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Communication

Monocytes in children with leukemias and lymphomas — down-regulation of HLA and costimulatory molecules★✪

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The aim of the study was to evaluate the function of monocytes in children with leukemias and lymphomas based on the expression of critical costimulatory, activatory and adhesion molecules (CD80, CD86, HLA-DR and CD54 = ICAM-1), estimated with tricolor flow cytometry. In comparison to the control group we found a lower percentage of monocytes with costimulatory molecules (CD80 before and CD86 after lipopolysaccharide stimulation) at the time of diagnosis and of monocytes with HLA-DR molecules after remission induction. We also noted a lower percentage of monocytes with HLA-DR expression in the group with severe or therapy resistant infections. The results of our investigation suggest some defect in costimulation and antigen presentation in lymphoproliferative diseases in children.

Damage of the immune system is a major side effect of chemotherapy in childhood. Immune deficiency in patients with neoplastic disease reduces the possibility of neoplasm

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Abbreviations: APC, antigen presenting cell; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HLA, human leukocyte antigen; ICAM, intercellular adhesion molecule; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; TNF, tumor necrosis factor.

eradication and results in frequent or severe infections and may be life-threatening. Antigen presenting cells (APCs) such as monocytes/macrophages and dendritic cells play a significant role in immunity against viral, bacterial and (especially) fungal infections. Among many surface markers, they express high levels of major histocompatibility complex class II antigens (MHC, HLA) which play an important role in antigen presentation. During the immune response, activation of T cells requires not only the interaction between T-cell receptor with HLA expressed on APCs but also a costimulatory signal. One of them is B7 family and CD28 or CTLA-4. B7 family molecules (CD80 and CD86) are ligands expressed on APCs, CD28 and CTLA-4 (CD152) are co-receptors present on T-cells. CD28 has an activating function, CTLA-4 an inhibitory one (Melichar *et al.*, 2000).

Monocytes/macrophages produce proinflammatory cytokines (TNF-α) in response to LPS, they phagocytose microorganisms and present antigens to T helper lymphocytes (Hoflich *et al.*, 2002). The number of dendritic cells in peripheral blood of patients with cancer receiving chemotherapy or recovering from stem cell transplantation is lower than that in healthy individuals (Savary *et al.*, 1998). Few authors found a normal number but impaired function of monocytes/macrophages/dendritic cells in patients with neoplastic diseases (Ratta *et al.*, 2002; Ugurel *et al.*, 2004). Unfortunately, there is no report regarding the function of monocytes in children suffering from neoplasms.

The aim of the study was to evaluate the function of monocytes in children with lymphoproliferative diseases, such as leukemias and lymphomas, based on the expression of critical costimulatory/activatory/adhesion molecules.

MATERIALS AND METHODS

The study group consisted of 56 children (31 boys), aged 2–18 years (mean 6.7 ± 3.9) treated for acute lymphoblastic leukemia – 40 and Hodgkin and non-Hodgkin lymphoma – 16, according to the protocols recommended by the Polish Pediatric Leukaemia/Lymphoma Study Group. Peripheral blood samples were taken at the time of diagnosis, after remission induction, during maintenance treatment, in the first year and more than a year after the end of the treatment. The examination was also performed in the case of fever or infection. The aetiology of chosen severe/therapy resistant infections is listed in Table 1.

Table 1. Separated group of patients with severe/resistant infecions (n = 11)

Aetiology (probable)	Disease, stage of treatment
Pneumonia of unknown aetiology, multiorgan damage and unfavourable outcome (n = 1)	Acute lymphoblastic leukaemia (ALL), maintenance treatment
Pneumonitis of unknown aetiology (n = 3)	Cases suffering from ALL (2), non-Hodgkin lymphoma (NHL-1) during intensive treatment
Gancyclovir resistant cytomegalovirus (CMV) pneumonitis (n = 1)	ALL, maintenance treatment
Disseminated CMV infection (n = 1)	Hodgkin disease, after autologous peripheral blood stem cell transplantation
Aspergillus fumigatus pneumonia (n = 1)	ALL, during remission induction
Prolonged fever of unknown origin (> 7d) (n = 2)	ALL, maintenance treatment
Varicella (n = 2)	NHL T-cell

The control group included 46 children (25 boys) from the Department of Pediatric Surgery subjected to minor surgical operations. The control children were free of infection during the period of 2 weeks preceding admission to the Department, had a negative history of allergic diseases, normal laboratory tests for leukocytosis, lymphocytosis and normal acute phase index markers. The study and control patients were age- and sex-matched ($P > 0.05$).

Peripheral blood mononuclear cells. PBMC were isolated by Ficoll-Hypaque density gradient centrifugation. Cells were washed twice in phosphate-buffered saline (PBS)/1% bovine serum albumin (BSA). A volume of 1.0 ml of cell suspension containing 1×10^6 cells were resuspended in RPMI-1640 medium (Gibco, Paisley, Scotland) supplemented with 5% fetal calf serum, penicillin 100 U/ml, streptomycin 100 μg/ml, 2 mM of L-glutamine, and LPS (*Escherichia coli*, 0111: B4 serotype, Sigma, U.S.A.) 10 μg/ml (complete medium). LPS is a major component of the outer membrane of Gram-negative bacteria. Monocytes in peripheral blood and macrophages possess an LPS receptor — CD14. LPS–CD14 interaction leads to the release of proinflammatory cytokines (Carrillo *et al.*, 2001). The cells were then incubated for 24 h at 37°C, in 5% CO_2. LPS stimulation was perfomed in all cases — examined and control.

Flow cytometry. After culture, the supernatant was removed and the cells were washed with PBS three times. Cell suspension (100 μl) was then stained with 10 μl monoclonal antibody to the following cell-surface markers: CD14-PerCP (IgG_{2b}), CD54-PE/IgG_{2b}(ICAM-1), CD80-PE/IgG_1, CD86-FITC/$IgG_{1\chi}$, HLA-DR-PE/IgG_{2a} (Becton Dickinson, San Jose, CA, U.S.A.) and incubated for 20 min at room temperature in darkness. Appropriate isotypic negative controls were used: IgG_{2b}/PerCP, IgG_{2b}/PE, IgG_1/PE, IgG_1/FITC. Immunofluorescence-stained cells were analyzed on an EPICS XL flow cytometer (Coulter). Live monocytes were identified by gating forward- and side-scatter parameters. For each sample, 10^4 events were acquired.

Statistics. 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 values and median (25th–75th percentiles). Significance levels were calculated according to the nonparametric Mann-Whitney U test (differences between control and examined group, differences between consecutive stages of therapy and also between group with and without infection), and the Wilcoxon test (comparison between results obtained before and after LPS stimulation). A level of $P < 0.05$ was regarded as significant.

RESULTS

The results obtained in the control and examined groups (without infection) are summarized in Table 2.

We found in the examined and control groups:

- the mean percentage of $CD14^+CD80^+$ and $CD14^+CD86^+$ cells was higher after LPS stimulation than before stimulation (3.37 *vs* 37.41, $P < 0.0001$, and 49.97 *vs* 72.34, $P = 0.0001$, respectively)

In children with malignancies we observed:

- at the time of diagnosis the mean percentage of $CD14^+CD80^+$ cells before LPS stimulation and $CD14^+CD86^+$ cells after LPS stimulation were lower in the examined group than in the control group (Fig. 1);
- after remission induction the mean percentage of $CD14^+CD86^+$ cells after LPS stimulation was lower in the examined group than in the control group; it was also lower than the percentage obtained in the group over a year after the end of treatment (Table 2);
- the mean percentage of $CD14^+$HLA-DR^+ cells was lower in the examined group after remission induction than in the control group (Fig. 2);

Table 2. Percentages of monocyte subpopulations in the control and examined group depending on stage of therapy (results from flow cytometry)

	Monocyte subpopulations (%)											
	$CD14^+CD80^+$ cells before stimulation		$CD14^+CD86^+$ cells before stimulation		$CD14^+CD80^+$ cells after stimulation		$CD14^+CD86^+$ cells after stimulation		$CD14^+HLA\text{-}DR^+$ cells		$CD14^+CD54^+$ (ICAM-1^+)cells	
	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	5.04	3.90 (2.4–7.8)	50.03	44.0 (29.0–71.3)	29.50	29.0 (13.9–44.0)	70.73	71.0 (60.0–81.0)	86.45	86.3 (77.0–95.8)	52.77	54.6 (47.0–72.0)
2. Time of diagnosis	1.72	0.7 (0.4–2.1)	42.68	45.0 (6.5–63.0)	27.67	30.8 (12.8–35.5)	56.03	55.4 (40.8–71.4)	72.91	80.6 (56.3–89.5)	55.43	60.8 (45.0–65.0)
3. After remission induction	2.9	3.1 (2.1–6.7)	44.70	48 (23.5–67.8)	25.20	25.0 (14.5–47.0)	49.46	46.0 (40.0–68.5)	56.36	55.8 (52.5–60.5)	61.32	59.2 (45.2–77.4)
4. During maintenance treatment	2.6	2.6 (1.0–4.2)	37.0	37.0 (29.0–45.0)	35.45	34.5 (16.0–50.5)	64.7	65.5 (59.4–72.0)	74.62	81.2 (64.3–88.6)	56.5	56.7 (43.3–74.8)
5 First year after end of treatment	2.2	2.4 (1.3–4.1)	75.8	62.0 (34.5–80.0)	31.36	33.0 (17.5–46.0)	76.3	75.3 (60.5–88.0)	87.10	89.9 (85.0–90.8)	68.2	64.0 (51.5–79.0)
6. More than one year after end of treatment	4.22	3.3 (1.1–7.35)	60.78	63.5 (50.5–71.2)	35.36	38.8 (3.4–65.0)	80.61	78.5 (70.0–92.0)	85.54	84.1 (80.6–93.3)	69.05	66.0 (46.0–89.4)
Statistics	2 *vs*. 1 P=0.005						2 *vs* 1 P=0.02 2 *vs* 6 P=0.02 3 *vs* 1 P=0.01 3 *vs* 6 P=0.02		3 *vs*. 1, 2, 4, 5, 6 P<0.001			

- there was no difference in the percentage of $CD14^+CD54^+$ (ICAM-1^+) cells between the control and the examined group in any stage of treatment.

We compared the results from group with and without infection with no respect to the stage of therapy. There was no difference for all variables, but in the separated group with severe or resistant infections (Table 1) compared to results in control group we found a lower percentage of $CD14^+HLA\text{-}DR^+$ cells during infection (86.48 *vs*. 37.38, P = 0.0002) (Fig. 2).

DISCUSSION

Changes in expression of cell surface molecules during anticancer treatment

At the time of diagnosis of a neoplastic disease we found a lower percentage of

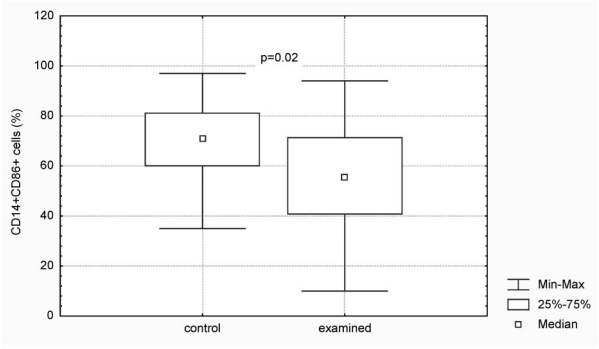

Figure 1. Percentage of $CD14^+CD86^+$ cells after LPS stimulation in control and examined group at the time of diagnosis.

Boxes indicate upper and lower quartile

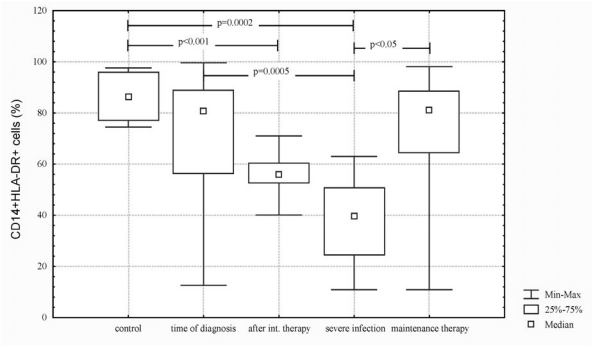

Figure 2. Percentage of $CD14^+HLA\text{-}DR^+$ cells depending on stage of treatment and/or infection.

Boxes indicate upper and lower quartile

monocytes with costimulatory molecule expression. Ugurel *et al.* (2004) showed a decreased expression of HLA-DR, HLA-DQ, HLA-DP and CD86 on monocytes from melanoma patients, but they did not find any differences in the expression of HLA class I antigens and CD80. This may be the cause of ineffective antigen presentation in melanoma and other neoplasms. This down-regulation of costimulatory molecules in cancer patients is not caused by IL-10, TGF-β and VEGF (Berthier-Vergnes *et al.*, 2001).

Among all APCs, the dendritic cells (DCs) are the most potent stimulators of T-cell response and play a crucial role in antitumor immunity. Ratta *et al.* (2002) demonstrated that DCs from multiple myeloma patients show lower expression of HLA-DR, CD40 and CD80 antigens and have impaired induction of T-cell proliferation. However, Brown *et al.* (2001) showed that up-regulation of CD80 expression on monocytes of patients with myeloma was the same as in the control group.

Lately, Orsini *et al.* (2003) have documented an inhibiting influence of leukemic cells on dendritic cells: addition of CLL cells induces lower expression of costimulatory molecules and reduce the allo-stimulatory ability of dendritic cells. Some authors consider that dendritic cells have an important role in response to IFN-α in CML: cells from patients without cytogenetic remission expressed lower levels of CD80, CD86, CD54 (ICAM-1), CD40 and class I HLA (Paquette *et al.*, 2002). It is possible that IFN-α stimulates DCs to present CML antigens or improves the capacity of dendritic cells to stimulate T-lymphocyte response (Wang *et al.*, 1999). In contrast, Rezvany *et al.* (2001) found similar expression of CD54 (ICAM-1), CD80 and CD86 on dendritic cells derived from CLL patients but at the gene level expression IL-10 was higher and expression of IFN-γ was lower in the examined group.

Infections

The role of the immune system in the pathogenesis of infection and septic shock has been widely examined. In our study we observed a lower percentage of monocytes with HLA coexpression in severe or therapy resistant infections and no changes in all the examined costimulatory/adhesion/activatory molecules during infections. Our findings concerning expression of costimulatory molecules are similar to those of Ampel and Christian (2000) – they found no difference in the expression of the B7 ligands CD80 and CD86 on $CD14^+$ cells in fungal infection, i.e. after incubation with the coccidioidal antigen toluene spherule lysate. In Balbo *et al.* (2001) opinion in peripheral blood CD86 molecules have a prevalent functional role in presentation of allergens/antigens by blood monocytes. Some authors postulate an involvement of costimulatory molecules in chronic infections. Amaraa *et al.* (2002) showed a higher percentage of $CD14^+CD80^+$ cells in patients with chronic hepatitis C virus infection. This phenomenon was accompanied by high IL-10 production by these cells. Rhinovirus also induced an increase of CD80 on monocytes and CD86 on B-cells (Papadopoulos *et al.*, 2002).

Sepsis is a biphasic event consisting of an initial hyperinflammatory phase and a following – anti-inflammatory one (immune-paralysis) (Contreras, 1999). Hoflich *et al.* (2002) defined "immunoparalysis" as percentage of $HLA\text{-}DR^+$ monocytes in peripheral blood below 30. This parameter has a very strong correlation with the clinical outcome. Monocytes with down-regulated MHC expression are not able to produce cytokine and to present antigens to T-cells (Hoflich *et al.*, 2002). Docke *et al.* (1997) restored monocytic function by IFN-γ application. Loercher *et al.* (1999) identified and HLA-DR-negative monocyte subset responsible for inhibiting T

cell proliferation and cytokine production in patients with ovarian carcinoma. These cells also do not express CD80 and CD86. HLA-DR is the strongest of the MHC class II molecules expressed on monocytes. However, Taylor and co-workers found that also HLA-DQ expression on monocytes is reduced in injured patients with infection – this suggested that these antigens play an important role in immune response (Taylor *et al.*, 2000; Hoflich *et al.*, 2002). Abendroth *et al.* (2000) observed inhibition of MHC class II expression on varicella-zoster-virus (VZV) infected fibroblasts. In their opinion this phenomenon may protect the infected cells from immune surveillance and facilitate virus replication and transmission.

In contrast to our findings, epithelial cells infected with respiratory syncytial virus showed upregulation of CD54 (ICAM-1) and MHC class I and II antigens (Wang *et al.*, 2000). In a study by Michałkiewicz *et al.* (1999) *Pseudomonas aeruginosa* Exotoxin A inhibited the expression of costimulatory and adhesion molecules on monocytes (CD80, CD86, CD54 = ICAM-1, HLA-DR). Those authors also found that supplementation with IFN-γ did not reverse this effect.

Monocytes play the most important role in immune response against fungi. Down-regulation of HLA II class molecules on these cells in cancer patients can explain therapy resistance and poor outcome in infections of this origin.

In summary, our study suggests that circulating monocytes from children with lymphoproliferative diseases may have an impaired capacity for T-cell stimulation and this may be one of the mechanisms whereby neoplastic cells can escape immune recognition.

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