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ALTERED VESSEL SIGNALLING MOLECULES IN SUBJECTS WITH DOWN'S SYNDROME

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Down's syndrome (DS) is the most frequent human chromosomal abnormality and is associated with mental retardation. Some evidence indicates that certain inflammatory molecules may be increased in DS. Proinflammatory and vasoactive molecules in the blood of non demented subjects with DS were measured in the present investigation. Plasma levels of interleukin-6 (IL-6), vascular endothelial growth factor (VEGF), monocyte chemoattractant protein-1 (MCP-1) and C reactive protein (CRP) were measured in child (2-14 years), adult (20-50 yrs) and elderly (> 60 yrs) DS subjects. Increased plasma levels of IL-6 and MCP-1 were present in DS. Plasma levels of VEGF were increased only in DS adults. Positive linear correlation between IL-6 and MCP-1 levels was present. However, no subclinical inflammation was apparent in DS, since neopterin and CRP levels were within the normal range. An altered regulation of these molecules might interfere with some processes involved in cognitive performances of DS subjects.

DS is the most frequent human chromosomal abnormality (1), occurring in 0.8 out of 1000 live births. The presence of an extra chromosome 21 leads to several clinical alterations, a variable degree of mental retardation (2) and an accelerated aging of different organs and tissues (3). By the fourth decade of life, the appearance of cognitive impairment and dementia further deteriorates mental performances of DS subjects (4-5). In fact, brains from subjects with DS show many neuropathological features, i.e. neuritic plaques, neurofibrillary tangles and degeneration of the basal forebrain cholinergic neurons (4-5), which are considered the pathological hallmarks of Alzheimer's disease (AD). Altered immune responses are frequently observed in DS subjects, even at an early age (6), and have been considered responsible for the high morbidity of the syndrome. Few data regarding blood cytokines and their relations with cognitive decline in DS are available. A recent investigation showed that plasma levels of the macrophage inhibiting protein-1 (MIP-1) was higher in a small group of adult DS than in non DS mentally retarded controls, and levels of IL-6 correlated with the degree of mental retardation only in DS subjects (7). Recent findings showed that elevated levels of IL-6, soluble IL-6 receptor (sIL-6R) and

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soluble vascular cell adhesion molecule-1 (sVCAM-1) were increased in children with DS (8) and suggested a possible remodelling of the molecule network with regulatory function upon vasculature in DS.

MCP-1 is a member of CC chemokine family and is involved in inflammatory processes (9) by binding to specific CC chemokine receptor-2 (CCR-2) leading to monocyte activation and chemotaxis. CCR2 is also expressed in endothelial cells of the arterial wall and MCP-1 activation by CCR2 induces endothelial regeneration after injury (10), angiogenesis (11) and collateral formation *in vivo* (12). Recent findings showed that MCP-1 induced angiogenesis was mediated through pathways involving VEGF (13). VEGF represents a key regulator of physiologic and pathologic angiogenesis and is associated with different diseases including cancer, chronic inflammation and diabetes (14).

DS subjects over-express DS critical region protein-1 (DSRC-1), which is one of 50-100 genes residing within the minimal region of human chromosome-21; this region is present in three copies in DS subjects (15). DSRC-1 belongs to a family of calcineurin-interacting proteins which is able to bind and modulate calcineurin (CnA) (16). DSRC-1, by regulating CnA, acts as a novel VEGF regulator and it has been suggested to regulate vessel maturation by affecting VEGF signaling (17). It is of interest that MCP-1 has been found to influence neurodegeneration and neuron repair both *in vitro* (18) and *in vivo* (19). Moreover, an over- expression of this cytokine has also been found in patients with neurodegenerative processes of the brain, such as AD and in an animal model of this disease (20-21). VEGF and IL-6 has also been claimed to play a critical role in neurodegeneration (22) and to be associated with AD (23-24). We hypothesize the presence of an altered vessel molecule network in DS in the absence of inflammation and with biological relevance on vessel morphogenesis, developing and remodelling. Therefore, we measured blood levels of IL-6, VEGF, MCP-1 and CRP in DS subjects from three different age groups.

MATERIALS AND METHODS

Patients

Three groups of male DS patients were studied: Group 1 consisted of 23 DS children (age 2-14 years, mean age 8 yrs), Group 2 of 14 DS adults (age 20-50 years, mean age 35 yrs) and Group 3 of 9 DS elderly patients (> 60 years, mean age 63 yrs). DS was assessed by clinical examination and karyotype analysis; all patients 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. The project was approved by the Ethics Committee of the University of Bologna and by the "Fondazione Antoniana" of Bologna, Italy.

Laboratory procedures

Blood samples were collected from DS subjects. Plasma was obtained by centrifugation (1500 *g* for 15 min), transferred into coded plastic tubes, rapidly frozen and stored at –20° C until analysis. IL-6, VEGF (isoform 165) and MCP-1 plasma levels were measured using cytokine assay with the Evidence Investigator (Randox Ltd., Rome, Italy). A sandwich chemiluminescent immunoassay was employed for the cytokine assays: increased levels of cytokines in a specimen leading to increased binding of horseradish peroxidase labelled antibody and a consequent increase in chemiluminescence emission. The light signal generated from each of the tested regions on the biochip was detected using digital imaging technology and was compared to that from a stored calibration curve. The concentration of analyte present in the sample was calculated from the calibration curve. Plasma CRP concentration was evaluated by LANIA (Latex Agglutination Nephelometric Immunoassay) technique (Biolatex, Spain). Samples were diluted 1:36 and results were calculated automatically by IMMAGE system. The minimum detectable concentration was 0.4 mg/dl. Plasma levels of neopterin were measured in order to assess activation of the reticular endothelial system (RES) by using commercially available ELISA kits (BRAHMS Elitest, Germany).

Statistical analysis

The results are given as mean ± standard deviation (SD). Comparisons between groups were assessed by one-way analysis of variance. Linear regression analysis between some experimental variables was also assessed (SPSS statistical package).

RESULTS

Levels of plasma IL-6, VEGF, MCP-1 and CRP in DS subjects of different age groups are reported in Table I. IL-6 levels were increased in all age groups when compared with reference values, however, an

Table I. *Plasma levels of IL-6 (pg/mL), VEGF (pg/mL), and CRP (mg/dl) in three age-cohorts of DS subjects.*

Age cohorts	2-14 yrs (n=23)	20-50 yrs (n=14)	> 60yrs (n=9)	p
IL-6	111 ± 22	66 ± 15	33 ±12	< 0.04
VEGF	27 ± 5	139 ± 35	56 ± 12	< 0.002
CRP	0.5 ± 0.1	0.6 ± 0. 1	0.4 ± 0.1	n.s.

Data are shown as mean ± S.D.; p = statistical comparisons by ANOVA; n.s. no statistical difference. Normal reference values: IL-6 = 3.12-12.5; VEGF = 1.5 – 110; CRP = 0.0-0.9.

age-related decrement was present ($p < 0.04$). Plasma levels of VEGF were increased in the adults with DS ($p < 0.002$). Circulating levels of MCP-1 were increased in DS, particularly in children and adults with the syndrome ($p < 0.0001$), as shown in Fig. 1. Plasma CRP levels in the three DS groups were within the normal range (Table I). IL-6 plasma levels positively correlated with those of MCP-1($R = 0.48$, $p = 0.013$). As stated previously, DS subjects were free from acute clinical illness. Moreover, to exclude the presence of subclinical inflammatory alterations which might activate macrophage responses, neopterin plasma levels were also determined in DS subjects and the levels of this metabolite were within the normal range (DS = 7 ± 2.0, controls 7 ± 2.3 nml/L).

DISCUSSION

IL-6 levels were increased in DS subjects, particularly in the youngest subjects, and these findings confirm previous observations showing elevated IL-6 and sIL-6R in children with DS assessed by different methods (6, 8). Elevated IL-6 circulating levels have been described in aging and have been correlated with the age-associated frailty and cognitive impairment (25).

Increased circulating IL-6 or sIL-6R has also been reported in patients with AD (26-27) and elevated plasma IL-6 predicted subsequent cognitive decline in subjects from the MacArthur Study of Successful Aging (28). Altered cytokine levels have been suggested to play a role in neuropsychiatric disorders (29) and altered IL-6 brain levels in AD patients have been found (24).

Previous investigations showing increased sICAM-3 and sVCAM-1 in DS plasma also suggest the presence of mild activation and /or moderate dysfunction of endothelial cells in these subjects (8).

Elevated levels of IL-6 and intercellular adhesion molecules also reflect endothelial dysfunction in different pathological conditions, such as atherosclerosis and its complications (30). However, DS is considered a human condition with low clinical atherosclerosis manifestation and low cardiovascular disease risk during adulthood and aging (31-33).

Here we showed that plasma MCP-1 was elevated in DS and VEGF levels were increased in adult DS. A positive linear correlation between IL-6 and MCP-1 levels ($R = 0.48$, $p = 0.013$) was also observed. These data further support the notion of an abnormal endothelial regulation in DS. The plasma level increases of these molecules could not be ascribed to clinical or subclinical inflammation, since the DS subjects studied were free of clinically apparent pathological conditions and both CRP and neopterin plasma levels were within the normal range in all DS subjects.

Increased levels of IL-6, VEGF and MCP-1 reported here may contribute to the pathophysiology of DS symptoms, such as tissue alterations and impaired mental performances associated with the syndrome.

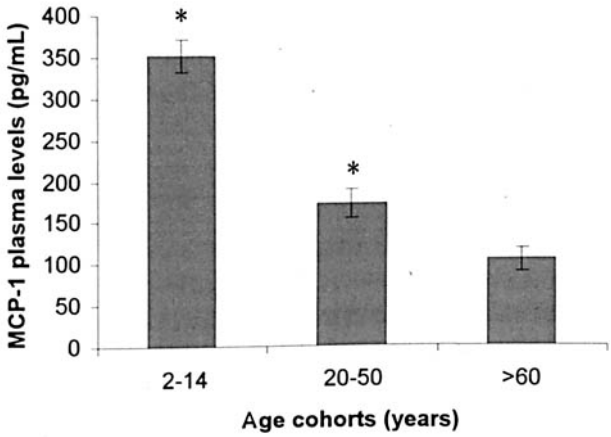

Fig. 1. *Plasma levels of MCP-1(pg/mL) in three age-cohorts of DS subjects. Normal reference values = 72-110 (pg/mL). * statistical analysis p < 0.0001.*

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