

Received: 2004.06.30
Accepted: 2004.12.03
Published: 2005.08.15

Assessment of selected co-stimulatory, adhesion and activatory molecules and cytokines of Th₁/Th₂ balance in acute lymphoblastic leukemia in children

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Włodzimierz Łuczyński^{1ABCDEF}, Anna Stasiak-Barmuta^{2ADE},
Maryna Krawczuk-Rybak^{1ADEG} and Iwona Malinowska^{3B}

¹ Department of Pediatric Oncology, Medical University of Białystok, Poland

² Flow Cytometry Unit, Medical University of Białystok, Poland

³ Department of Pediatrics, Hematology and Oncology, Medical University of Warsaw, Poland

Source of support: by the State Committee for Scientific Research (KBN, Poland) grant no. P05E08025.

Summary

Introduction:

Recent years have seen a rise in the importance of cytokine production and co-stimulatory/activatory molecule expression in the immune response in leukemia. The aim of our study was to assess the function of T lymphocytes in children with acute lymphoblastic leukemia (ALL) during remission induction based on selected cytokine and co-stimulatory/activatory molecule expression.

Materials and Methods:

The study group consisted of 50 children with ALL (B cell precursor). Peripheral blood samples were taken before treatment (day 0), after the prednisone prophase (day 8), and during (day 15) and after (day 33) remission induction. The percentages of T cells with interferon (IFN)- γ (Th₁), interleukin (IL)-4 (Th₂) and IL-2 receptor (IL-2R), CD28, CTLA-4, CD38, ICAM-1, and HLA-DR expression were assessed by tricolor flow cytometry.

Results:

At the time of diagnosis we noted higher percentages of T cells with adhesion molecule ICAM-1, activation molecule CD38 expression, and an increased population of Th₂ cells (IL-4) compared with the control group. During and after remission induction we observed a decreased population of CD38⁺ T cells, elevated percentages of helper T lymphocytes with IL-2R expression, and a rise in helper T lymphocytes producing IFN- γ (Th₁). During fever/infection, higher levels of activated T lymphocytes (CD4⁺HLA-DR⁺, CD8⁺HLA-DR⁺), a rise in Th₁, and no change in Th₂ populations were observed.

Conclusion:

The results suggest T cell activation and Th₂ predominance at the time of diagnosis and during remission induction in ALL in children. These results confirm the involvement of cellular immunity in the leukemic process and can be used in immune therapy in leukemia.

Key words:

immunosuppression • cancer • IFN- γ • IL-4 • co-stimulatory molecules

Abbreviations:

ALL – acute lymphoblastic leukemia, **B-CLL** – B cell chronic lymphocytic leukemia, **HLA-DR** – human leukocyte antigen class II (DR), **ICAM-1** – intercellular adhesion molecule 1, **IFN- γ** – interferon gamma, **IL-2** – interleukin-2, **IL-2R** – IL-2 receptor, **TGF- β** – transforming growth factor beta.

Full-text PDF:

http://www.aite-online/pdf/vol_53/no_4/7746.pdf

Author's address:

Włodzimierz Łuczyński, M.D., Department of Pediatric Oncology, Medical University of Białystok, Waszyngtona 17, 15-274 Białystok, Poland, tel./fax +48 85 7450-846; e-mail: vlodek@amb.edu.pl

INTRODUCTION

Oncohematological ailments represent a unique group of proliferative diseases in which neoplastic cells arise from the immune system and thus have a potential immunomodulatory effect. Leukemic 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. In recent years a role has been ascribed to the function of co-stimulatory molecules in the immune response in leukemia. Signal transmission from an antigen-presenting cell (APC) to a T lymphocyte involves the T cell receptor complex (TCR/CD3) and the co-stimulatory molecules of the B7 family (CD80 and CD86) on the APC and CD28 and CD152 (CTLA-4) on the surface of the T cell. The adhesion molecules CD54 (ICAM-1), CD102 (ICAM-2), CD11a (LFA-1), and CD58 (LFA-3) play an adjunctive role. The presence of histocompatibility antigens (HLA-DR), IL-2 receptor (CD25), CD69, CD28, and CD38 indicates T cell activation¹⁷.

On the other hand, the study of the profiles of cytokine secretion by T lymphocytes reveals their functional diversity and allows their subdivision into Th₁ (interleukin (IL)-2, interferon (IFN)- γ , cellular response: antiviral and antineoplastic), Th₂ (IL-4, humoral response), Th₃ (transforming growth factor (TGF)- β , immunological tolerance, immunosuppression), and Tr₁ (IL-10, TGF- β , immunosuppression). Some authors report a Th₂ polarization profile in hematological malignancies and a normalization of the Th₁/Th₂ balance after remission^{7, 12}. A complex analysis of the immune response in neoplastic diseases in children has not been conducted previously.

The study's objective was to evaluate the function of T lymphocytes in acute lymphoblastic leukemia (ALL) in children at the time of diagnosis, in the course of inductive therapy, and after remission, also during concomitant infections. The assessment was based on the expressions of co-stimulatory/activatory molecules and T cell-produced cytokines, i.e. the Th₁/Th₂ balance. This study was authorized by the local ethics committee and all subjects gave their informed consent for participation.

MATERIALS AND METHODS

The study group consisted of 50 children (27 boys, 23 girls, mean age: 6.50 ± 4.89 years) suffering from newly diagnosed B cell precursor ALL being treated in pediatric oncology centers in Białystok and Warsaw from September 2002 to September 2003.

All patients were treated according to the international protocol ALL IC 2002. During remission induction, the following drugs were administered: prednisone (60 mg/m², days 1–33), vincristine (4×1.5 mg/m²), daunorubicin (4×20 mg/m²), and L-sparaginase (8×5000 U/m²). The control group included 50 children (25 boys and 25 girls, mean age: 7.32 ± 4.09 years) from the Department of Pediatric Surgery (Białystok) subjected to scheduled operations for hernia. The control children had been free of infection during the 2 weeks preceding admission to the department, had a negative history of allergic diseases, normal laboratory tests for leukocytosis and lymphocytosis, and normal acute phase index markers. The study and control patients were age- and sex-matched ($p > 0.05$). The study was approved by the Ethics Committee of the Medical University in Białystok and informed consent was obtained.

Co-stimulatory and activation molecules

One milliliter of whole blood was taken to EDTA probe. Ten μ l of each monoclonal antibody (mAb) were added to 100- μ l samples of whole blood. Assessment of co-stimulatory and activation molecules was based on the analysis of peripheral blood mononuclear cells by flow cytometry and using the following antibodies: CD3-PerCP, CD4-PerCP, CD8-PerCP, HLA-DR-FITC, CD28-PE, CD152-FITC, CD54-FITC, CD25-FITC, and CD38-FITC (Becton Dickinson, USA).

Th₁/Th₂ balance

Cells and cell culture. Whole blood samples, diluted to 1:1 with pyrogen-free RPMI 1640 medium, were cultured for 4 h at 37°C in 5% CO₂. The cells were stimulated with PMA (50 ng/ml; Sigma Chemical, St. Louis, MO, USA) and ionomycin (1 μ M; Sigma) in the presence of the protein transport inhibitor Brefeldin A (3 μ mol/ml; Sigma) as described by Mori et al.¹². For the proliferative cell response evaluation, whole blood without Brefeldin A was used.

Staining. Cultured cells were washed twice in phosphate-buffered saline (PBS)/1% bovine serum albumin and then stained with 10 μ l mAb to the following cell-surface markers: CD3-PerCP, CD4-PerCP, and CD8-PerCP (Becton Dickinson, San Jose, CA, USA). Ten μ l of CD69-FITC (Becton Dickinson) was added to the control blood. The cells were then fixed and permeabilized with IntraStain (Dako, Glostrup, Denmark) according to the manufacturer's instructions and incubated with 20 μ l mAb against the cytokines IL-4 and IFN- γ (Coulter, USA). Finally, the cells were washed twice in PBS and resus-

pended in 250 µl of PBS containing 0.5% formaldehyde.

Flow cytometry. Phenotypic analysis of the cultured cells was performed by 3-color flow cytometry using a Coulter EPICS XL flow cytometer. A minimum of 10^4 lymphocytes were evaluated. Confirming isotypic negative controls were used.

T lymphocyte function was assessed on days 0 (before any treatment), 8 (after the prophase with prednisone), 15 (in the course of remission induction, i.e. after 15 days with prednisone and 1 dose each of vincristine and daunorubicin) and 33 (after remission induction, i.e. 33 days with prednisone, 4 doses each of vincristine and daunorubicin, and 8 doses of L-asparaginase). On days 8, 15, and 33 the therapeutic response was evaluated by assessing blast count in the peripheral blood and/or bone marrow. All episodes of elevated temperature and infection were recorded. In the study group, 21 fever/infection episodes were noted, including fever of unknown etiology (20 episodes) and *E. coli* sepsis (1 episode). The results were compared between the groups of patients with and without fever/infection.

Statistical analysis was performed using Statistica 5.0 for Windows. The results were not normally distributed when examined using the Lilliefors statistic and normal plot and are expressed by mean and median values (25th–75th percentiles). Significance levels were calculated according to the nonparametric Mann-Whitney U test. A level of $p < 0.05$ was regarded as significant. The Spearman correlation coefficient was used to determine the association between the obtained flow cytometry results and clinical data (e.g. therapy response: the percentage of blasts in peripheral blood or bone marrow).

RESULTS

In the control group, absolute lymphocyte counts were significantly higher than in all the assessment stages in the study group (3.500 ± 2.429 vs. 2.137 ± 1.551 , $p = 0.01$). However, as no differences were found in the absolute lymphocyte counts between the respective stages of the therapy ($p > 0.05$), percentages were used to evaluate the lymphocyte subpopulations.

Table 1 presents the T cell subpopulations in patients without fever or infection.

Co-stimulatory and activatory molecules

The mean percentages of $CD3^+$ cells in the group of ALL children both at the time of diagnosis and on days 8 and 15 of treatment did not differ from that

noted in the control group (controls: 69.23%, day 0: 68.22%, day 8: 71.31%, day 15: 64.90%). Their highest level was observed in ALL children after remission induction, i.e. on day 33 (83.74%, compared with the controls and day 15: $= 0.01$). The percentages of helper cells with receptor co-expression for IL-2 ($CD4^+CD25^+$) at the time of diagnosis and on day 8 approached control levels, but were higher after remission induction (day 33; $p < 0.04$). The percentages of $CD3^+$ cells with co-expression of CD28 or CTLA-4 (CD152) revealed no differences between the mean values in the control subjects and those at the respective therapy stages in the study group ($CD3^+CD28^+$ control vs. ALL patients: 27.80% vs. 23.39%; $CD3^+CD152^+$: 9.30% vs. 12.17%). Subpopulations of $CD3^+$ cells with co-expression of CD54 (ICAM-1) were higher than in controls ($p = 0.04$) both at diagnosis and during remission induction, with the most pronounced increase on day 8 compared with day 33 ($p = 0.0003$).

The mean percentage of $CD8^+$ cells with CD38 co-expression was significantly higher in the study group at ALL diagnosis than in healthy children ($p = 0.0005$; Fig. 1). During chemotherapy, a decrease was observed in the percentage of $CD8^+CD38^+$ cells to values approaching those of the controls ($p < 0.01$).

Because of the small number of fever/infection episodes, the results were evaluated irrespective of the therapy stage, i.e. all the results with vs. without infection/fever were compared. The mean values of $CD4^+$ and $CD8^+$ cells with co-expression of HLA-DR during infection were significantly higher than in the infection-free period ($p = 0.011$ and $p = 0.02$, respectively; Table 2 and Fig. 2). No such differences were found with respect to other co-stimulatory, adhesion, and activatory molecules evaluated in the study.

Th₁/Th₂ balance

No statistically significant differences were found in the percentages of $CD4^+$ and $CD8^+$ lymphocytes with IFN- γ co-expression between the control and study group at ALL diagnosis (Fig. 3 and 4). Likewise, no differences were observed in the percentages of the Th₀ ($CD4^+IFN-\gamma^+IL-4^+$) and Th_{double-negative} ($CD4^+IFN-\gamma^-IL-4^-$) subpopulations. During therapy a gradual increase was noted in the percentage of Th₁ lymphocytes (day 0 vs. day 33, $p = 0.0004$), which on day 33 was higher than in the control group. The percentage of T lymphocytes with IL-4 co-expression (both helper and cytotoxic/suppressor: Th₂ and Tc₂) at diagnosis and during therapy (except for day 15) was significantly higher than in controls ($p < 0.05$).

Table 1. Percentages of T lymphocytes with IFN- γ and IL-4 and co-stimulatory and activatory molecule co-expression

T lymphocyte subpopulations																						
	CD4 ⁺ CD25 ⁺			CD3 ⁺ CD54 ⁺			CD8 ⁺ CD38 ⁺			CD4 ⁺ IFN- γ ⁺ (Th1)			CD8 ⁺ IFN- γ ⁺ (Tc1)			CD4 ⁺ IL-4 ⁺ (Th2)			CD8 ⁺ IL-4 ⁺ (Tc2)			
	mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		
1. Control group	9.46	8.90 (7.9–12.0)		2.86	2.3 (1.7–3.7)		48.66	51.0 (40.0–60.0)		7.08	3.20 (2.1–10.5)		6.76	4.15 (2.2–10.4)		2.12	0.8 (0.5–2.4)		1.37	0.55 (0.1–1.75)		
2. At time of diagnosis	9.37	7.1 (6.0–14.2)		7.05	3.35 (2.5–7.1)		67.06	68.2 (54.5–78.1)		3.24	3.10 (1.1–4.4)		4.93	3.5 (1.2–5.9)		3.38	2.3 (2.1–4.6)		3.00	2.35 (0.9–5.3)		
3. Day 8	9.33	8.2 (6.6–11.8)		10.02	10.0 (3.0–14.8)		40.27	40.1 (26.7–57.5)		5.21	4.15 (2.5–6.3)		7.12	4.9 (2.3–11.6)		4.60	2.75 (1.1–6.5)		3.02	2.0 (1.6–4.4)		
4. Day 15	4.62	3.75 (2.5–6.78)		8.62	8.7 (1.6–10.1)		35.02	29.0 (18.0–50.5)		11.00	5.90 (3.0–6.8)		6.04	5.9 (3.5–7.1)		1.44	0.85 (0.1–2.8)		1.57	1.5 (0.3–2.7)		
5. Day 33	12.25	12.35 (10.3–14.2)		5.04	4.7 (3.2–6.0)		48.36	46.0 (29.3–67.5)		10.30	6.10 (4.4–13.3)		9.35	6.5 (3.9–13.2)		4.27	3.2 (1.1–6.85)		2.97	1.6 (0.5–4.2)		
Statistics	1, 2, 3, 4 vs. 5 p<0.04		1 vs. 2, 3, 4, 5 p<0.04		1 vs. 2 p=0.0005		1 vs. 2 p=0.02		2 vs. 5 p=0.0004		1 vs. 5 p=0.02		1 vs. 2 p=0.054		1 vs. 3 p=0.03		1 vs. 5 p=0.04		1 vs. 2 p=0.01		1 vs. 3 p=0.002	
			3 vs. 5 p<0.0003		2 vs. 3, 4, 5 p<0.01		2 vs. 5 p=0.0004														1 vs. 5 p=0.04	

Table 2. Subpopulations of T lymphocytes depending on the presence of fever/infection

	CD4 ⁺ HLA-DR ⁺ (%)			CD8 ⁺ HLA-DR ⁺ (%)			CD4 ⁺ IFN- γ ⁺ (Th1)			CD8 ⁺ IFN- γ ⁺ (Tc2)			CD4 ⁺ IL-4 ⁺ (Th2)			CD8 ⁺ IL-4 ⁺ (Tc2)		
	mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)		mean	median (25–75 pc)	
No fever or signs of infection	7.42	4.2 (2.1–7.1)	9.30	7.05 (3.55–11.8)	7.00	4.3 (2.4–7.4)	6.36	4.35 (2.3–8.8)	3.22	2.05 (0.6–4.3)	2.25	1.5 (0.3–3.2)						
Presence of fever/ infection	24.52	17.25 (5.0–44.0)	23.25	22.5 (12.3–34.2)	17.76	7.4 (12.2–13.3)	21.90	17.7 (8.4–28.6)	2.93	2.75 (0.6–4.7)	1.28	1.3 (0.9–2.0)						
Statistics	p=0.0011		p=0.02		p=0.005		p=0.01		–		–							

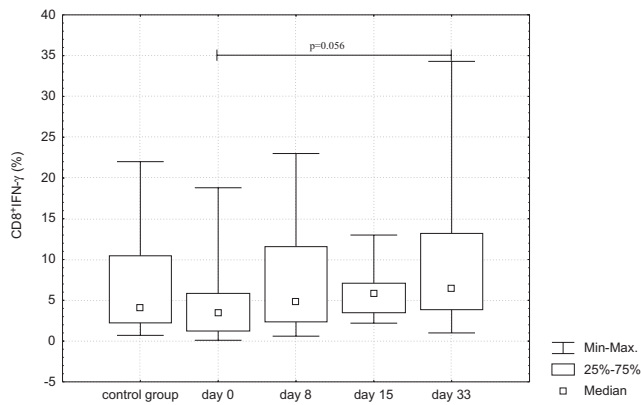


Figure 1. CD8⁺ lymphocytes with co-expression of IFN- γ .

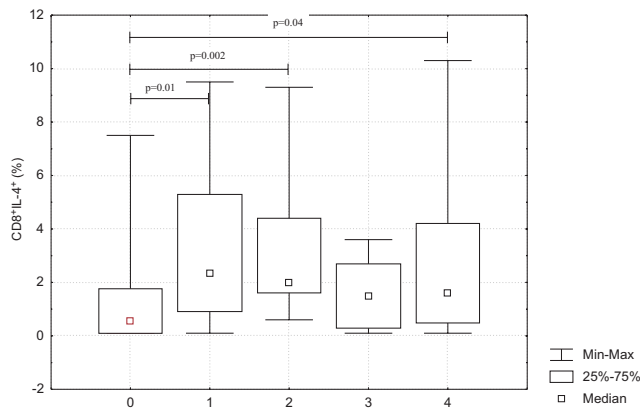


Figure 2. CD8⁺ lymphocytes with co-expression of IL-4.

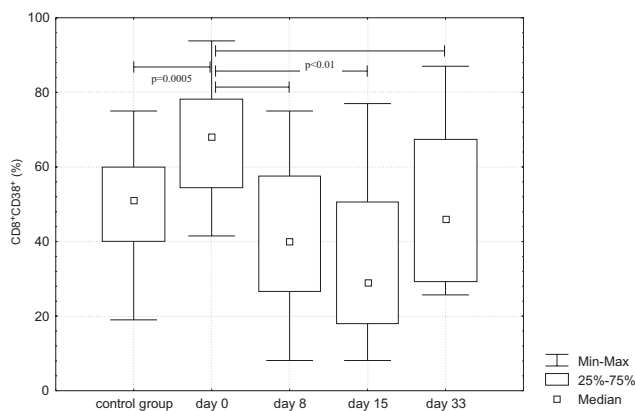


Figure 3. CD8⁺ lymphocytes with CD38 co-expression.

No correlation was found between the blast count in bone marrow/peripheral blood and the percentage of Th₁ and Th₂ cells (and Tc₁, Tc₂), both at the time of diagnosis and during the subsequent examinations.

In the course of infection (analysis of all the results irrespective of therapy stage, as in the case of activatory molecules), the percentage of T cells with IFN- γ co-expression (both CD4⁺ and CD8⁺ subpopula-

tions, $p=0.005$ and $p=0.01$, respectively) was statistically significantly higher during fever/infection; however, no such differences were observed in the percentage of cells with IL-4 co-expression (Table 2 and Fig. 2).

DISCUSSION

Our analysis of the host T cell response to the leukemic clone and therapy showed an increased percentage of lymphocytes with co-expression of adhesion molecules ICAM-1 at disease diagnosis. Pui et al.¹⁵ found higher serum ICAM-1 levels in children with ALL at the time of diagnosis, which decreased after remission. According to these authors, higher serum ICAM-1 levels in patients with advanced malignancy may result either from the increased response of the host immune system to neoplastic cells or reflect high tumor mass. Banks et al.¹ suggest that the higher serum ICAM-1 level observed by many researchers in adult cancer patients may originate from neoplastic cells. Scrivener et al.¹⁷ at the time of B cell chronic lymphocytic leukemia (B-CLL) diagnosis observed weaker expression of CD25, CD28, and CD152 and a higher percentage of T cells (CD3⁺) with histocompatibility antigen co-expression (HLA-DR). However, the percentage of T cells with adhesion molecules remained normal. According to these authors, it is the CD3⁺HLA-DR⁺ subpopulation that acts as the immune system's activation index at the moment of diagnosis¹⁷. Frydecka et al.⁴ noted lower percentages of CD28⁺ (costimulatory molecule) and higher CD152⁺ (downregulatory molecule) T cells in B-CLL patients than in controls. In the authors' opinion these alterations may lead to hyporesponsiveness or anergy in this disease.

We found a higher percentage of T cells (CD8⁺) with co-expression of CD38⁺ molecules at ALL diagnosis, which in our opinion may suggest the activation of the immune system at the onset of leukemia. CD38 molecules are expressed in certain forms of B-CLL and are considered to be an unfavorable prognostic factor¹⁰. In patients with HIV infection, an increased percentage of CD8⁺CD38⁺ lymphocytes indicates the active form of the disease¹⁴, just as in Epstein-Barr virus (EBV)-induced mononucleosis⁸.

The present evaluation of the Th₁/Th₂ balance in ALL children revealed disorders in the form of increased percentages of CD4⁺ and CD8⁺ T cells with IL-4 co-expression (Th₂), and no significant differences between the control and study groups in the percentage of T cells with IFN- γ co-expression (Th₁). The study of the Th₁/Th₂ balance in children with ALL conducted by Zhang et al.²⁰ revealed an

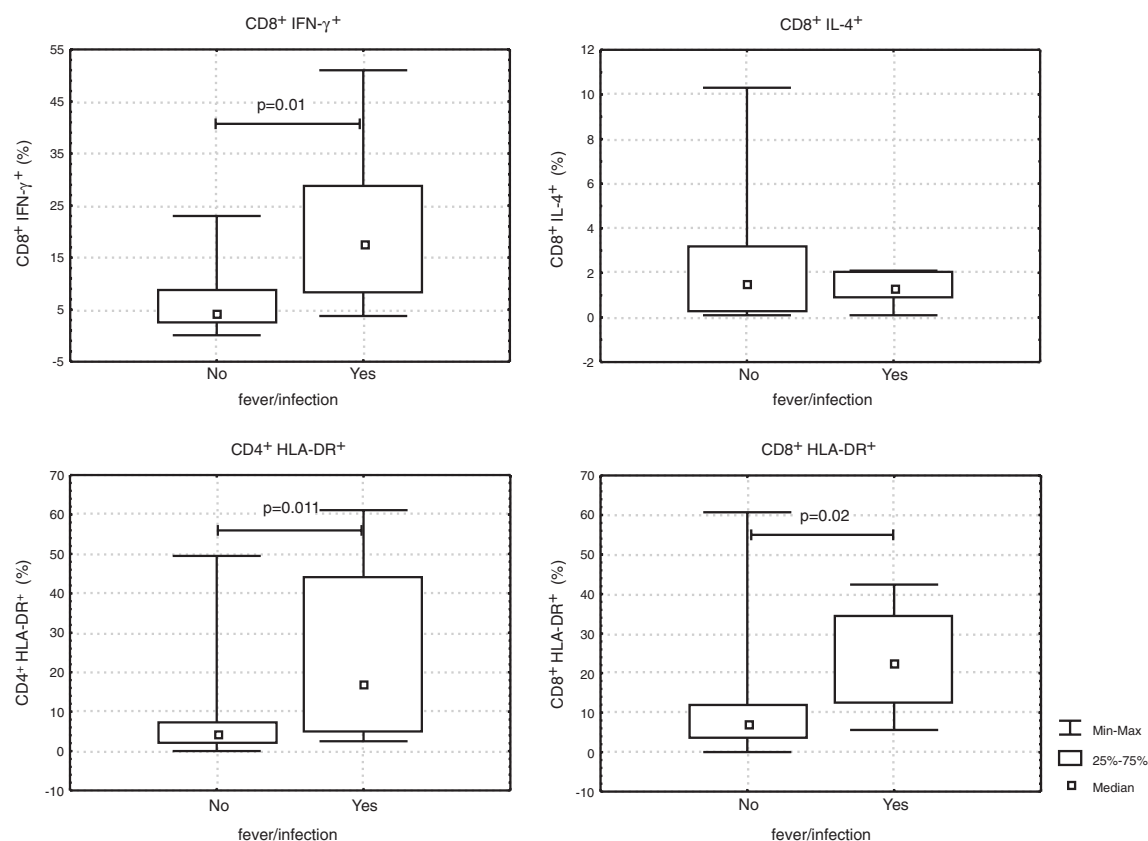


Figure 4. T lymphocytes with co-expression of IFN-γ, IL-4 and HLA-DR in patients with or without fever/infection.

increased percentage of T cells with IL-4 co-expression at the time of diagnosis and, additionally, a reduction in the percentage of lymphocytes with co-expression of IFN-γ, which is consistent with our observations (in our group the difference was statistically insignificant) and confirms the predominance of a Th₂ profile. However, in the follow-up examination 6 months after therapy termination, these authors observed normal capability of IFN-γ production²⁰.

The significance of IL-4 in the immunological balance in hematological diseases is emphasized by many authors. Similar to our results, Wołowicz et al.¹⁹ found higher IL-4 and IL-10 concentrations in B-CLL patients. However, Kamińska et al.⁵ found levels of IL-4 and IFN-γ in supernatants from whole blood cell cultures of adult patients with ALL comparable with those of controls. According to Rossi et al.¹⁶, in acute myeloid leukemia with CD30L expression (AML CD30L⁺), the T cell subpopulation is capable of IL-4 synthesis, which seems to be one of the main blast growth factors. In B-CLL it may be the subpopulation of CD8⁺CD28⁺CD30⁺ cells that is involved in IL-4 production³. Kay et al.⁶ observed the expression of IL-4 not only in T cells, but also in the B-CLL blasts themselves. The authors believe that it is IL-4 that inhibits apoptosis of abnormal cells, while

fludarabine administration significantly inhibits IL-4 production by T cells. During chemotherapy we observed a gradual increase in the percentage of T cells with IFN-γ co-expression. A similar phenomenon, likely an antineoplastic response, was observed by Matar et al.¹¹ after a single dose of cyclophosphamide to lymphoma rats.

In the group of patients with infection we found a significant increase in the percentage of T cells with co-expression of class II histocompatibility antigen molecules (CD4⁺HLA-DR⁺, CD8⁺HLA-DR⁺) and IFN-γ (CD4⁺IFN-γ⁺, CD8⁺IFN-γ⁺). This observation has been confirmed by many authors, mainly while assessing viral infection mechanisms. Activation of cytomegalovirus is one of the viral infections that appear in patients in immunosuppression. Van Dam et al.¹⁸ observed T cell activation (both CD4⁺ and CD8⁺) in the form of increased expression of HLA-DR molecules in rats with impaired function of the immune system. Ohga et al.¹³ showed that the HLA-DR⁺ T cell subpopulation contains a greater number of EBV-DNA copies than do HLA-DR⁻ cells.

Consistent with our findings is the observation of Bruserud et al.² that T cells become activated (pres-

ence of HLA-DR, receptor for IL-2 (CD25) and receptor for transferrin (CD71)) during infection in patients with neutropenia despite their considerably lower count. These authors believe that during bacterial infections the concentration of IL-4, but not of IFN- γ , is increased². T cell activation (mainly an increased percentage of HLA-DR⁺ cells) in children with infection in the course of antineoplastic therapy is confirmed by our earlier observations⁹.

In conclusion, at the onset of ALL and during remis-

sion induction, children show disturbances in the immune balance, including T cell function, i.e. the activation and predominance of the Th₂ profile. The identification of autoreactive T cells that recognize leukemic cells and stimulation of their antineoplastic response may be used in the future to increase the survival rates of patients with hemopoietic system neoplasms. Another path would involve increasing the immunogenicity of blasts to serve as efficient APCs and thus be eliminated by immunocompetent cells.

REFERENCES

1. Banks R. E., Gearing A. J., Hemingway I. K., Norfolk D. R., Perren T. J. and Selby P. J. (1993): Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular adhesion molecule (VCAM-1) in human malignancies. *Br. J. Cancer*, 68, 122–124.
2. Bruserud O., Ulvestad E., Halstensen A., Berentsen S., Bergheim J. and Nesthus I. (1997): Interleukin 4 responses in acute leukaemia patients with severe chemotherapy-induced leucopenia. *Eur. J. Haematol.*, 59, 269–276.
3. de Toter D., Reato G., Mauro F., Cignetti A., Ferrini S., Guarini A., Gobbi M., Grossi C. E. and Foa R. (1999): IL4 production and increased CD30 expression by a unique CD8⁺ T-cell subset in B-cell chronic lymphocytic leukaemia. *Br. J. Haematol.*, 104, 589–599.
4. Frydecka I., Kosmaczewska A., Bocko D., Ciszak L., Wolowiec D., Kuliczowski K. and Kochanowska I. (2004): Alterations of the expression of T-cell-related costimulatory CD28 and downregulatory CD152 (CTLA-4) molecules in patients with B-cell chronic lymphocytic leukaemia. *Br. J. Cancer*, 90, 2042–2048.
5. Kamińska T., Hus I., Dmoszyńska A. and Kandefer-Szerszeń M. (2001): Cytokine production in whole blood cell cultures of patients with B-lineage acute lymphoblastic leukemia. The influence of granulocyte-macrophage colony-stimulating factor. *Arch. Immunol. Ther. Exp.*, 49, 71–77.
6. Kay N. E., Han L., Bone N. and Williams G. (2001): Interleukin 4 content in chronic lymphocytic leukaemia (CLL) B cells and blood CD8⁺ T cells from B-CLL patients: impact on clonal B-cell apoptosis. *Br. J. Haematol.*, 112, 760–767.
7. Lee P. P., Zeng D., McCaulay A. E., Chen Y.-F., Geiler C., Umetsu D. T. and Chao N. J. (1997): T helper 2-dominant anti-lymphoma immune response is associated with fatal outcome. *Blood*, 90, 1611–1617.
8. Lepej S. Z., Vince A., Rode O. D., Remenar A. and Jeren T. (2003): Increased numbers of CD38 molecule on bright CD8⁺ T lymphocytes in infectious mononucleosis caused by Epstein-Barr virus infection. *Clin. Exp. Immunol.*, 133, 384–390.
9. Łuczyński W., Stasiak-Barmuta A., Muszyńska-Roslan K., Krawczuk-Rybak M. and Kasprzycka E. (2002): T-cell and monocyte activation during infection in children with neoplastic disease [Aktywacja limfocytów T i monocytów w przebiegu infekcji u dzieci z chorobą nowotworową]. *Med. Wieku Rozwoj.*, 6, 31–41.
10. Manocha S., Matrai Z., Osthoff M., Carter A. and Pettitt A. R. (2003): Correlation between cell size and CD38 expression in chronic lymphocytic leukaemia. *Leuk. Lymphoma*, 44, 797–800.
11. Matar P., Rozados V. R., Gervasoni S. I. and Scharovsky O. G. (2002): Th₂/Th₁ switch induced by a single low dose of cyclophosphamide in a rat metastatic lymphoma model. *Cancer Immunol. Immunother.*, 50, 588–596.
12. Mori T., Takada R., Watanabe R., Okamoto S. and Ikeda Y. (2001): T-helper (Th)₁/Th₂ imbalance in patients with previously untreated B-cell diffuse large cell lymphoma. *Cancer Immunol. Immunother.*, 50, 566–568.
13. Ohga S., Nomura A., Takada H., Ihara K., Kawakami K., Yanai F., Takahata Y., Tanaka T., Kasuga N. and Hara T. (2001): Epstein-Barr virus (EBV) load and cytokine gene expression in activated T cells of chronic active EBV infection. *J. Infect. Dis.*, 183, 1–7.
14. Papasavvas E., Sandberg J. K., Rutstein R., Moore E. C., Mackiewicz A., Thiel B., Pistilli M., June R. R., Jordan K. A., Gross R., Maino V. C., Nixon D. F. and Montaner L. J. (2003): Presence of human immunodeficiency virus-1-specific CD4 and CD8 cellular immune responses in children with full or partial virus suppression. *J. Infect. Dis.*, 188, 873–882.
15. Pui C. H., Luo X., Evans W., Martin S., Rugg A., Williams J., Crist W. M. and Hudson M. (1993): Serum intercellular adhesion molecule-1 in childhood malignancy. *Blood*, 82, 895–898.
16. Rossi F. M., Degan M., Mazzocco F. T., Francia R. D., Aldinucci D., Poletto D., Vellenga E., Pinto A. and Gattei V. (2002): Co-expression of CD30 ligand and interleukin-4 (IL-4) receptors by acute myeloid leukaemia blasts is associated with the expansion of IL-4-producing CD30⁺ normal T cells. *Br. J. Haematol.*, 117, 59–69.
17. Scrivener S., Kaminski E. R., Demaine A. and Prentice A. G. (2001): Analysis of the expression of critical activation/interaction markers on peripheral blood T cells in B-cell chronic lymphocytic leukaemia: evidence of immune dysregulation. *Br. J. Haematol.*, 112, 959–964.
18. van Dam J. G., Damoiseaux J. G., Christiaans M. H. and Bruggeman C. A. (2000): Acute primary infection with cytomegalovirus (CMV) in kidney transplant recipients results in the appearance of a phenotypically aberrant CD8⁺ T cell population. *Microbiol. Immunol.*, 44, 1011–1017.
19. Wolowiec D., Frydecka I., Kosmaczewska A., Boćko D., Ciszak L., Urbaniak-Kujda D., Kapelko-Słowik K., Kuliczowski K. and Kochanowska I. (2003): IL-4 and IL-10 production by T cells in patients with B-cell chronic lymphocytic leukemia [Wydzielanie interleukiny 4 oraz interleukiny 10 przez limfocyty T chorych na przewlekłą białaczkę limfatyczną B-komórkową]. *Adv. Clin. Exp. Med.*, 12, 45–49.
20. Zhang X. L., Komada Y., Chipeta J., Li Q.-S., Inaba H., Azuma E., Yamamoto H. and Sakurai M. (2000): Intracellular cytokine profile of T cells from children with acute lymphoblastic leukemia. *Cancer Immunol. Immunother.*, 49, 165–172.