mRNA for chosen pro- and anti-inflammatory cytokines in T-lymphocytes in paediatric leukemias and lymphomas - a preliminary report

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Abstract

We assessed mRNA for chosen pro- and anti-inflammatory cytokines in T-lymphocytes of peripheral blood in paediatric patients with leukemias and lymphomas. Levels of four different cytokine mRNAs (IFN-γ, IL-10, IL-4, TGF-β) were determined by the real-time PCR technique. In the whole examined group, at the time of diagnosis, we noted lower amounts of mRNA for TGF-β_1, comparing to respective values in the control patients. In the ALL group, we observed the following: 1) at the time of diagnosis: lower amounts of mRNA for IL-4 and for TGF-β_1, comparing to respective values in the control group; 2) lower amounts of mRNA for IL-10 after remission induction, comparing to the time of diagnosis. In our opinion, "immunedysregulation" in lymphoproliferative diseases in children is not caused by IFN-γ deficiency. The deficit of anti-inflammatory cytokines, i.e., IL-4, TGF-β, with higher amounts of IL-10, suggests their role in cancer development.

Key words: leukaemia, lymphoma, children, mRNA, cytokines, T-lymphocytes

Introduction

Haematological malignancies represent a unique group of proliferative diseases, in which neoplastic cells arise from the immune system and thus, they exert a potential immunomodulatory effect. Leukaemic blasts, like normal lymphocytes, are produced in bone marrow and these two types of cells may interact. It is likely that neoplastic cells produce substances, which impair the immune response, thus promoting cancer expansion. The main role in antitumour immunity is played by T-lymphocytes. According to produced cytokines, T-cells can be differentiated into Th_1/Tc_1 subsets (producing IL-2 and IFN-γ), Th_2/Tc_2 (IL-4, IL-5, IL-10) and Th_3/Tr_1 (TGF-β, IL-10). Most of the authors report a Th2 polarization profile in haematological malignancies and normalization of the Th_1/Th_2 balance in remission [1]. A type 1-to-type 2 cytokine shift may be responsible for reduced antitumor immunity and is correlated with disease progression. We previously found Th_2 (the percentage of $CD4^+IL\text{-}4^+$ cells - flow cytometry) predominance at the time of diagnosis of acute lymphoblastic leukaemia (ALL) [2]. In the present, study we assessed mRNA for chosen pro- and anti-inflammatory cytokines at the time of diagnosis of most common lymphoproliferative diseases in children.

Material and methods

Twenty (20) patients with ALL (n=13), Hodgkin lymphoma (n=4) and non-Hodgkin lymphoma (n=3) were prospectively enrolled into this study. Peripheral blood (1 ml) was taken at the time of diagnosis and in ALL - after prednisone prophase (day 8th), during (day 15th) and after remission induction (day 33rd). The control group included 30 children from the Department of Paediatric Surgery, subjected to minor surgical operations.

$CD4^+$ and $CD8^+$ 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's instructions. First strand cDNA was synthesized, using random hexamers as primer and a High Capacity cDNA Archive Kit by AppliedBiosystems. Levels of four different cytokines mRNAs (IFN-γ, IL-10, IL-4, TGF-β) were determined by the real-time PCR technique with the TaqMan chemistry, using ready-to-use

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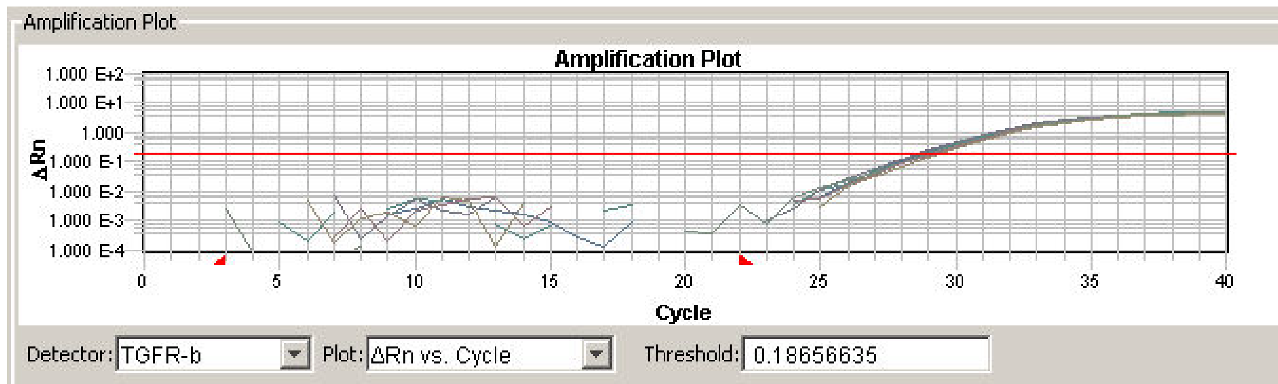
Figure 1. Amplification plot (log view) of TGF-b mRNA



Assays-on-Demand Gene Expression Products by AppliedBiosystems, which contain target-specific primers and probe, and a TaqMan Universal Master Mix, containing AmpErase uracil-N-glycosylase (UNG) to prevent re-amplification of carryover PCR products. PCR amplification and fluorescence data collection were performed with the ABI PRISM 7900HT Sequence Detection System (AppliedBiosystems). In order 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 of the four cytokines mRNA and GAPDH mRNA. For the calculation of our data we used the Comparative Ct method for relative quantification (ΔΔCt method), which describes the change in expression of the target gene in a test sample relative to a calibrator sample and provides an accurate comparison between the initial levels of template in each sample. We used Total Raji RNA by AppliedBiosystems, as a calibrator sample, which was processed in the same way as the test samples. The obtained data were analysed with the Sequence Detector System (SDS) software, version 2.1 (AppliedBiosystems).

Results

In the examined groups and in the control group, we found the following: a) in $CD4^+$ cells: positive correlations between mRNA for IFN-γ and: IL-4 (r=0.83, p<0.0001), TGF-β_1 (0.91, p<0.0001); and between IL-4 and TGF-β_1 (r=0.86,p<0.0001); b) in $CD8^+$ cells: a negative correlation between mRNA for IFN-γ and IL-10 (r=-0.44, p=0.0007); a positive correlation between IFN-γ and IL-4 (r=0.43, p=0.01), IFN-γ and TGF-β_1 (r=0.81, p<0.0001); also a positive correlation between IL-4 and TGF-β_1 (r=0.7, p<0.0001). In the whole examined group, at the time of diagnosis, we noted lower amounts of mRNA for TGF-β_1 in $CD4^+$ cells (the difference not statistically significant) and in $CD8^+$ cells (p=0.01) comparing to the control patients; the amounts of other cytokines (IFN-γ, IL-4, IL-10) did not differ between those in the whole examined group and those in the control patients. In the ALL group, we observed: a) at the time of diagnosis: lower amounts of mRNA for IL-4 (both in $CD4^+$ and $CD8^+$ cells, p=0.02 and p=0.04) and for TGF-β_1 (also in both subpopulations p=0.03 and p=0.01), comparing to respective values in the control group; b) lower amounts of mRNA for IL-10 after remission induction, comparing to the time of diagnosis (p=0.05). An example of amplification plot for TGF-β is presented on Figure 1.

Discussion

The only report, concerning Th_1/Th_2 balance in children with ALL, was performed by Zhang et al. [3]. The authors found lower Th_1/Th_2 and Tc_1/Tc_2 ratios at the time of diagnosis and recovery after achieving complete remission. Regarding IFN-γ, we did not find any difference between the examined group and the control. This confirms our previous results, obtained with flow cytometry, and the results obtained by Kiani et al. in CML patients [2, 4]. Podhorecka et al. found a higher Th_1 percentage in patients with B-cell chronic lymphocytic leukaemia (B-CLL) than in controls and the Th_2/Tc_2 shift during disease progression [5]. The amounts of mRNA for IL-4 were lower in ALL patients at the time of diagnosis. Similar results, concerning IL-4, were obtained by Kiełbiński et al. in acute leukaemias in adults [6]. In contrast to our results, Kamińska et al. observed, comparable with the control levels of IL-4 in supernatants from the whole blood cell cultures of adult patients with ALL [7]. Mori et al. reported polarization to Th_2 in untreated B-cell diffuse large cell lymphoma patients and to Th_1 in complete remission [1]. We noted a tendency (not significant) to higher amounts of mRNA for IL-10 at the time of diagnosis of leukemias and lymphomas. IL-10 production in patients with cancer was higher than that in healthy controls, so it is possible that tumour cells secrete cytokines, shifting T-cells to produce more IL-10 [8]. Kebelmann-Betzing et al. suggest that the secretion of IL-10 (from leukaemic blasts) and the lack of down-regulation of adhesion and costimulatory molecules determine the mechanism of escape from immune surveillance in relapsed ALL [9]. In the whole examined group and, especially in ALL patients, we observed lower amounts of mRNA for TGF-β, comparing to the values in the control patients. Tatsumi et al. found Th_2, but not Th_3, type response against renal cell carcinoma or melanoma [10]. In our opinion "immunedysregulation" in lymphoproliferative diseases in children is not caused by IFN-γ deficiency. The deficit of anti-inflammatory cytokines, i.e., IL-4, TGF-β and the higher amounts of IL-10, revealed in that group of children, suggests their role in cancer development, but it should be confirmed in a greater cohort of patients. The very strong correlations, observed among all the assessed substances, suggest their

interdependence in cytokine network in the human organism. Determination of the factors, that affect Th and Tc profiles, may lead to a better understanding of the immunological status and development of immunotherapy.

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