Int* 2006; 48: 433–435.
- [12] Weijerman M.E., de Winter J.P.: Clinical practice. The care of children with Down syndrome. *Eur J Pediatr* 2010; 169: 1445–1452.
- [13] Gibson P.A., Newton R.W., Selby K., et al.: Longitudinal study of thyroid function in Down's syndrome in the first two decades. *Arch Dis Child* 2005; 09: 575–578.
- [14] Wouters J., Weijerman M.E., Furth A.M., et al.: Prospective HLA, EMA, and TGA testing celiac disease in children with Down syndrome. *J Pediatrics* 2009; 154: 239–242.
- [15] Zwaan M.C., Reinhardt D.R., Hizler J., et al.: Acute leukemias in children with Down syndrome. *Pediatr Clin North Am* 2008; 55: 53–70.
- [16] Kenis G., Teunissen Ch., De Jongh R., et al.: Stability of interleukin 6, soluble interleukin 6 receptor, interleukin 10, and CC16 in human serum. *Cytokine* 2002; 19: 228–235.
- [17] Carta M.G., Serra P., Ghiani A., et al.: Chemokines and pro-inflammatory cytokines in Down's syndrome: an early marker for Alzheimer-type dementia? *Psychother Psychosom* 2002; 71: 233–236.
- [18] Sutinen E.M., Pirttilä T., Anderson G., et al.: Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid- β production in human neuron-like cells. *J Neuroinflammation* 2012; 9: 199–212.

- [19] Licastro F., Mariani R.A., Faldella G., et al.: Immune-endocrine status and coeliac disease in children with Down's syndrome: relationships with zinc and cognitive efficiency. *Brain Res Bull* 2001; 55: 313–317.
- [20] Heyser C.J., Masliah E., Samimi A., et al.: Progressive decline in avoidance learning paralleled by inflammatory neurodegeneration in transgenic mice expressing interleukin-6 in the brain. *Proc Natl Acad Sci USA* 1997; 94: 1500–1505.
- [21] Kalman J., Juhasz A., Laird G., et al.: Serum interleukin-6 levels correlate with the severity of dementia in Down syndrome and in Alzheimer's disease. *Acta Neurol Scand* 1997; 96: 236–240.
- [22] Park E., Alberti J., Mehta P., et al.: Partial impairment of immune functions in peripheral blood leukocytes from aged men with Down's syndrome. *Clin Immunol* 2000; 95: 62–69.
- [23] Meguid N.A., Basheer H.H., Ismail E.A., et al.: Immune dysfunction in Egyptian children with Down syndrome. *Int J Ch Neuropsychiatry* 2004; 1: 65–74.
- [24] Trotta M.B.F., Azul J.B.S., Wajngarten M., et al.: Inflammatory and immunological parameters in adults with Down syndrome. *Immunity and Ageing* 2011; 8: 4–10.
- [25] Dogliotti G., Galliera E., Licastro F., Corsi M.M. Age- related changes in plasma levels of BDNF in Down syndrome patients. *Immunity and Ageing* 2010; 7: 2–7.
- [26] Corsi M.M., Dogliotti G., Pedroni F., et al.: Plasma nerve growth factor (NGF) and inflammatory cytokines (IL-6 and mcp-1) in young and adults subjects with Down syndrome: An interesting pathway. *Neuroendocrinology letters* 2006; 27: 773–778.
- [27] Griffin W.S., Stanley L.C., Ling C., et al.: Brain interleukin 1 and S-100 immunoreactivity are elevated in Down syndrome and Alzheimer disease. *Proc Natl Acad Sci USA* 1989; 86: 7611–7615.
- [28] Dinarello C.A.: The biological properties of interleukin-1. *Eur Cytokine Netw* 1994; 5: 517–531.
- [29] Castle S.C.: Clinical Relevance of Age-Related Immune Dysfunction. *Clin Infect Dis.* 2000; 31: 578–585.
- [30] Chandra R.K.: Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. *Lancet* 1992; 340: 1124–1127.
- [31] Miller R.A.: Aging and immune function. *Int Rev Cytol* 1991; 124: 187–215.
- [32] Burns E.A., Leventhal E.A.: Aging, Immunity, and Cancer. *Cancer Control* 2000; 7: 513– 522.
- [33] Licastro F., Chiappelli M., Ruscica M., et al.: Altered cytokine and acute phase response protein levels in the blood of children with Downs syndrome: relationship with dementia of Alzheimer's type. *Int J Immunopathol Pharmacol* 2005; 18: 165–172.
- [34] Tartaglia L.A., Weber R.F., Figari I.S., et al.: The two different receptors for tumor necrosis factor mediate distinct cellular responses. *Proc Natl Acad Sci USA* 1991; 88: 9292–9296.
- [35] Godfried M.H., van der Poll T., Jansen J., et al.: Soluble receptors for tumor necrosis factor: a putative marker of disease progression in HIV infection. *AIDS* 1993; 7: 33–36.
- [36] Śmigielska-Kuzia J., Boćkowski L., Sobaniec W., et al.: Anti-inflammatory plasma cytokines in children and adolescents with Down syndrome. *Folia Histochemica et Cytobiologica* 2010; 48: 667–670.

Adres do korespondencji:

Joanna Śmigielska-Kuzia, Klinika Neurologii i Rehabilitacji Dziecięcej, ul. Waszyngtona 17, 15-274 Białystok, e-mail: jsmig1@poczta.onet.pl