Soluble form of TRAIL, Fas and FasL in the serum of patients with B-CLL

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Abstract

Purpose: Although many studies demonstrated expression of TNF family members in the course of B-CLL, there is a little known about relationships between soluble forms of these proteins. Furthermore, there is no study reported on effects of used therapy on this relation. The present study was designed to asses the relationships between the serum concentrations of sFas, sFasL and sTRAIL in patients with B-CLL regarding their correlation with clinical stage and used therapy.

Material and methods: We studied 40 patients with B-cell chronic lymphocytic leukemia (B-CLL) at diagnosis, before treatment and four weeks after therapy. To measure sFas, sFasL and sTRAIL levels in serum commercially available ELISA kits were used.

Results: We found increased concentrations of sFas in sera of all patients with B-CLL before treatment in comparison to the control group. There were no significant differences in concentrations of sFasL and sTRAIL between patients and control group. Increased sFasL concentrations after FC and CC therapy as well as decreased concentrations after 2CdA therapy in comparison to values before treatment were found. The concentrations of sTRAIL after FC and CC therapy were higher than those in patients before treatment.

Conclusions: Results obtained suggest that relationship between sFas, sFasL and sTRAIL in sera of patients with B--CLL before treatment may facilitate the growth B leukemic cells. Changes in these relations after therapy with FC and CC can make a contribution to inhibit B cells growth on the apoptosis way in this patient group.

Key words: B-cell chronic lymphocytic leukemia, sFas, sFasL, soluble TNF-related apoptosis-inducing ligand.

Introduction

TNF superfamily proteins such as TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL-R and Fas ligand (FasL)/Fas play a role in cancer development of the lymphoid system [1]. B-cell chronic lymphocytic leukemia (B-CLL) is a clinically heterogenous disease characterized by the accumulation of a clonal population of B lymphocytes. This accumulation is considered to result from the prolonged survival of B-CLL cells arrested in the G_0 stage of the cell cycle and by resistance toward apoptosis-inducing agents [2,3]. TRAIL and FasL cooperate in limiting lymphocyte proliferation following activation and use common pathways leading to apoptotic cell death [1,4].

TRAIL is present as a type II membrane protein and a soluble protein in culture supernatants and interacts with five distinct receptors: TRAIL-R1 (DR4), TRAIL-R2 (DR5), TRAIL-R3 (DcR1), TRAIL-R4 (DcR2) and osteoprotegerin (OPG) [5,6]. The ability to transmit apoptosis is restricted to TRAIL-R1 and TRAIL-R2 which contain "death domain" responsible for transducing the death signal [5-7]. The other receptors, TRAIL-R3, TRAIL-R4 and OPG, lack a functional death domain, are decoy receptors unable to transduce a death signal [5,8]. These receptors compete with TRAIL-R1 and TRAIL-R2 for TRAIL binding [7]. It has been observed that B cells from B-CLL expressed levels of the TRAIL-R1 and TRAIL-R2 [9,10]. Despite TRAIL death receptor expression B-CLL cells were relatively resistant to induction of apoptosis by rhTRAIL [10].

Soluble TRAIL is generated by deletion of the transmem-

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***Table1*. Serum levels of TNF molecules in patients with B-CLL before treatment**

	Control	Patients before treatment			
		Stage I n=8	Stage II n=4	Stage III n=7	Stage IV n=5
sFas pg/ml	179±84.36	293.6*±129	297.5*±42.7	345*±197	378*±168
sFasL ng/ml	60±18.0	87.7±44.79	75±37.4	46.6±25.3	44.7±21.7
sTRAIL pg/ml	79.6±25.20	55.6±28.65	54.5±15.2	44.1±21.8	45.6±33.19

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

brane and intracellular domains of mTRAIL by immune cells including lymphocytes, NK cells, monocytes, dendritic cells, macrophages and recently by neutrophils [6,8,11].

Although TRAIL signaling appears similar to Fas signaling, TRAIL induces apoptosis more efficiently than FasL. In contrast to TRAIL expressed on many tissues, the expression of FasL is restricted to activated lymphocytes and sites of immune privilege [12]. FasL (APO-1, CD95L) is a type II integral membrane protein and Fas (APO-1, CD95) is a type I membrane protein [1].

FasL and Fas expression were found on the surface of neoplastic plasma cells [13,14]. Binding FasL to Fas induces apoptosis of the Fas positive cells [1,13,14]. Both Fas and FasL exist as a soluble forms. Soluble Fas is generated by differential splicing via deletion of an exon encoding the transmembrane domain of Fas. In contrast, sFasL, a 26-kDa glycoprotein, is generated by proteolytic cleavage of the membrane bound form [13,15].

Although many studies demonstrated expression of TNF family members in the course of B-CLL, there is a little known about relationships between soluble forms of these proteins that may influence the B cells growth [13-16]. Furthermore, there is no study reported on effects of used therapy on the concentrations of these proteins.

The present study was designed to asses the relationships between the serum concentrations of sFas, as inhibitor of apoptosis, and sFasL and sTRAIL, as inducers of apoptosis, in patients with B-CLL regarding their correlation with clinical stage and used therapy.

Material and methods

Patient group

We studied 40 patients with B-cell chronic lymphocytic leukemia (B-CLL) at diagnosis, before treatment and four weeks after therapy. The average of these patients was 62.1 years, and the male to female ratio was 22/18. The diagnosis of leukemia was established on the basis of clinical observation, morphological composition of peripheral blood, marrow puncture and cytochemical tests, such as the reaction of alpha-naphtyl and black Sudan-B for peroxidases (POX), PAS and esterases. Leukemic B-CLL, according to generally accepted criteria was confirmed by flow cytometry immunophenotypic analysis of CD5, CD19, CD23, CD3, CD4 and CD8 using a EPIX XL analyzer (Coulter, USA). Patients were graded according to Rais' staging system as follows: stage I [8], stage II [10], stage III [14], stage IV [8]. Patients with active infection or allergic reaction 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-h i.v. for 3 days and mitoxantrone 10 mg/m^2 on day 1st and cyclophosphamide 650 mg/m^2 i.v. on day 1st (CMC); cyclophosphamide 650 mg/m^2 i.v. on 1st day (CC), fludarabine 25 mg/m2 i.v. for 3rd day and cyclophosphamide 250 mg/m^2 i.v. for 3rd day (FC).

The study was approved by the Local Ethical Committee and all patients gave written informed consent.

Control group

Control subjects (n=15) were normal healthy volunteers of the same age (workers and students of the Medical University of Bialystok).

Peripheral venous blood was drawn from into pyrogen-free blood collection tubes without additives. The serum was collected after centrifugation at 2500 rpm for 10 min and then was stored at -70°C until analyzed.

To measure sFas, sFasL and sTRAIL levels in serum commercially available ELISA kits were used (R&D Systems, Minneapolis, USA), according to the manufacturer's instructions. Recombinant sFas, sFasL and sTRAIL were used as respective standards.

The results are expressed as mean ± standard deviation. Data was analyzed according to the nonparametric U Mann-Whitney test. Correlations were calculated using the Pearson's test. A p value less than 0.05 was considered statistically significant.

Results

In sera of all patients with B-CLL before treatment we found increased concentrations of sFas in comparison to the control group of healthy person ($p<0.05$) (*Tab. 1*). There were no significant differences in concentrations of sFasL and sTRAIL between sera of these patients and the control group (*Tab. 1*). The concentrations of all parameters were analyzed according to Rais' stages. However, there was no significant differences between patients in different stages (*Tab. 1*)

To answer the question of which kind of therapy used in patients with B-CLL leads to specific changes in concentrations

Table 2. **The mean concentrations of parameters examined in the serum of patients with B-CLL before and after treatment**

	Patients with B-CLL			
	Before treatment	After treatment		
	$\bar{x}$±SD		*n*	$\bar{x}$±SD
sFas pg/ml	328.2±136.8	2CdA	13	368±15.3
		CMC	10	309±10.4
		FC	9	335.0±39.5
		CC	8	325±78.2
sFasL ng/ml	63±31	2CdA	13	37.6[a]±1.42
		CMC	10	58.3±1.60
		FC	9	87.5[a]±1.31
		CC	8	126[a]±0.84
sTRAIL pg/ml	49.5±16.1	2CdA	13	46.8±9.42
		CMC	10	60±8.65
		FC	9	84.7[a]±6.40
		CC	8	81[a]±8.91

[a] – statistical difference between patients before and after treatment ($p<0.05$)

of mediators examined their values we analyzed in detail due to 2CdA, CMC, FC or CC therapy.

We didn't find significant differences between concentrations of sFas in sera of patients after 2CdA, CMC, FC and CC therapy in comparison to the values obtained before treatment (*Tab. 2*). In contrast, the sera concentrations of sFasL in patients after used therapy were changed. Patients treated with FC and CC showed an increase in sFasL concentrations as compared to examinations before treatment ($p<0.05$) (*Tab. 2*). We also found that concentrations of sFasL in patients after 2CdA therapy were lower in comparison to examinations before treatment ($p<0.05$) (*Tab. 2*). In contrast to 2CdA therapy, CMC treatment didn't significantly influence on the sFasL concentrations in patients with B-CLL. In the present study we also observed absence of changes in sTRAIL concentrations in sera of patients after 2CdA and CMC treatment. In contrast, FC and CC therapy led to increased sTRAIL concentrations in patients with B-CLL (*Tab. 2*).

In this study we analyzed relationship between the concentrations of parameters examined in sera of patients before and after treatment. However, there were no correlation between these molecules.

Discussion

Results of the present study revealed an unfavorable relations between the soluble form of TNF superfamily molecules in sera of patients with B-CLL before treatment. Increased sFas and simultaneous decreased sFasL and sTRAIL concentrations can make a contribution to B leukemic cells growth and progression of B-CLL.

High concentrations of sFas, observed in the present study, can block death of B leukemic cells by inhibiting the interaction between Fas and FasL and can help B-CLL cells to escape from induction of apoptosis by FasL positive cells, such as autologous T cells [13,15].

Decreased concentrations of sFasL and sTRAIL as inducers of apoptosis may also be responsible for progression of B-CLL. Membrane – bound (mTRAILl) and soluble TRAIL (sTRAIL) were shown to rapidly induce apoptosis in susceptible tumor cells upon trimerization of its receptor and subsequent activation of the caspase cascade, leading to fragmentation of DNA [8].

It has been demonstrated that FasL and TRAIL synergistically induce apoptosis of chronic lymphocytic leukemia B cells [16]. Dicker et al. indicated that two or more death receptors are more effectively than one of these. This is similar to the interactions to achieve optimal immune co-stimulation during the immune response to antigen. Similarly, a requirement for co-ligation of multiple death receptors via FasL and TRAIL, could be an important mechanism for enhancing the specificity of killing by immune mechanisms [16]. Since sFasL and sTRAIL are generated by proteolytic cleavage of the membrane bound FasL and TRAIL, decreased sFasL and sTRAIL in the sera of these patients may be associated with high expression of their membrane forms. In fact, Tinhofer et al. demonstrated that the CD19+ B cells fraction from PBMC B-CLL patients were found to significantly express FasL [14].

Changes in sFasL and sTRAIL concentrations, besides a direct effect on B leukemic cells growth, may have different implications for the immune response in patient with B-CLL. For example, it has been demonstrated that Fas/FasL and TRAIL-R/TRAIL regulate myelopoiesis and dendritic cell functions [1]. Furthermore, sFasL has been shown to induce activation of NF-kB and its low concentration can be responsible for impaired synthesis of different molecules [6].

Elevated concentrations of sFas according to progression disease appear to confirm its role in pathogenesis of B-CLL and to indicate that sFas in patients' sera is, at least partly, derived from B leukemic cells. This observation is in agreement with data presented by Osorio et al. who found sFas in supernatants from *in vitro* cultured B-CLL cells. They also demonstrated a correlation between sFas serum levels and clinical progression [13].

The key question of this study is whether therapy used may change the relations between sFas, sFasL and sTRAIL in sera of patients with B-CLL. Results obtained revealed that any kind of therapy didn't significantly influence on the sFas concentrations in sera of patients. In contrast, increased sFasL and sTRAIL concentrations in sera of patients after FC and CC therapy were observed. Additionally, in sera of patients treated with CMC increased concentrations of sTRAIL without effect on sFasL levels were observed. Absence of changes in high concentrations of sFas after FC and CC therapy appear to be balanced by simultaneous increased concentrations of sFasL and sTRAIL. Alterations above can make a contribution to enhance B leukemic cells apoptosis and lead to inhibit progression of B-CLL in patients after treatment.

Since sTRAIL-induced apoptosis is more effectively than sFasL, soluble recombinant derivatives of TRAIL are considered as novel tumors therapeutics because of their selective apoptosis inducing activity in a variety of human tumors but not in normal

cells [17]. On the other hand, elevated sTRAIL concentrations may have unfavorable influence on the T cell function. Soluble TRAIL has been shown to inhibit T cell activation and proliferation without inducing T cell death [8].

Summarizing, our results indicate that relationship between sFas, sFasL and sTRAIL in sera of patients before treatment may facilitate the growth B leukemic cells in patients with B-CLL. Therapy with FC and CC lead to favorable changes in these relations. However, larger prospective studies, involving more molecules of TNF superfamily, are needed to explain a diagnostic value of these observations and to design appropriate therapeutic strategies for B-CLL.

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