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Decrease of interleukin (IL)17A gene expression in leucocytes and in the amount of IL-17A protein in CD4+ T cells in children with Down Syndrome

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ABSTRACT

Background: Down Syndrome is by far the most common and best known chromosomal disorder in humans. It expresses multiple systemic complications with both structural and functional defects as part of the clinical manifestation. The mechanisms of immune changes occurring in Down Syndrome are complex and include an extra gene copy of chromosome 21 and secondary dysregulation of numerous intercellular interactions. Recent studies suggest a role of interleukin 17A (IL-17A), a pro-inflammatory cytokine located on 6p12 chromosome, in the pathogenesis of inflammatory and autoimmune diseases. We aimed to analyze IL17A gene expression in peripheral white cells and IL-17A intracellular expression on CD4+ T-cells.
Methods: The research was carried out on a group of 58 children aged 6–12 years including a group of 30 children with Down Syndrome (simple trisomy of chromosome 21 only) and a reference group of 28 healthy children. We evaluated gene IL17A expression using real-time PCR and intracellular IL-17A analyzed by flow cytometry.
Results: We found significantly decreased gene expression in white cells and significantly decreased expression of IL-17A levels on CD4+ T-cells in Down Syndrome.
Conclusions: Our data indicate that decreased IL-17A expression may play a significant role in the etiology of infections in Down Syndrome. Moreover, we demonstrated that in Down Syndrome the other gene located outside the extra chromosome 21 is also affected.

Introduction

IL-17A is mainly expressed by an activated cluster of differentiation CD4+ T cells, which are classified as T helper 17 (Th17) cells [1]. Recent studies suggest a role of Th17 cells in the pathogenesis of inflammatory and autoimmune disease. Th17 cells are functionally involved in immune responses against extracellular pathogens and have the ability to secrete cytokines: IL-17A, IL-17F, IL-21 and IL-23. Interleukin 17A (commonly referred as IL-17) belongs to the interleukin 17 cytokine family along with five other cytokines: IL-17B, IL-17C, IL-17D, IL-17E (also known as IL-25), and IL-17F. From all of them, the biological function and regulation of both IL-17A and IL-17F are identified to a large extent [2].

Besides Th17 cells, several different types of innate immune cells can also produce IL-17A. Among of them, γδT cell population is widely researched. These cells are mainly localized in mucosal tissues, which secrete IL-17A, and play an important role in early defense against bacterial and fungal infections [3]. During inflammation, several other innate immune cell types can also produce IL-17A. They are lymphoid tissue inducer cells (LTi) such as, natural killer and natural killer T cells, macrophages and Paneth cells. Their functional importance is not yet very well characterized [3,4].

IL-17A is encoded by the *IL17A* gene, which is located on chromosome locus 6p12.

Functionally pro-inflammatory mediate IL-17A play protective roles in host defense against pathogens at epithelial and mucosal

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barriers of the extracellular bacteria which infects the skin, colon and lung, respectively. In addition to this role, IL-17A is involved in fungal infection control which is generated during acute inflammation against *Candida albicans* [5].

Down Syndrome (DS) is by far the most commonly known chromosomal disorder in humans. It expresses multiple systemic complications with both structural and functional defects as a part of the clinical manifestation. DS is also accompanied by increased prevalence of infections as well as is prone to recurrent bacterial and viral infections. Moreover, it co-exists with enhanced incidence of autoimmune diseases and increased risk to develop leukemia.

The mechanisms of immune dysfunction in DS are complex and include an extra gene copy of chromosome 21 and secondary dysregulations of numerous intercellular interactions [6–9]. The number of the dysregulated expressed genes in DS causes immunodeficiency in DS patients. Most of these genes are poorly investigated [10]. It is suggested that cytokine IL-17A produced by Th17 cells may play a significant role in the development of these disturbances in DS. In DS individuals, IL-17A have not been studied so far. Therefore, in the current study we, for the first time, have aimed to analyze *IL17A* gene expression in peripheral white cells and additionally intracellular expression of IL-17A on CD4+ T-cells in children with DS.

Materials and methods

Patients

For the study we recruited thirty children with DS (18 boys and 12 girls) with pure trisomy of chromosome 21 only with confirmed karyotype 47, XY, +21 and 47, XX, +21 (aged 8.1 ± 2.1; range: 6–12 years). The control group included twenty-seven age- and gender-matched healthy children (16 boys and 11 girls) (aged 9.1 ± 1.8; range: 6–12 years) based on physical and laboratory examinations. Controls were recruited from volunteer patients. All patients had no infection before the research and all were clinically and laboratory examined [11]. All examined children were of Caucasian origin. The study was approved by the Ethical Committee of the Medical University of Białystok. The children's parents or legal guardians gave their written informed consent.

Laboratory investigations

A blood sample of 2.4 mL was taken from the left cubital vein after an overnight fast (8–12 h). Mononuclear cells from peripheral blood samples were isolated after centrifugation over a Ficoll density gradient (Histopaque 1077, Sigma–Aldrich, St. Louis, USA), washed three times in phosphate-buffered saline (PBS) and immediately cryopreserved in RMPI-1640 50%, fetal bovine serum (FBS) 40% and dimethyl sulfoxide (DMSO) 10% (all purchased from Sigma) at −80 °C for 24 h and stored in liquid nitrogen until analyses were performed.

RNA isolation and real-time PCR

Real-time PCR

Total RNA from frozen blood white cells was isolated using the ISOLATE RNA Mini Kit (Bioline, Newcastle University, UK), quantified on a spectrophotometer (ThermoScientific, Waltham, MA, USA) and stored at −80 °C. Real-time PCR assay performed in the CFX96 Real-Time System (Bio-Rad, Berkeley, California, USA) was used to quantify mRNA levels of IL-17A. Housekeeping *GAPDH* gene was evaluated. 30 ng of total RNA in a total volume of 20 μL was reverse transcribed using the Tetro cDNA Synthesis Kit (Bioline, Newcastle University, UK) and 1 μL oligo(dT) primer. Real-time PCR was carried out using 2 μL of the cDNA product, 400 nM each primer and the SensiFAST™ SYBR Kit (Bioline, Newcastle University, UK). The following primers were used: IL-17A forward, 5′-CAT CCA TAA CCG GAA TAC CAA TA-3′, IL-17A reverse, 5′-TAG TCC ACG TTC CCA TCA GC-3′; GAPDH forward, 5′-AGT CAA CGG ATT TGG TCG TAT T-3′ and GAPDH reverse, 5′-ATG GGT GGA ATC ATA TTG GAA C-3′ [12]. The cycling conditions were as follows: 95 °C for 2 min to activate the DNA polymerase followed by 40 cycles of 95 °C for 20 s, 60 °C for 20 s, and 72 °C for 30 s. The reactions were then subjected to a melting protocol from 55 °C to 95 °C with a 0.2 °C increment and 1 s holding at each increment to check the specificity of the amplified products. Single product formation was confirmed by melting point analysis and agarose gel electrophoresis. The negative water and RT controls showed no amplification. The relative expression of the IL-17A gene normalized to GAPDH was determined by the delta–delta Ct method [13].

Flow cytometry analysis

Using flow cytometry, we evaluated the percentage (%) and mean fluorescence intensity (MFI) levels of intracellular IL-17A on CD4+CD196+IL17+ cells. Compared were four samples from single patient: fresh whole blood with frozen mononuclear cells (including fresh isolated and frozen mononuclear cells, frozen mononuclear cells and cultured without stimulation and frozen mononuclear cells and cultured with stimulation). Taking into account the results (percentage and MFI) collated in Table 1 a conclusion was drawn that the methods of cell isolation and cryopreservation, thawing and culturing can be used in flow cytometry test method.

After thawing, the mononuclear cells were cultured with a complete medium containing RPMI-1640 90% and FBS 10% at 37 °C in a 5% CO_2. Two parallel cultures were performed for 24 h each per patient. The first unstimulated cells culture was cultured on the culture medium without the addition of stimulating agents. The second stimulated cells were cultured on a culture medium with the addition of ionomycin (Sigma–Aldrich, St. Louis, USA) (1 μg/mL), PMA (Sigma–Aldrich, St. Louis, USA) (50 ng/mL). After 20 h of incubation, brefeldin A (Sigma–Aldrich, St. Louis, USA) (20 μg/mL) was added to both cultures for the 4 remaining hours.

The samples (100 μL) were incubated for 30 min at room temperature with 10 μL of the following monoclonal antibody mAbs from BD Biosciences: anti-CD4, anti-CD161, anti-CD196, and anti-IL-17A (eBioscience, San Diego, CA, USA). After incubation of cells with antibodies to 500 μL was given after permeabilization fluid (BD) for 10 min. After this time was added anti-IL-17A (eBioscience, San Diego, CA, USA). Next washed twice with phosphate-buffered saline, and fixed with CellFix (BD Immunocytometry Systems, San Jose, CA, USA). Flow cytometry analysis was performed using the FACSCalibur cytometer (BD Immunocytometry Systems, San Jose, CA, USA). Cell analysis was performed using the FACSCalibur cytometer (BD Immunocytometry Systems, San Jose, CA, USA). Flow cytometry data were collected in list mode and analyzed using CellQuest software (BD Immunocytometry Systems, San Jose, CA, USA). Analysis of EPCs was performed using flow cytometry based on surface expression of the following markers: CD4, CD161, CD196, and IL-17A on cells localized in the lymphocyte gates. During the conduct of assays lymphocyte population was tested for monocytes (presence of CD14, which is

Table 1
Comparative analysis of percentage (%) and MFI (mean fluorescence intensity) intracellular expression of IL-17A on CD4+CD196+IL17+ T-cells relationships samples.

Samples	%	MFI
Fresh whole blood	4.46	31.28
Fresh isolated and frozen mononuclear cells	3.78	22.54
Frozen mononuclear cells and cultured without stimulation	3.59	25.44
Frozen mononuclear cells and cultured with stimulation	6.6	26.23