Assessment of immune function in Down syndrome patients

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Abstract In Down syndrome (DS), trisomy 21 leads to overexpression of gene coding for specific enzymes. This overexpression translates directly into biochemical aberrations that affect multiple interacting metabolic pathways which culminates in cellular dysfunction and contributes to the unique pathogenesis of DS. The aim of this study is to evaluate parameters of immune response in terms of cytokines [tumor necrosis factor-α (TNF-α) and interleukin-2 (IL-2)] together with the quantitative expression of cystathionine beta synthase (CBS), whose transsulfuration pathway generates cysteine and hydrogen sulfide (H2S). H2S is known to boost host defense at physiological concentrations and to display cytotoxic activity at higher concentrations. Calcineurin activity (CAN) was also measured as its dysregulation has been shown to cause immune suppression. Subjects were 60 DS patients vs. 30 age and socioeconomic matching healthy controls. In their blood, the cytokines: TNF-α and IL-2, together with CBS and its by product H2S as well as CAN activity, were measured.

Results showed that CBSmRNA relative expression (0.56 ± .06 vs. 0.32 ± .02), plasma H2S (72 ± 8.5 vs. 50.8 ± 4.1) and TNF-α (8.11 ± .01 vs. 3.6 ± 0.9) were significantly higher among DS patients compared to controls, while CAN (0.27 ± 0.1 vs. 0.45 ± 0.2 units), was significantly decreased in blood of DS patients compared to controls. IL-2 (36.4 ± 2.6 vs. 37.4 ± 0.9) showed no significant difference between DS patients and controls. Accordingly it can be concluded that excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 may be the cause for decreased immunity in DS patients compared to controls.

1. Introduction

The molecular basis for DS is the presence of an extra copy of chromosome 21, referred to as trisomy 21 (Ts21). Chromo-
interacts physically and functionally with CAN. It was found that the 1.5-fold increase in dosage of RCAN1 destabilizes a regulatory circuit, responsible for many of the features of Down syndrome [5]. It is suggested that RCAN1 overexpression, inhibits CAN [6]. Recently, it has been reported that the gene RCAN1 contained within the DSCR, acts to prevent the nuclear occupancy of transcription factors responsible for CAN expression. CAN plays a critical role in regulating T cell development and activation [7]. In activated T lymphocytes, CAN dephosphorylates the nuclear factor of activated T cells and promotes its translocation into the nucleus and the up-regulation of early T-lymphocyte genes such as the Th1-cytokine interleukin IL-2 [8,9].

Situated on DSCR1 is the gene controlling CBS, an enzyme that catalyzes the first of two steps in the transsulfuration pathway that converts homocysteine into cysteine. A 157% increase in CBS enzyme activity has been previously documented in individuals with DS and has been associated with reduced levels of homocysteine and production of H2S [10]. Physiological functions of endogenous H2S have been found to play an important role as a signal molecule in regulating cell survival [11]. It appears paradoxical that, on one side, H2S acts as a physiological intercellular messenger to stimulate cell growth, and on the other hand H2S at higher concentration is known to be cytotoxic. Proliferating T cells depend on their endogenous capacity to generate H2S, and this capacity is enhanced by T cell activation [12]. In addition to this autocrine role, H2S may have a paracrine function in T cell activation at normal concentration. This idea is supported by the report of increased H2S output from activated antigen presenting cells [13].

TNF-α is an early and potent pro-inflammatory cytokine that stimulates the inflammatory response and is involved in the apoptosis pathway which is injurious [14]. IL-2 is also a key factor in multiple processes involved in the survival of T lymphocytes and in the regulation of T cell responses. It plays an important role in the regulation of anergy because the anergic CD4+ T cells are characterized by their inability to proliferate and express IL-2 following TCR-specific stimulation in the presence of adequate costimulation [15,16]. It has been proposed that anergy results from TCR activation in the absence of IL-2 production [17,18].

Accordingly, CAN activity and CBS mRNA expression and H2S together with the cytokines: TNF-α and IL-2, were measured in blood of DS patients and compared to those of controls.

2. Patients and methods

Patients were 60 Down syndrome cases (33 males and 27 females). Age ranged from 2 to 15 years (mean 9.3 ± 4.1 years). Controls were 30 matched healthy normal children (18 males and 12 females), their age ranged from 2 to 14 years (mean 9.1 ± 4.2 years). DS patients were selected from cases already diagnosed by chromosomal karyotyping in the genetics unit, pediatric department, Cairo University. Blood was drawn after an informed consent of the parents in accordance with the current revision of the 1975 Helsinki declaration.

3. Measurement of plasma H2S concentration

Plasma sample (0.1 ml) was added to a test tube containing 0.5 ml of 1% zinc acetate and 2.5 ml of distilled water, then 0.5 ml of 20 mmol/L N,N-dimethyl-p-phenylenediaminedihydrochloride in 7.2 mol/L HCl and 0.4 ml of 30 mmol/L FeCl3 in 1.2 mol/L HCl were also added to the same test tube for 20 min of incubation at room temperature. The protein in the plasma was removed by adding 1 ml of 10% trichloroacetic acid to the solution and centrifuging it. The optical absorbance of the resulting solution at 670 nm was measured with a spectrometer [19].

4. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis for CBSmRNA

Phytohemagglutinin stimulated lymphocytes were used for RNA extraction since unstimulated leukocytes contain no detectable CBS activity [20]. Total RNA was extracted from lymphocytes using QIAGEN RNA extraction kit (QIAGEN Inc., USA). The RNA samples were reverse transcribed using a blend of Sensiscript and Omniscript reverse transcriptases; using QIAGEN One step RT-PCR kit (QIAGEN Inc. USA). The cDNA product was amplified using 2.5 units of HotStarTaq DNA polymerase, 1 mmol MgCl2/l, and 0.1 umol of the CBS primer 5′-GGG CAC ACC ATC GAG ATC CTC-3′ and 5′-AGA GCC TGC CCA GGC TGT C-3′ [21]. β-actin primers were: 5′-GTG GGG GCC CCC AGG CAC CA-3′; and 5′-CTC CCT ATG AAT GTC ACG CAC GAT TTC-3′. Five microlitre of RT reaction of each cDNA was processed for PCR. Ten microlitre from each PCR reaction product was separated on 2% agarose gel then stained with ethidium bromide. The appearance of specific bands at 205 and 540 bp for CBS and β-actin, respectively, using a 50 bp molecular weight marker, was evaluated under ultraviolet light and photographed.

5. ELISA IL-2

The amounts of IL-2 in circulating lymphocytes were determined by a sandwich enzyme linked immunosorbent assay (ELISA) Predicta IL-2 Kit [22].

TNF-α plasma analysis of the cytokine TNF-α was performed using ELISA R & D kit [23].

5.1. Calcineurin activity

The assay was carried out using the AK-804 BIOMOL GREEN Quantizyme Assay System (Biomol) Bueno et al. [24].

6. Results

Results showed that calcineurin activity (0.27 ± 0.1 vs. 0.45 ± 0.2 units) was significantly decreased among DS patients compared to controls, while CBSmRNA expression (0.56 ± 0.06 vs. 0.32 ± 0.02) (Fig. 1) and H2S (72 ± 8.5 vs. 50.8 ± 4.1) were significantly higher among DS patients compared to controls (Table 1). TNF-α (8.11 ± 0.1 vs. 3.6 ± 0.9) was significantly higher in blood of DS patients compared to controls and IL-2 (36.4 ± 2.6 vs. 37.4 ± 0.9) showed no significant difference.

![Figure 1](image-url)
significant differences between DS patients and controls (36.4 ± 2.6 vs. 37.4 ± 0.9) as shown in Table 2.

7. Discussion

The excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 is thought to underlie both the dysmorphic features and the pathogenesis of the neurological, immunologic, endocrinial, and biochemical abnormalities that are characteristic of DS. The successful management of the clinical problems and unique pharmacological sensitivities of these children is a major medical challenge and depends on understanding of the unique metabolic imbalance induced by overexpression of the constitutively expressed genes on chromosome 21 [2].

Results of the present study revealed a significant decrease in calcineurin activity among DS patients compared to controls. The reason for this decrease is attributed to a gene located on the region DSCR1 inhibiting calcineurin [2]. It has been demonstrated that RCAN1 protein is overexpressed in the brain of Down syndrome fetuses, and interacts physically and functionally with calcineurin [2]. Overexpression of RCAN1 and RCAN inhibits calcineurin-dependent gene transcription through the inhibition of its transcription factor (NAFT). CAN plays a critical role in regulating T cell development and activation [7].

There was a significant increase in CBSmRNA among DS patients compared to controls. A 157% increase in CBS enzyme activity has been previously documented in individuals with DS and has been associated with reduced levels of homocysteine and increased level of H2S [25, 26]. An increased level of H2S is known to cause memory and motor function deficits and is cytotoxic to T-cells [27, 11].

In the present study there was a significant increase in cytokine TNF in DS patients compared to controls. This result although contradictory to the study of Cetiner et al., who showed decreased levels in TNF among DS children [28], is in agreement with previous studies that demonstrated the same as our results [29, 30]. Also in vitro incubated DS blood stimulated with 2.5 × 10^3 TCID50/ml influenza A virus showed a significant rise in TNF compared with control children. It has been assumed that the production of higher levels of pro-inflammatory cytokines (TNF) may be responsible for a more severe clinical course of viral disease in these children [31], since TNF is involved in the apoptosis pathway [14].

Previous studies are controversial regarding IL-2 production by stimulated lymphocytes in DS patients. While a study showed decreased levels of IL-2 among stimulated lymphocytes [32], another study showed increased levels of IL-2 among DS patients compared to controls [33]. Results of the present study showed no significant differences in IL-2 in DS patients compared to controls. Other studies showed that the decreased immunity among DS patients is due to decreased lymphocyte subsets and that the primary immune defect in DS is in part a depressed number and function of helper T cells which stimulate IL-2 [34].

Accordingly it can be concluded that excessive synthesis of multiple gene products derived from overexpression of the genes present on chromosome 21 may lead to decreased immunity in DS patients compared to controls.

### Table 1
Calcineurin and cystathionine beta synthase in blood of DS patients.

<table>
<thead>
<tr>
<th>Down syndrome</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcineurin activity</td>
<td>0.27 ± 0.1 units</td>
<td>0.45 ± 0.2 units</td>
</tr>
<tr>
<td>CBSmRNA relative expression</td>
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<td>0.32 ± 0.02</td>
</tr>
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<td>H2S μmol/L</td>
<td>72 ± 8.5</td>
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</tbody>
</table>

### Table 2
Cytokines in blood of DS patients.

<table>
<thead>
<tr>
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### References


