

## Lower expression of mRNA for interferon-gamma in T helper cells of children with newly diagnosed lymphomas

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**Abstract:** The complex interactions between cancer and host cells are far from being fully elucidated. Assessment of Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>3</sub>/Tr<sub>1</sub> balance is an interesting approach to explain immunological disturbances in lymphomas. The aim of our study was to assess mRNA for pro- and anti-inflammatory cytokines in T-cells in 20 children with Hodgkin- and non-Hodgkin lymphomas. CD4<sup>+</sup> and CD8<sup>+</sup> cells were isolated from whole peripheral blood and four different cytokine mRNA levels (IFN- $\gamma$ , IL-10, IL-4, TGF- $\beta$ ) were determined by real-time PCR technique. Comparing to the control group, we found lower expression of mRNA for IFN-gamma in CD4<sup>+</sup> cells at the time of lymphoma diagnosis. It may be one of the pathogenetic mechanisms of impaired immunity in these patients.

**Key words:** Lymphoma - Children - T-cells - Interferon-gamma - Immunosuppression

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### Introduction

Leukemias and lymphomas are the most frequent neoplastic diseases in children. The complex interactions between cancer and host cells are far from being fully elucidated. One of the approaches to understand these relationships can be assessment of cytokines produced by both neoplastic and host cells. We, as well as the other authors, showed anti-inflammatory cytokine predominance (Th<sub>2</sub>, Th<sub>3</sub>, Tr<sub>1</sub> subpopulations of T-cells) at protein and mRNA levels at the time of leukemia diagnosis in children [5, 7, 12]. Data concerning Th<sub>1</sub>/Th<sub>2</sub>/Th<sub>3</sub>/Tr<sub>1</sub> balance in lymphomas are not very clear. The aim of our study was to determine pro- and anti-inflammatory cytokine mRNA expression profiles in T cells of children with Hodgkin- and non-Hodgkin lymphomas.

### Materials and methods

Ten patients with Hodgkin lymphoma (HL) and 10 with non-Hodgkin lymphoma (NHL) were prospectively enrolled in this study. Peripheral blood (1 ml) was taken at the time of diagnosis before the treatment. Control group included 30 children from the Department of Paediatric Surgery subjected to minor surgical operations. The study was approved by the Ethics Committee of the Medical University of Białystok. Informed consent was obtained for collecting the materials for this study.

CD4<sup>+</sup> and CD8<sup>+</sup> cells were isolated from whole peripheral blood by immunomagnetic bead separation (Dynabeads, DYNAL Biotech, Oslo, Norway). mRNA was isolated from sorted lymphocyte subpopulations using Dynabeads mRNA Direct Micro Kit (DYNAL) according to the producer instructions. First strand cDNA was synthesized using random hexamers as primer and High Capacity cDNA Archive Kit by Applied Biosystems. Four different cytokine mRNAs level (IFN- $\gamma$ , IL-10, IL-4, TGF- $\beta_1$ ) were determined by real-time PCR technique with the TaqMan chemistry using ready-to-used Assays-on-Demand Gene Expression Products by Applied Biosystems which contain target-specific primers and probe and TaqMan Universal Master Mix, containing AmpErase uracil-N-glycosylase (UNG) to prevent the re-amplification of carryover PCR products. The PCR amplification and fluorescence data collection were performed with ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). To normalize the amount of expressed cytokine mRNAs, the internal housekeeping gene GAPDH was used and each complementary DNA (cDNA) product was tested in triplex for each

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**Table 1.** Cytokine mRNA expression revealed by real-time PCR ( $\Delta\Delta C_t$  method) in CD4 and CD8 cells of children with lymphoma (median values)

	CD4 <sup>+</sup> cells (median $-\Delta\Delta C_t$ )				CD8 <sup>+</sup> cells (median $-\Delta\Delta C_t$ )			
	IFN- $\gamma$	IL-4	IL-4	TGF-b	IFN- $\gamma$	IL-4	IL-10	TGF-b
control group	10.02	-0.70	6.22	4.11	12.25	-1.17	6.69	4.15
lymphoma group	8.52	-2.25	4.62	4.47	12.03	-2.39	5.72	4.70
significance	p=0.03	(-)	(-)	(-)	(-)	(-)	(-)	(-)

of four cytokines mRNA and GAPDH mRNA. To calculate our data we used comparative  $C_t$  method for relative quantification ( $\Delta\Delta C_t$  method) which describes the change in expression of the target gene in a test sample relative to a calibrator sample and provides accurate comparison between the initial level of template in each sample. As a calibrator sample we used Total Raji RNA (Applied Biosystems) which was processed in the same way as the test samples. Data were analyzed with Sequence Detector System (SDS) software version 2.1 (Applied Biosystems).

Significance levels were calculated according to the nonparametric Mann-Whitney U-test (difference between the lymphoma and the control group) and the Spearman correlation coefficient (correlation between mRNAs for the examined cytokines). Level of  $p < 0.05$  was regarded as significant.

## Results and discussion

We found lower mRNA levels for IFN- $\gamma$  in CD4<sup>+</sup> cells in children with newly diagnosed lymphomas in comparison to the control group ( $-\Delta\Delta C_t$ : 10.02 vs. 8.52,  $p=0.03$ ). There were no statistically significant differences in mRNA levels between the control and the lymphoma group for the other assessed cytokines, *i.e.* IL-10, IL-4 and TGF- $\beta_1$  ( $p > 0.05$ , Table 1).

In the lymphoma and control groups (analysed separately and together), we noted positive correlations between mRNA levels for all the assessed cytokines in CD4<sup>+</sup> and CD8<sup>+</sup> cells (*e.g.* IFN- $\gamma$  and IL-10, IL-4, TGF- $\beta$ ,  $r=0.71$ ,  $r=0.61$ ,  $r=0.60$ , respectively).

These preliminary results demonstrate that levels of expression for IFN- $\gamma$  mRNA in T helper cells are lower in children with lymphomas than in healthy children. Similarly, IFN- $\gamma$  production in peripheral blood mononuclear cells was at low levels in *Helicobacter pylori*-induced low-grade gastric mucosa-associated lymphoid tissue (MALT) lymphoma [11]. However, opposite results were obtained by Gergely *et al.* [2] - they found increased percentage of IFN- $\gamma$ -producing T cells ( $Th_1$ ) in untreated lymphoma cases. Their study was performed at protein level (flow cytometry) and our study - at mRNA level. This may be one of possible explanations for different results. Interestingly, Mori *et al.* [9] found increased percentages of  $Th_1$  and  $Th_2$  cells in patients with untreated B-cell diffuse large cell lymphoma but the mean  $Th_1/Th_2$  ratio was lower than in patients in remission - is it a sign of IFN- $\gamma$  deficiency? The authors

suggest that  $Th_1/Th_2$  imbalance could play a significant role in lymphoma genesis and durable remission.

Some authors postulate a relationship between type of immune response and prognosis in lymphomas: tumor-resistant hosts developed  $Th_1$  dominant response, whereas susceptible hosts -  $Th_2$  dominant response [4]. Moreover, in cases positive for  $Th_1$  and  $Th_2$  profile chemokines (*i.e.* ST2(L), CCR5 and CXCR3 expression), favourable prognosis was shown in comparison to cases negative for  $Th_1$  or  $Th_2$  profile [10].

We have not noted any differences in mRNA levels for IL-10, IL-4 and TGF- $\beta$  between the control and lymphoma group. In Hodgkin disease, Mainou-Fowler *et al.* [8] observed increased production of IL-4 by CD8<sup>+</sup> cells from peripheral blood. Kaizu *et al.* [3] reported "disruption of the cytokine network", *i.e.* increase in IFN- $\gamma$  and IL-10 after splenectomy in aggressive NK cell leukemia/lymphoma. Correlations between all the assessed mRNAs found in our experiment suggest their interdependence in cytokine network in the human organism.

Results of our investigation could be useful in the development of new therapeutic strategies using  $Th_1$  cytokines. First reports in this field demonstrate induction of humoral immune response after adenovirus-mediated intralesional interferon-gamma gene transfer [1].

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