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Prognostic significance of ligands belonging to tumour necrosis factor superfamily in acute lymphoblastic leukaemia

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ABSTRACT

Altered activities of ligands belonging to tumour necrosis factor (TNF) superfamily, namely B-cell activating factor (BAFF), a proliferation-inducing ligand (APRIL) and apoptosis inducing ligand (TRAIL) were demonstrated in several haematological diseases including acute lymphoblastic leukaemia (ALL). BAFF, APRIL and TRAIL provide crucial survival signals to immature, naive and activated B cells. These ligands are capable of activating a broad spectrum of intracellular signalling cascades that can either induce apoptosis or protect from programmed cell death. BAFF and APRIL, which can directly activate the NF-κB pathway, have been identified as crucial survival factors for ALL cells. Here, we have analyzed serum BAFF, APRIL and TRAIL concentrations in 48 patients with newly diagnosed ALL and 44 healthy volunteers. The levels of APRIL and BAFF were significantly higher in ALL patients as compared to healthy volunteers. In contrast, concentrations of TRAIL were significantly lower in ALL patients. Moreover, following induction, the levels of APRIL, but not BAFF or TRAIL, were significantly lower in a group of patients with complete remission (CR) as compared to non-respondent (NR) ALL patients. Furthermore, we demonstrated statistically significant differences in concentrations of APRIL between CR MRD-negative and CR, MRD-positive ALL patients. Notably detection of higher concentrations of APRIL was associated with shorter leukaemia-free survival and overall survival. Altogether, our data indicate that APRIL can play an important role in the pathogenesis of ALL and the measurement of APRIL levels can improve prognostication in ALL patients.

1. Introduction

Acute lymphoblastic leukaemia (ALL) is currently ranked as the most common malignant disease in children and a major cause of mortality due to hematopoietic malignancies in adults [1]. Notwithstanding high rates of remission induction and survival achieved through current treatment regimens, the response to therapy is still poor in a subset of patients. This fact necessitates a thorough comprehension of the survival signals and microenvironment that contribute to the establishment of the leukaemic clone and its resistance to therapy. Implications of the dysregulation, which is capable of providing survival and co-stimulatory signals, are still broad. The ability to manipulate such a powerful axis could increase or even grant total independence of environmentally regulated homeostatic control.

Tumour necrosis factor-alpha (TNF-α) is a pleiotropic cytokine that is capable of exerting numerous biological effects. In particular, TNF-α can have proliferative and/or survival effects on 'normal' untransformed cells and some human tumour cells in contrast to anti-proliferative, apoptotic and cytotoxic effects exerted against other tumours (both *in vitro* and *in vivo*) [2]. Both B-cell activating factor (BAFF), and a proliferation-inducing ligand (APRIL) are members of the TNF superfamily that provide crucial survival factors for immature, naive and activated B cells [3,4]. BAFF and APRIL are produced as type II transmembrane proteins, (likewise many ligands belonging to the TNF family), and are then proteolytically cleaved at a furin protease site and released in a soluble form [5]. Previous studies demonstrated that APRIL and BAFF share two TNF receptors

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superfamily members: B-cell maturation antigen (BCMA), transmembrane activator and calcium-modulating cyclophilin ligand interactor (TACI) [6–8]. In addition, APRIL binds to heparin sulfate proteoglycan, which serves as a platform to mediate ligand multimerization and cross-linking [9].

Our relatively limited understanding of the role of BAFF, APRIL and their receptors in normal B-cell homeostasis and in several tumour models does not preclude the possibility of their involvement in the pathogenesis of haematological malignancies. Certain studies reported aberrant expressions of BAFF and APRIL by tumour B cells isolated from a subset of patients with chronic lymphocytic leukaemia [10,11]. BAFF and APRIL were found to protect B-CLL cells from spontaneous and drug-induced apoptosis and to enhance cell survival [10]. Previous performed studies have already shown promise for BAFF as a potential prognostic marker in CLL, especially when used in combination with CD38, ZAP70 expression and the mutational status [12]. Similarly, in follicular lymphoma, expressions of both BAFF and BAFF-R are elevated and coincide with inferior progression-free survival (PFS). The overexpression of BAFF, suggested being secondary to the elevated expression of BAFF-R, apparently increased sensitivity to BAFF [13]. Furthermore, the most recent studies found that myeloma cell lines and primary myeloma cells express BAFF, APRIL and their receptors, and that both BAFF and APRIL act as growth factors for myeloma cells [14]. BAFF was also demonstrated to protect myeloma cells from dexamethasone-induced apoptosis [15]. In addition, the possible use of BAFF and APRIL as markers of disease activity and progression [16] as well as their role in angiogenesis [17] has recently been investigated.

TNF-related apoptosis inducing ligand (TRAIL) belongs to the TNF superfamily produces an effect opposite to that of BAFF and APRIL and participates in the elimination of neoplastic cells including leukaemic cells [18,19]. TRAIL, also known as Apo-2L, can be biologically effective as an integral membrane protein (mTRAIL, 32 kDa) as well as a soluble cytokine (sTRAIL, 24 kDa) [19]. To date, several important functions of TRAIL have been reported. Firstly, TRAIL-mediated cytotoxicity plays an important role in innate and adaptive immune responses [20]. Secondly, TRAIL exerts a regulatory function on erythroid and myeloid maturation in normal haematopoiesis [21–24]. Moreover, senescent neutrophils can be eliminated by TRAIL-induced apoptosis upon their return to the bone marrow [25]. Importantly, TRAIL was demonstrated to induce apoptosis of leukaemic cells in the course of haematological malignancies, including multiple myeloma cells and Philadelphia chromosome-positive leukaemia [26,27]. In contrast, AML blasts were shown to exhibit a very low sensitivity to the pro-apoptotic effects of TRAIL [28].

The principal objective of the present study was to evaluate serum levels of BAFF, APRIL and TRAIL in healthy volunteers and in ALL patients with varying severity of the disease. In addition, we intended to analyze mutual relationships among baseline BAFF, APRIL and TRAIL levels and other known prognostic parameters in the cohort of ALL patients. Finally, we aimed to evaluate whether measuring serum APRIL, BAFF and TRAIL concentrations could improve prognostication of ALL patients.

2. Patients

Forty-eight patients with newly diagnosed acute lymphoblastic leukaemia B linage were enrolled in the study. Their median age at the time of sample collection was 31.6 and the range was 18–56. Twenty-three subjects were male and 25 female. Patients who received corticosteroids before the beginning of the treatment course had been excluded from the study. Diagnoses were established according to the 2008 WHO recommendation [29]. Blood counts, flow cytometry, molecular study, FISH and cytogenetic analysis were performed, reviewed, and classified. Patients were treated in the Haematology Department of the Medical University of Bialystok from 2007 to 2013 with induction chemotherapy regimens corresponding to the standard therapy based on the Polish Adult Leukaemia Group: therapy consisted of prednisone pretreatment p.o. (PDN) 60/40* mg/m^2 (* for patients over 40 years old) for 7 days followed by 4 weeks induction therapy: prednisone for 28 days with the same dose as pretreatment with daunorubicine i.v. 50/40* mg/m^2 and vincristine i.v. 2 mg days 1, 8, 15 and 22, together with Peg-Asparginase i.v. 1000 IE/m^2 on day 13. After induction, the response was evaluated following the recommendation by NCCN Guidelines. Thirty-eight patients after 1st induction achieved complete remission (CR), which was defined as the absence of physical signs of leukaemia or detectable leukaemia cells on blood smears, a bone marrow with active haematopoiesis and <5% leukaemia blast cells, and normal cerebrospinal fluid. Eight patients were non-responders with the levels of blastic cells at least 20%, [4 patients with normal karyotype, 2 with t(v;11q23) 1 with hypodiploidy and 1 with t(12;21)(p13;q22)]. Patients under 40 who achieved CR but were MRD positive ($\geq$0.1%) and all non-responders were administered a second-line induction, FLAM (fludarabine, cytarabine and mitoxantrone). For all patients who achieved complete remission after one or two induction therapies consolidation therapy included a course of high-dose cytarabine plus cyclophosphamide and a cycle of high-dose methotrexate plus etoposide and dexamethasone. Central nervous system (CNS) prophylaxis consisted of intrathecal therapy with Depocyte and prednisolone administered twice during the induction therapy (during pretreatment −7 to −3 days) and on day 10 as well as once during each consolidation. For all patients with t(9;22)(q34;q11.2), (BCR-ABL positive gene fusion) the standard treatment was supplemented with Glivec at 600 mg/per day. Upon the termination of consolidation, patients were stratified into either a standard risk (SR) or a high-risk (HR) group. HR was defined as the presence of at least one of the following factors: age $\geq$35 years, WBC at diagnosis $\geq 30 \times 10^9$/L, adverse immunophenotype, cytogenetic and molecular abnormalities defined by the presence of at least one of the following parameters: pro-B, early-T or mature-T phenotype, two courses of induction required to achieve CR, t(9;22) and t(4:11) [BCR-ABL and MLL] and low hypodiploidy, or CR achieved after two induction cycles. Patients with none of the above features were assigned to the SR group. Subjects from the HR group were intended for either alloHSCT or autologous (autoHSCT) haematopoietic stem cell transplantation, depending on the availability of an appropriate donor. A search for a matched unrelated volunteer for patients without a human leukocyte antigen identical sibling was initiated. Patients allocated to the SR group were given 2-year maintenance therapy consisting of epirubicin, vincristine, prednisone, mercaptopurine and methotrexate. The characteristics of ALL patients are listed in Table 1.

The control group was population-based and comprised forty-four age-matched healthy volunteers (22 males, 22 females, median of age 32, range: 20–57), who had no history of acute or chronic diseases and received no medications. The analysis of their complete blood counts revealed no abnormalities, either.

All patients' samples as well as the samples from healthy volunteers were collected under the Ethics Committee of the Medical University of Bialystok upon signing an approved protocol and a written informed consent form in accordance with the Declaration of Helsinki, No. R-I-002/218/2007.

3. Methods

The cytokine measurement was done following the manufacturers' instructions. Three independent sets of experiments were performed. Each experiment included the kit's standards and samples both from the ALL patients and healthy controls run in triplicate. No significant variations were observed among the experiments.