



### Original Contribution

## AGE-DEPENDENT GENE EXPRESSION PROFILE IN ATOPIC DERMATITIS

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### ABSTRACT

**PURPOSE:** Atopic dermatitis (AD) is an immune-mediated disease which usually begins during the childhood. The major regulators of Th1/Th2 balance are cytokines and T-regulatory cells. The aim of the present study was to compare gene expression profiles in children and adults with AD. **METHODS:** Total RNA was isolated from blood samples of 19 AD patients composed of 6 children and 13 adult AD patients. We analyzed the gene expression of *IL12A*, *IL12B*, *IL23A*, *IL10* and *Foxp3* mRNA in patients' blood, by quantitative real-time PCR. Relative quantitative evaluation of mRNAs was performed by the comparative  $\Delta\Delta C_t$  method. **RESULTS:** The gene expression, on mRNA level, of all investigated genes was higher in children than in adult AD patients. However, the major result from this study is that *IL12A* (RQ=4.14; p=0.0029), *IL23A* (RQ=3.53; p=0.006), and *Foxp3* (RQ=3.51; p=0.023) mRNAs are significantly increased in children's blood compared to adult AD patients, in contrast to *IL12B* and *IL10* mRNAs. **CONCLUSION:** On the basis of these preliminary results, we could conclude that the altered Th1/Th2 cytokine balance in AD depends on the age of patients. Our results demonstrated that IL-12p70, IL-23 and Treg cells could play different roles in pathogenesis of atopic dermatitis in younger compared to older ages.

**Key word:** IL12-related cytokine, IL10, Foxp3, gene expression, T regulatory cells

### INTRODUCTION

Atopic dermatitis (AD) is a genetically determined increased reactivity of the skin, with dryness, itching and tendency to develop acute and chronic inflammatory skin reaction, with increasing incidence in recent decades. Although AD can start at any age, usually it begins during infancy and childhood. A total of 85% of all cases of atopic dermatitis begin before 5 years of age and many of these children have a spontaneous remission before adolescence (1). It is well known that early childhood represents a critical period of immune development, and that children's immune systems are not equivalent to adults'. This fact contributes to higher susceptibility of children to immune-mediated diseases including AD, which is a preferentially Th2-mediated disease with altered Th1/Th2 cytokine production. The major regulators of Th1/Th2 immune response are

cytokines, particularly IL-10 and IL-12 (2). Bioactive IL-12p70 (p35/p40) is produced by antigen presenting cells such as dendritic cells (DC), monocytes/ macrophages and is a powerful promoter of Th1 cell development by induction of IFN- $\gamma$  and suppression of Th2-immune response. In addition, the IL-12p40 subunit exists in three other forms: a heterodimeric cytokine IL-23 (p19/p40), a homodimeric IL-12p80 and a monomeric form, which also possesses significant impact on the regulation of Th1/Th2 immune response (2-3). The finding of new members of IL-12-related cytokines, rise many questions about their role in the pathogenesis of variety of immune-mediated diseases including atopic dermatitis. IL-10 is an important immunoregulatory cytokine which suppresses production of pro-inflammatory cytokines including IL-12p40-containing cytokines (3-4). IL-10 is secreted by a wide variety of cell types, like Th cells, monocytes, macrophages, dendritic cells. In addition, recently IL-10 has been shown to be produced also by specialized subsets of

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regulatory T cells (Treg), marked as IL-10–producing T reg cells or Tr1. T reg cells express exclusively the forkhead-winged helix transcription factor gene (*Foxp3*), which is a master control gene for their development. Recently, some studies have demonstrated the important role of Treg cells in pathogenesis of allergic and atopy, although these data are inconsistent (5-6).

Furthermore, recent research has revealed that the capacity of immune cells, isolated from patients with AD, to produce regulatory cytokines in childhood and adulthood are quite different, although these data are rare. Mandron et al. (7) have demonstrated that monocytes from children with AD were able to respond to LPS stimulation and produced amounts of IL-12p70 within the same range as that observed in non-atopic adult individuals, in contrast to monocytes from adult patients. Negatively correlation between serum level of total IL-12 (p70+p40) and age among patients with AD have been also reported by Piancatelli D et al (8).

The aim of the present study was to determine and compare gene expression profiles of peripheral blood cells in children and adults with AD. We sought to determine mRNA level of *IL12A*, *IL12B*, *IL23A* genes encoding IL-12p35, IL-12p40 and IL-23p19 subunits respectively; *IL10* gene and *Foxp3* gene typical for Treg cells in attempt to clarify their significance for pathogenesis of atopic dermatitis.

## MATERIALS AND METHODS

**Patients:** A total group of 19 AD patients were included in the present study. Patients, attending to the Clinic of dermatology and venerology, University Hospital, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria, between October 2007 and March 2009 were selected. The diagnosis Atopic dermatitis was set by the criteria of Hanifin and Rajka (9). The group of AD patients was divided into two subgroups: 6 children with age between 3-14 years and 13 adult 23 – 70 years old. Disease history and informed consent was taken from patients or their parents. Patients who received phototherapy or oral immunotherapy (e.g. corticosteroids) were excluded. Individuals with any other autoimmune, allergic, parasitic or dermatologic disease were also excluded. This study was conducted according to the ethical standards of the Ethics Review Board of the Faculty of Medicine, Trakia University, Stara Zagora

**Gene expression assay:** Total RNA was isolated from blood samples by using a column-based illustra RNAspin mini RNA isolation kit (GE Healthcare, UK) following the manufacturer's instructions. Synthesis of cDNA was performed manually according to manufacturer's instructions with High-Capacity cDNA Archive kit (Applied Biosystems, USA) which uses random hexameres and MutliScribe™ MuLV reverse transcriptase enzyme. Incubation conditions for reverse transcription were 10 min at 25°C followed by 2 hours at 37°C and incubation was performed on a GeneAmp PCR System 9700 (Applied Biosystems, USA)

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed on a 7500 Real - Time PCR System (Applied Biosystems, Foster City, CA, USA). The following validated PCR primers and TaqMan probes were used: *IL12B* (assay ID: Hs00233688\_m1); *IL12A* (Hs00168405\_m1); *IL23A* (Hs00372324\_m1); *IL10* (Hs00174086\_m1) and *Foxp3* (Hs00203958\_m1). Eukaryotic 18S ribosomal RNA (Hs99999903\_m1) was used as endogenous control.

An aliquot of 5 µl of the RT reaction was amplified in duplicate at a final volume of 20 µl, using a TaqMan Universal PCR Master Mix and Gene Expression Assay mix, containing specific forward and reverse primers and labeled probes for target genes and endogenous control (Applied Biosystems, USA). The thermocycling conditions were: initial 10 min incubation at 95°C followed by 40 cycles of denaturation for 15 s at 95°C and extension for 1 min at 60°C. PCR data were collected with Sequence Detection System software, version 1.3.1.

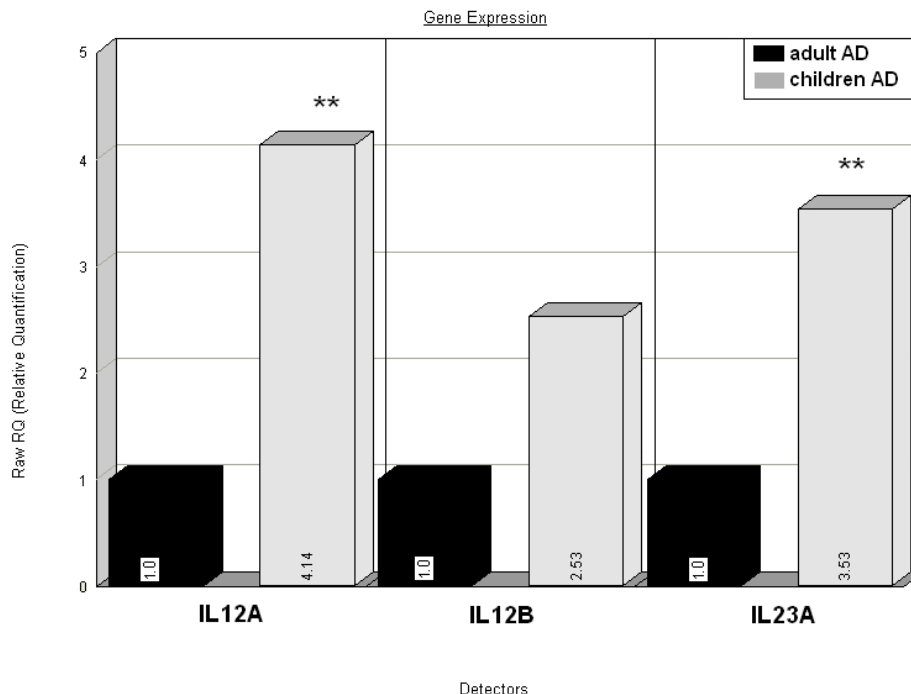
**Statistical and data presentation:** Relative quantitative evaluation of mRNAs was performed by the comparative  $\Delta\Delta C_t$  method. The mean  $\Delta C_t$  obtained in adults AD patients for each target gene was used as calibrator, after normalization to endogenous control-18S rRNA. The results are also presented as an n-fold mean difference relative to calibrator ( $RQ=2^{-\Delta\Delta C_t}$ ). The average  $\Delta C_t$  values of children's and adult's blood were compared by Mann-Whitney test. Differences were considered significant when the P value was less than 0.05.

## RESULTS AND DISCUSSION

To evaluate whether the gene expression of immune regulatory genes depends on age of patients with AD, we compared two main groups of patients with AD – children aged between 3-14

years and adults aged between 23 – 70 years. The investigated genes was higher in children with AD than in adult AD patients. However, a statistical significance was reached only for *IL12A*, *IL23A* and *Foxp3* mRNAs. As shown in **figure 1**, the gene expression of all three IL12-related genes (*IL12A*, *IL12B* and *IL23A*) was elevated in children compared to adult patients. The elevation of *IL12A* mRNA level was the highest (RQ=4.14; p=0.0029), followed by *IL23A* (RQ=3.53; p=0.0066) and *IL12B* (RQ=2.53; p=0.25) mRNA levels. Gene expression of specific subunits for both cytokine - IL-12p35 and IL-23p19 encoded respectively by *IL12A* and *IL23A* genes was significantly up-regulated in children's blood. These results indicate that both heterodimeric cytokine IL-12p70 and IL-23 have a different role in pathogenesis of atopic dermatitis among

gene expression, on mRNA level, of all children in contrast to adult. These results are in concordance with previously reported data about negatively correlation between serum levels of total IL-12 (p70+p40) and age of AD patients (8, 10). On the basis of ours and others' results, we could hypothesize that Th1 immune response is predominantly developed in atopic dermatitis during childhood. In addition, expression of *IL10* mRNA was not statistically different between children and adult AD patients. Because, it is well documented, that IL-10 not only stimulates the differentiation toward Th2 cells, but also inhibits Th1- cell development, we could assume that the reduced ability to produce IL-10 in children with AD might have direct or indirect effects on their elevated expression of IL-12-related genes.

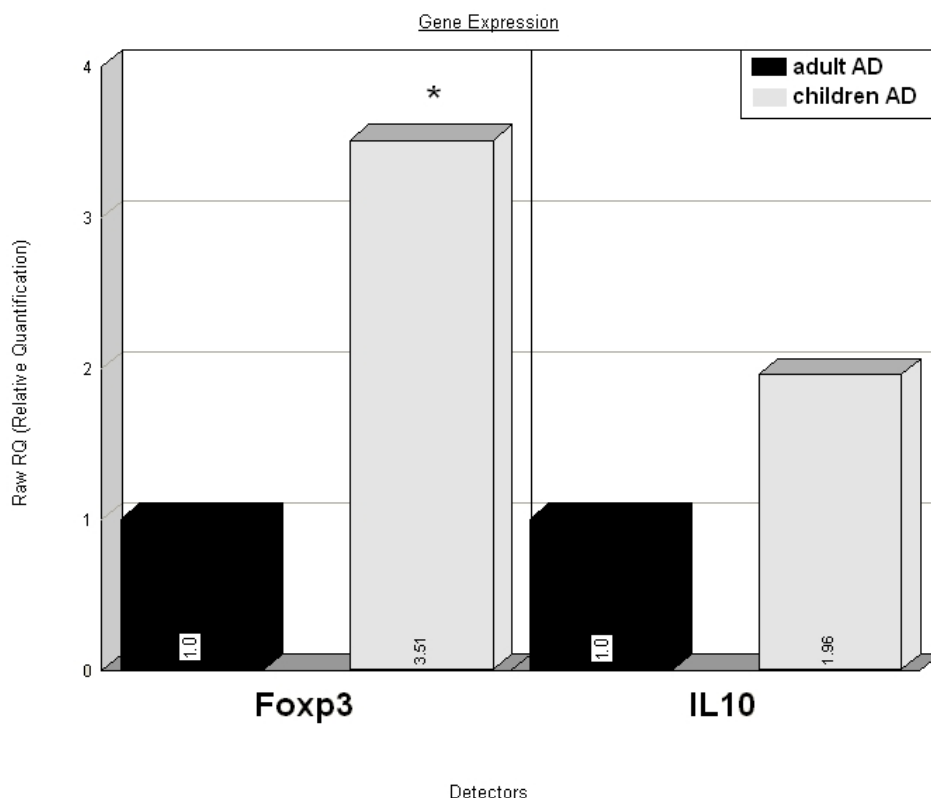


**Figure 1.** The relative expression levels of mRNAs for *IL12A*, *IL12B* and *IL23A* in blood from children with AD and adult patients with AD, which was used as calibrator (RQ=1.00). The results are presented as mean RQ value.

\*\* p<0.01, children with AD vs. adult AD patients

The mRNA level of *Foxp3* gene was also elevated in children with AD compared to adult patients (RQ=3.51; p=0.023) at similar level to *IL23A* mRNA expression (**Fig. 2**). The increased gene expression of *Foxp3* in blood cells isolated from children with AD, demonstrates that Treg cells could play a pivotal role in immunopathogenesis of AD in children. Other authors have demonstrated higher induced *Foxp3* expression after stimulation with both house dust mite and

ovalbumin in children who developed atopic dermatitis than those did not develop the disease (11). However, for the first time we demonstrated systemic up-regulation of *Foxp3* mRNA in children's blood, which was not associated with *IL10* up-regulation. This result suggests that in early life of AD patients, natural and adaptive Tregs- cells which express *Foxp3* are predominant over T regulatory 1 cells, which lack *Foxp3* expression and mediated their effects largely through IL-10.



**Figure 2.** The relative expression levels of mRNAs for *Foxp3* and *IL10* in blood from children with AD and adult patients with AD, which was used as calibrator ( $RQ=1.00$ ). The results are presented as mean RQ value.

\*  $p<0.05$ , children with AD vs. adult AD patients

Our results suggest that the traditional Th1/Th2 theory to explain the complexity of the immune response in atopic dermatitis is oversimplified. Other cell types, including Treg cells should be considered as important players in immunopathogenesis of atopic dermatitis. In addition, it seems likely that the immune response and its regulation in children and adult patients with AD should be considered as different and age-specific. Although, AD is generally accepted as a preferentially Th2-mediated disease, in children with AD we observed up-regulation of Th1-cytokines genes, *IL12A* and *IL23A*, in contrast to typical Th2 cytokine gene – *IL10*. We could hypothesize that balance of Th1/Th2 cytokines depends on age of patients with AD and the activity of Tregs may be also influence the balance of allergen specific Th1 and Th2 cells.

In addition, we have investigated the gene expression on mRNA level of all investigated target genes in two subgroups of children with AD, precisely pre-school children at 3-6 years of age and school-aged children with age of 7-14 years. There were no significant differences in

gene expression level of all investigated genes between these two subgroups. It should be noted that this result has a limitation, regarding the relatively small number of children patients which was included in two subgroups of children with pre-school and school ages.

On the basis of these preliminary results, we could conclude that the altered Th1/Th2 cytokine balance in atopic dermatitis depends on the age of patients. The increased expression of *IL12A* and *IL23A*, but not *IL12B* or *IL10* indicated that systemic Th1 immune response is predominantly developed in atopic dermatitis during childhood. In addition, we could assume that Treg cells which express *Foxp3p* have an important role on regulation of the balance between Th1/Th2 cytokines in atopic dermatitis during childhood.

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