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Original Research Article

Expression of IL-1 and IL-6 and their natural regulators in leukocytes of B-cell chronic lymphocytic leukaemia patients

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ABSTRACT

Purpose: The purpose of the study was the assessment of the expression of IL-1β and IL-6, and the proteins regulating their biological activity, namely IL-1RII, IL-1Ra, as well as sIL-6Rα, sgp-130 in leukemic lymphocytes and autologous neutrophils of B-CLL patients.
Material/methods: The study involved a group of B-cell chronic lymphocytic leukemia patients and healthy volunteer blood donors. The presence of chosen proteins and their natural regulators was confirmed by Western blot.
Results: Western blot analysis showed a decreased expression of IL-1β and IL-6 in the leukocytes of B-CLL patients. Decreased expression of sIL-6Rα has been observed in lymphocytes, with a simultaneous increase of expression in PMNs. Lower expression of sgp-130 was found in B cells while its expression was elevated in the neutrophils of patients in early stages of the disease.
Conclusions: The changes observed in the expression of IL-1 and IL-6 seem to exclude their immediate involvement in the progress of B-CLL. However, the presented changes in the expression of proteins regulating IL-1β and IL-6 in PMNs indicate a potential role of early immune response cells also in advanced stages of the disease.

1. Introduction

B-cell chronic lymphocytic leukaemia is defined as a monoclonal lymphocytosis of B-cells [1]. Apart from progressing lymphocytosis, the expansion of neoplastic B-cells is characterised by bone marrow infiltration, splenomegaly, lymphadenopathy, and immune disorders expressed in immunodeficiency and autoimmune syndromes [2–4]. Proliferation and increased survival of dysfunctional B lymphocytes is regulated by endogenous cytokines via autocrine signalling, but also by paracrine signals, whose source may come from immunocompetent cells, including neutrophils (PMNs) [5–9].

Cytokines, low molecular mass intercellular protein mediators, are involved in every stage of cell life and death, and therefore play a key role in immune and inflammatory responses and the process of development, regeneration, and maintenance of homoeostasis in the body. The first identified cytokines were interleukins (IL) – regulators of proliferation, differentiation and functioning of leukocytes – and among those, IL-1 and IL-6 [10].

Interleukin 1 (IL-1) is the central mediator of immunological response. The IL-1 family includes groups of pro-inflammatory agonist ligands (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, IL-36γ), three antagonist receptors, and the anti-inflammatory IL-37. The receptor group in this family is composed of six receptor chains constituting four receptor signalling complexes, decoy receptors: IL-1 RII (IL-1R2), IL-18BP, and negative regulators: TIR8/SIGIRR, IL-1RAcPb. Precise regulation by the antagonists, decoys, and signalling inhibitors determines the maintenance of balance between enhancing the immune response and uncontrollable inflammation [11].

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IL-6 is also considered to be a key mediator of the body's defence responses, having a strong pleiotropic action. It exercises its effect on target cells by conjugating with a class I receptor, an IL-6Rα (CD126) subunit which binds ligands, and glycoprotein 130 (gp130), responsible for signal transduction [12,13]. IL-6Rα occurs in a membrane-bound form, (mIL-6Rα), and a soluble form (sIL-6Rα), both transmitting activation signals. A sub-unit of gp130 also has a soluble form – sgp130, which functions as an antagonist [14–16].

B-CLL is characterised by an extremely heterogeneous course in individual patients, despite its similar clinical picture and B-cell morphology. In a large percentage of patients, the disease progresses slowly and does not require cytotoxic treatment, while in some people its course is very aggressive [17]. The decisions on treatment are made at diagnosis and therefore there is a constant search for new biological predictors, also among cytokines, whose assessment might find its place in clinical practice, contributing to a more individual approach to B-CLL patients.

This is the context of the research done to assess the expression of IL-1β and IL-6, along with the proteins that regulate their activity, IL-1RII and IL01Ra respectively, as well as sIL-6Rα and sgp-130 in leukemic B lymphocytes, as potential diagnostic and prognostic factors.

Growth and proliferation of leukemic cells can also be influenced by mediators of other cells, such as neutrophils – first line of immune defence – which can easily identify neoplastically-transformed cells, leading to their elimination. The role of neutrophils in the course of chronic B-cell lymphocytic leukaemia is still being discussed. It was determined that these cells are capable of releasing a range of cytokines which can affect the neoplastic process [18,19]. In the light of this information, it was undertaken to confront the results obtained from leukemic B-cells with the data obtained from autologous neutrophils of patients.

2. Materials and methods

2.1. Participants

The study involved a group of 23 B-cell chronic lymphocytic leukaemia patients treated at the Department of Haematology of the Medical University of Bialystok. The assays were done before the initiation of treatment. The diagnosis of leukaemia was based on clinical observation, morphological composition of peripheral blood, bone marrow puncture, trepanobiopsy, lymph node biopsy and cytochemical examinations. A flow cytometer, EPIX XL (Coulter, USA), was used to identify the immunophenotypes of leukemic cells. CD5, CD19, CD23 and CD20 monoclonal antibody panels were used to differentiate the B cells. More than 55% of B lymphocytes were positive for CD19+/CD5+. The patients were graded according to the modified Rai staging system [20,21]. Patients with accompanying acute inflammatory bacterial, viral, mycotic or allergic states were excluded from the study.

The control group consisted of 13 healthy volunteer blood donors. Healthy blood donors were sought in the group of about 50 years old, taking into account the criteria: overall good health, lack of chronic diseases, permanent drug noncompliance, no smoking.

The incidence of B-cell chronic lymphocytic leukaemia falls mostly on the fifth/sixth decade of life. Healthy blood donors were sought in the group of about 50 years old, taking into account the criteria: overall good health, lack of chronic diseases, permanent drug noncompliance, no smoking. It was failed to collect the whole group of healthy persons at the sought aged. However, no differences in the results obtained in a group of healthy subjects according to age.

The clinical data on the patients and healthy persons are presented in Table 1.

Approved by the Bioethics Committee of the Medical University of Białystok no. R-I-002/41/2013 and R-I-002/584/2013.

2.2. Cell isolation

Upon the donor's consent, blood samples were extracted from the basilic vein and preserved with EDTA for cell isolation. Cells were isolated from whole blood by density centrifugation, using Polymorphprep™. This method enables simultaneous separation of two highly purified leucocyte fractions: polymorphonuclear cells, involving neutrophils (PMNs) and mononuclear cells (PBMCs). After initial isolation with Polymorphprep™, the cells were sorted using a magnetic MACS® Separator with CD16 Microbeads (PMNs) and CD19 MicroBeads (B lymphocytes).

2.3. Cell purity assessment

The purity of the collected cell suspensions was assessed in the so-called "thick drop" preparations, using May-Grünewald-Giemsa staining. The purity of all of the cell suspensions was at 99.75% (in the range of 98–100% for individuals, neutrophils and lymphocytes B).

2.4. Cell viability assessment

The viability of the cells was assessed immediately after separation in a light microscope using trypan blue. The proportion of viable cells in the control group was on average 97% for PMNs and B lymphocytes, and 96% for PMNs and 98% for B cells in the study group.

2.5. Protein isolation

Immediately following the separation of cells in the patient and control groups, the protein was isolated in the presence of a protease

Table 1
The clinical data on the patients and healthy persons.

Patients $n=23$ Modified Rai classification	Age [years]	WBC [$\times 10^3$ cells/μl]	Blood smear [%]		
			Stick figure neutrophils	Polymorphonuclear cells	Mononuclear cells
Early/intermediate stages					
0° $n=1$ (M)	54–65	23.8–189	0–10	2–45	50–98
I° $n=4$ (1 M, 3 F)					
II° $n=7$ (4 M, 3 F)					
Advanced stages					
III° $n=3$ (2 M, 1 F)	52–89	13.55–510.5	0	5–30	70–95
IV° $n=8$ (4 M, 4 F)					
Healthy persons $n=13$ (7 M, 6 F)	20–50	4.8–9.5	0–2	45–81	16–60

n – number of persons, M – men, F – females.