TRAIL receptors in the serum of patients with B-cell chronic lymphocytic leukemia

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Great importance in the course of chronic B-cell lymphocytic leukemia (B-CLL) has been ascribed to cytokines belonging to the superfamily of the tumor necrosis factor (TNF), including TRAIL (TNF-related apoptosis inducing ligand) and its specific receptors: TRAIL receptor 1 (TRAIL-R1), TRAIL receptor 2 (TRAIL-R2), TRAIL receptor 3 (TRAIL-R3), TRAIL receptor 4 (TRAIL-R4) and osteoprotegerin (OPG). Both the molecule and the receptors may occur in membrane and soluble forms, except for OPG which has only a soluble form. The aim of the study was to assess the levels of sTRAIL molecule and soluble TRAIL receptors – sTRAIL-R2 and OPG in the serum of patients with B-CLL. The findings revealed reduced concentrations of sTRAIL both before and after treatment and elevated levels of sTRAIL-R2 and OPG in patients before treatment. After treatment with CC (2CdA/Cladrybin and Cyklofosfamid) and FC (Fludarabin and Cyklofosfamid) we observed an increase in sTRAIL and a decrease in sTRAIL-R2. OPG levels were found to increase after treatment with CHOP (Vincristini, Cyklofosfamid, Adriamycin and Prednisol) and they decreased after administration of Leukeran (Chlorambucyl) and CMC (2CdA/Cladrybin, Mitoxanton and Cyklofosfamid).

The relationships between TRAIL and its natural regulators in the serum of BCLL patients prior to treatment may impair apoptosis of leukemic B cells. Changes in these relationships after treatment with CC and FC seem to promote enhancement of apoptosis in these cells.

Key words: B-cell chronic lymphocytic leukemia, soluble TNF-related apoptosis-inducing ligand, soluble TNF-related apoptosis-inducing ligand receptor type 2 (sDR5), osteoprotegerin

B-cell chronic lymphocytic leukemia (BCLL) is the most common form of all lymphocytic leukemias in the 50-60 year-old population, characterized by an increased absolute count of lymphocytes in the peripheral blood and bone marrow, enlargement of lymph nodes and spleen, impairment of cellular and humoral type immunity [1].

Leukemic B cells have a prolonged survival time associated with apoptotic disturbances, which in consequence leads to their accumulation [1, 2].

An important role in the course of chronic B-cell lymphocytic leukemia has been ascribed to cytokines belonging to the superfamily of the tumor necrosis factor (TNF), such as TNF-related apoptosis-inducing ligand (TRAIL/Apo-2L) [3, 4, 5]. TRAIL molecule is in 28% of the amino acid sequence homologous with FasL/CD95L molecule [5, 6]. Studies have shown that 60% of various neoplastic cell lines are sensitive to TRAIL-induced apoptosis, while non-transformable cells are resistant to it [6, 7].

Five receptors have been identified for TRAIL: TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (Dc1), TRAIL-R4 (Dc2) and osteoprotegerin (OPG). TRAIL-R1 and TRAIL-R2 receptors have an intracellular region with a complete death domain (DD)[5, 8].

TRAIL-R3, TRAIL-R4 and OPG belong to the so called decoy receptors. TRAIL-R3 does not have a cytoplasmic DD and TRAIL-R4 has an incomplete DD [5]. Osteoprotegerin (OPG) occurs only in a soluble form and its gene is located on chromosome 8q23-24 [9]. OPG has been found to affect the development and growth of B cells [9, 10].

These decoy receptors bind TRAIL molecule but are not able to induce apoptosis. Their high expression is the likely

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Table 1. The mean value of evaluated parameters in the serum of patients with B-CLL according to Rai' classification.

		Patients before treatment				
	Control n =15 x± SD	All patients before treatment n =24 x± SD	Patients in stage I n=6 x± SD	Patients in stage II n=6 x± SD	Patients in stage III n=6 x± SD	Patients in stage IV n=6 x± SD
sTRAIL pg/ml	88.38 ±16.20	52.4 ± 26.22	53.75*±12.20	54.5*±15.19	42.4*±16.33	39.5*±28.39
sDR5 pg/ml	31.11 ±15.36	83.41 ± 36.67	105.81*±23.45	77.5 *±18.92	70* ±14.14	62.5*±15
OPG pg/ml	75.66 ±15.51	141.5 ± 97.28	158*± 50.42	76.66 ± 28.96	113*±18.93	76.66 ±30.74

*statistical difference with control ($p< 0.05$)

cause of resistance of healthy cells to the apoptotic action of TRAIL [10, 11]. Recent reports seem to confirm a significant role of OPG in TRAIL binding and inhibition of apoptosis induction in the Jurkat line cell [9].

TRAIL molecules were found to play a role in the survival of B cells in BCLL. Olsson et al.2001 [12] observed the expression of TRAIL receptors (TRAIL-R1, TRAIL –R2, TRAIL-R3 and TRAIL –R4) on most leukemic cells. Even in the presence of death domain-containing receptors, B cells are relatively resistant to the induction of apoptosis by recombinant human TRAIL molecule (rhTRAIL). In spite of the majority of B-CLL lymphocytes expressed variable surface levels of "death receptors" TRAIL-R1 and TRAIL-R2, the presence of mTRAIL in culture increased leukemic cell survival in 11/44 of the B-CLL samples [12].This may be caused by soluble forms of TRAIL receptors (sTRAIL-R) such as sDR5 and OPG in the microenvironment of B-CLL. Soluble TRAILs receptors like the "decoy receptors" can block biological availability of the TRAIL molecule and thus inhibit its activity [6, 13, 14].

The study objective was to assess the levels of the soluble receptors sTRAIL-R2 (sDR5) and OPG in comparison to sTRAIL ligand in the serum of patients with chronic B-cell lymphocytic leukemia. The knowledge of the relationships between these molecules may help elucidate one of the mechanisms responsible for impaired apoptosis of B cells in the course of BCLL

Methods

Patient group. Serum samples were collected from 40 patients with BCLL in stage I, II, III and IV according to the RAI classification, hospitalized in the Department of Hematology, Medical University of Bialystok (aged 40-75 years, mean 62.1). The samples were investigated before and after treatment.

The diagnosis of leukemia was based on clinical observation, morphological composition of the peripheral blood, bone marrow puncture, trepanobiopsy, lymph node biopsy and cytochemical examinations. A flow cytometer EPIX XL (Coulter, USA) was used to identify immunophenotypes of leukemic cells. The monoclonal antibody panels of CD19 and CD20 for B cells and CD3, CD7 and CD8 for T cells were applied to differentiate between these cells.

Patients were graded according to Rai's staging system as follows: stage I, stage II, stage III, stage IV. Patients with accompanying acute inflammatory bacterial, viral, mycotic or allergic states were excluded from the study.

All patients with clinical stage III and IV disease were eligible for treatment. The treatment course consisted of 2CdA (Biodrybin) given at dose of 0.12 mg/kg/d in a 2-h intravenous infusion (i.v.) for 5 days; 2CdA 0.12 mg/kg/d in a 2-hour i.v. for 3 days and mitoxantrone 10 mg/m^2 on 1st day and cyclophosphamide 650 mg/m^2 i.v. on 1st day (CMC); cyclophosphamide 650 mg/m^2 i.v. on 1st day (CC), fludarabine 25 mg/m2 i.v. for 3rd day and cyclophosphamide 650 mg/m^2 i.v. for 3rd day (FC); vincristini 2 mg i.v. on 1st day, cyclophosphamide 750 mg/m^2 i.v. on 1st day, adriamycin 50 mg/m^2 i.v. on 1st day, prednisone 100 mg p.o. for 5 days (CHOP); chlorambucil (Leukeran) 4 mg p.o. 3 times every 4 days for a maximum 6 cycles.

Control group. The control group consisted of 15 healthy subjects (10 women and 5 men) of the same age (workers of Medical University of Bialystok)

Methods. Five ml samples of venous blood were collected once to obtain serum, which was stored at –25^{o}C. Serum concentrations of sTRAIL, sTRAIL-R2 (sDR5) and OPG molecules were determined by commercially available ELISA kits (R&D Systems, Minneapolis, USA).

Results were subjected to statistical analysis using a nonparametric U-Mann-Whitney test. Pearson's test was applied for the analysis of correlations.

Results

The results obtained in patients with BCLL before and after treatment were analyzed with regard to the RAI classification and type of treatment.

Prior to treatment (at baseline), serum sTRAIL concentrations were lower in patients as compared to controls (Table 1). The levels of sTRAIL-R2 (sDR5) were higher in BCLL patients, being the highest in stage I patients. In stage I and III patients, OPG levels were also increased in comparison to the control group (Table 1).

After treatment, no significant changes were observed in the mean serum concentrations of sTRAIL and OPG in comparison to the baseline values. Concentrations of sTRAIL were lower in stage II and III patients than in healthy subjects.

Table 2. The mean value of evaluated parameters in the serum of patients with B-CLL according to Rai'classification.

		Patients after treatment				
	Control n =15 x± SD	All patients after treatment n =24 x± SD	Patients in stage I n=6 x± SD	Patients in stage II n=6 x± SD	Patients in stage III n=6 x± SD	Patients in stage IV n=6 x± SD
sTRAIL pg/ml	88.38 ±16.20	58.31 ± 36.25	70.28 ± 27.94	59.75*±13.52	33.23*±13.32	78.66 ± 10.83
sDR5 pg/ml	31.11±15.36	43.10 ±16.66	35.71 ± 15.11	52*±16.43	43.42*±17.49	42.85 ± 11.12
OPG pg/ml	75.66 ±15.51	142.52 ±83.47	154* ± 38.62	116.5*±43.2	165* ± 68.26	181.66*±54.33

*statistical difference with control (p< 0,05)

Table 3. The mean value of evaluated parameters in the serum of patients with B-CLL before and after treatment.

		n	sTRAIL pg/ml x ± SD	sDR5 pg/ml x ± SD	OPG pg/ml x ± SD
Before treatment		24	52.4 ± 26.31	83.41 ± 35,67	141.67 ± 97.28
After treatment	2CdA	4	53.44 ± 19.44	35.45* ± 12.13	165 ± 57.19
	FC	4	76*± 18.19	35*± 19.14	131.33 ± 102.14
	CC	4	80*± 28.7	45*± 12.19	127 ± 29.00
	Leukeran	4	42 ± 12.16	63.33*± 11.54	92.66* ± 24.19
	CMC	4	57.57 ± 24.38	45.8* ± 17.97	102.44* ± 29.33
	CHOP	4	46.5 ± 31.81	46* ± 5.47	178*± 59.39

*statistical difference before and after treatment with control (p< 0,05)

However, OPG levels in all the patients were still lower as compared to the control group (Table 2). Statistically lower were also the levels of sTRAIL-R2 as compared to those found at baseline, although still higher in stage II and III patients than in the control group.

The analysis of TRAIL concentrations in BCLL patients revealed differences with respect to the type of treatment. In patients treated with CC and FC, sTRAIL concentration increased after treatment (Table 3). Administration of Leukeran, 2CdA, CMC and CHOP did not cause any significant changes. However, sTRAIL-R2 (concentrations were reduced irrespective of the therapy applied (Table 3).

Administration of CHOP resulted in a significant increase, while treatment with Leukeran and CMC caused a decrease in OPG levels as compared to the values obtained at baseline (Table 3).

No significant correlation was found between sTRAIL and its soluble receptors – sTRAIL-R2 and OPG.

Discussion

There are many literature reports describing changes in the expression and secretion of TNF superfamily cytokines in the course of chronic B-cell lymphocytic leukemia (BCLL), which may lead to disturbances in the process of apoptosis of leukemic B cells [4, 15].

Low serum concentrations of sTRAIL observed in the current study in BCLL patients both at baseline and after treatment are a likely cause of impaired apoptosis of neoplastic B cells. It has been shown that both the membranous and soluble form of TRAIL are capable of initiating apoptosis [6, 16]. The results seem to be especially significant, most of all in the light of the data reported by Kim *et al*., who have demonstrated that sTRAIL exceeds other molecules of this superfamily in its ability to induce apoptosis of many neoplastic cell types [17].

Impairment of apoptosis of the leukemic B cells in BCLL patients before treatment can also be caused by the presence of sTRAIL-R2, which occurs in their serum in high concentrations. Additionally, the resistance of leukemic cells to apoptosis may be enhanced by the reduced expression of the membrane receptor TRAIL-R2 associated with a release of its soluble form. This has been suggested by the findings of Ma *et al.*, who observed that apoptosis induced by TRAIL molecule is proportional to the level of TRAIL-R2 expression on neoplastic cells [18]. Also McFarlane *et al*., reported a correlation between low expression of TRAIL-R2 and resistance of B cells to TRAIL action [4].

High serum OPG level can be another cause of apoptotic disturbances in leukemic B cells in BCLL patients before treatment. It has been shown that OPG through the competitive binding to TRAIL molecule prevents apoptosis induction in the TRAIL-R1 and TRAIL-R2 dependent pathway [9].

As the modulation of neoplastic B cell apoptosis disturbances is the major aspect of BCLL therapy, we have undertaken also to assess the effect of chemotherapy on TRAIL family molecules. At the same time, evaluation of the behavior of TRAIL molecules and their natural regulators after therapy may reveal the causes of changes in the process of TRAIL-dependent apoptosis of B cells [19].

Elevated serum concentration of sTRAIL after CC and FC administration and a simultaneous decrease in sTRAIL-R2 levels may lead to the enhancement of leukemic B cell apoptosis. The observed reduction in sTRAIL-R2 and OPG

levels after Leukeran and CMC therapy may intensify B cell apoptosis, although low sTRAIL concentration following this therapy suggests only minor role of the changes. Lower serum sTRAIL-R2 concentrations observed after treatment are a likely result of the increased expression of the membrane receptor TRAIL-R2 on B cells raising their sensitivity to the action of TRAIL molecule. Kobylinska et al. revealed that cladribine alone, besides cytotxic effect, affected the events associated with apoptosis [20].

Johnston et al. observed the effect of standard treatment with FC enhancing the expression of mRNA and TRAIL-R1 and TRAIL-R2 protein on leukemic lymphocytes in BCLL patients [19].

CHOP therapy has also a substantial effect on TRAIL-dependent apoptosis. A reduction in sTRAIL-R2 that blocks apoptosis and a simultaneous increase in OPG that inhibits this process are likely to suppress apoptosis of leukemic B cells.

A positive role of changes in the expression of TRAIL-R1 and TRAIL-R2 following therapy has also been reported for other neoplasms. Younes and Kadin have described an increase in TRAIL activity through elevated expression of TRAIL-R1 and TRAIL-R2 after chemo-and radiotherapy in cancers of breast, colon and urinary bladder [21]. Recent studies have shown that anti-TRAIL-R1 and anti-TRAIL-R2 monoclonal antibodies, similarly to TRAIL, have the potential to destroy neoplastic but not healthy cells and its promising novel biotherapeutic agents for cancer therapy [22].

Summing up, the relationships between sTRAIL, sTRAIL-R2 and OPG in BCLL patients prior to treatment are likely to impair apoptosis of leukemic B cells. Among different therapeutic methods, CC and FC seem to have a beneficial effect on these relationships and promote apoptosis of B cells in BCLL patients.

References

[1] ROLIŃSKI J. Chronic B-cell lymphocytic leukemia -disorders of proliferation and apoptosis. Acta Haematol Pol 1998; 1: 19–24 (In Polish).

[2] PODHORECKA M. Apoptosis in pathogenesis of chronic B-cell lymphocytic leukemia. Post Hig Med Dośw 2004; 58: 236–242.

[3] ELSAWA S F, NOVAK AJ, GROTE DM, et.al. B-lymphocyte stimulator (Blys) stimulates immunoglobulin production and malignant B-cell growth in Waldenström macroglobulinemia. Blood 2006; 107: 2882–8.

[4] MacFARLANE M, HARPER N, SNOWDEN RT, et.al. Mechanisms of resistance to TRAIL-induced apoptosis in primary B cell chronic lymphocytic leukemia. Oncogene 2002; 21: 6809–18.

[5] KELLY SK, ASHKENAZI A. Targeting death receptors in cancer with Apo2L/TRAIL. Curr Opin Pharm 2004; 4: 333–339.

[6] ERLICH S, INFANTE-DUARTE C, SEEGER B, et.al. Regulation of soluble and surface- bond TRAIL in human T cells, B cells and monocytes. Cytokine 2003; 24: 244–253.

[7] GRIFFITH TS, LINCH D. TRAIL: a molecule with multiple receptors and control mechanisms. Curr Opin Immunol 1998; 10: 559–563.

[8] WANG S, EL-DEIRY WS. TRAIL and apoptosis induction by TNF-family death receptors. Oncogene 2003; 22: 8628–8633.

[9] EMERY JG, MCDONNEL P, BURKE MB, et.al. Osteoprotegerin is receptor for the cytotoxic ligand TRAIL. J Biol Chem 1998; 273: 14363–14367.

[10] YUN TJ, TALLQUIST MD, AICHER A, et.al. Osteoprotegerin, a crucial regulator of bone metabolism also regulated B cell development and function. J Immunol 2001; 166: 1482–1491.

[11] DEGLI-ESPOSTI M. To die – or not to die- the quest of the TRAIL receptors. J Leuc Biol 1999; 65: 535–542.

[12] OLSSON A, DIAZ T , AGUILAR-SANTELISES M, et.al. Sensitization to TRAIL- induced apoptosis and actinomycin D. Leukemia 2001; 15: 1868–1877.

[13] HERBEUWAL JP, LAMBERT C, SABIDO O, et.al. Macrophages from cancer patients: analysis of TRAIL, TRAIL receptors, and colon tumor cell apoptosis. J Natl Cancer I, 2003; 958: 611–21.

[14] WAJANT H, MOOSMAYER D, WUEST T, et.al. Differential activation of TRAIL-R1 and –R2 by soluble and membrane TRAIL allows selective surface antigen-directed activation of TRAIL-R2 by a soluble TRAIL derivative. Oncogene 2001; 20: 4101–4106.

[15] SECCHIERO P, TRIBELLI M, BARBAROTTO E, et.al. Aberrant expression of TRAIL in B chronic Lymphocytic Leukemia (BCLL) cells. J Cell Physiol 2005; 205: 246–252.

[16] TECCHIO C, HUBER V, SCAPINI P, et.al. IFN-a -stimulated neutrophils and monocytes Release a soluble form of TNF-related apoptosis-inducing ligand (TRAIL/Apo-2ligand) displaying apoptotic activity on leukemic cells. Blood 2004; 103: 3837–3844.

[17] KIM CY, JEONG M, MUSCHIACK H, et.al. Cancer gene theraphy using a novel secretable trimeric TRAIL. Gene Ther 2005; 13: 330–8.

[18] MA YF, ZHANG J, ZHAO YP, et.al. Correlation between sensitivity to TRAIL and expression level of DR5 on surface of tumor cells. Zhonguhua Zhong Liu Za Zhi 2004; 26: 528–30.

[19] JOHNSTON JB, KABORE AF, STRUTINSKY J, et.al. Role of the TRAIL/APO-L death receptors in Chlorambucyl- and Fludarabine-induced apoptosis of primary chronic lymphocytic leukemia (CLL) cells. Oncogene 2003; 22: 8356–69.

[20] KOBYLINSKAA, BEDNAREK J, BLONSKI JZ, et.al. In vitro sensivity of B-cell chronic lymphocytic leukemia to cladrybine and its combinations with mafosfamide and or mitoxantone. Oncol rep 2006; 16; 1389–95.

[21] YOUNES A, KADIN ME. Emerging. Aplications of the tumor Necrosis Factor Family of ligands and Receptors in Cancer Theraphy. J Clin Oncol 2003; 21: 3526–3534.

[22] CRETNEY E, TAKEDA K, SMYTH MJ. Cancer: Novel therapeutic strategies that exploit the TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL receptor pathway. Inter J Bioch Cell Biol 2007; 39: 280–286.