Abstract

Essential thrombocythemia (ET), is an acquired myeloproliferative disorder, characterized by persistent, non-reactive thrombocytosis and a slow, progressive course. Despite the fact that the understanding of the molecular basis of ET has significantly improved the diagnostic possibilities and has become an important element in the prognosis of the course of the disease, the available therapies are still based primarily on reducing the number of circulating platelets and, what is worse, do not prevent the further evolution of the disease. For this reason, the search for new, previously unknown mechanisms conditioning the development and evolution of essential thrombocythemia continues.The subject of intensive research are, inter alia, signaling pathways involving pro-inflammatory cytokines, including IL-1β (interleukin 1β) and IL-6 (interleukin 6), and factors belonging to the tumor necrosis factor family, including the relatively recently discovered APRIL (A Proliferation-Inducing Ligand) and BAFF (B-cell Activating Factor) proteins. These factors, apart from immunomodulatory activities, also seem to play an important role in the process of megakaryopoiesis. However, their role in the production of platelets remains unclear.

Therefore, the aim of the study was to i) quantify the concentration of APRIL and BAFF proteins in peripheral blood and bone marrow supernatant, ii) assess the concentration of pro-inflammatory cytokines – IL-1β and IL-6 in peripheral blood and bone marrow supernatant, iii) assess the direct effect of APRIL and BAFF on the pro-inflammatory response of T and B lymphocytes and iv) evaluate the direct influence of APRIL and BAFF proteins on the process of megakaryopoiesis in the *in vitro* environment.

The research material consisted of peripheral blood and bone marrow aspirate obtained from patients diagnosed with ET and from age- and gender-matched healthy control. Concentrations of APRIL, BAFF and their soluble receptors (sTAC1 and sBCMA), as well as IL-1β and IL-6 in peripheral blood and marrow supernatant were determined by enzyme immunoassay. Percentage of T and B lymphocytes producing IL-6 and IL-1β after 24-hour stimulation in the presence of BAFF and APRIL, was assessed by multicolor flow cytometry. CD34 + progenitor cells from bone marrow were isolated using a cell sorter and then stimulated with TPO in the presence or absence of the TAC1 fusion protein to assess the level of expression of APRIL and BAFF genes and their receptors (quantitative PCR), protein concentration in the supernatant and the amount of platelets produced.

The analysis of the obtained results showed increased levels of APRIL and BAFF in plasma, regardless of the presence of the JAK2(V617F) mutation and a prior history of thromboembolic and haemorrhagic episodes. Moreover, in studies of the bone marrow supernatant, an increased concentration of APRIL and its receptor – sBCMA as well as a decreased concentration of BAFF and sTACl were observed in the group of ET patients compared to the bone marrow supernatant obtained from the control group. In addition, it has been proven that APRIL plays an important role in the maturation of megakaryocytes, acting as a growth factor, regardless of the presence of the JAK2 (V617F) mutation. At the same time, the neutralization of APRIL activity using the rhTACl fusion protein was associated with a significant reduction in the number of platelets. Based on the above observations, it should be assumed that the assessment of APRIL and BAFF may prove helpful in the diagnosis of patients with ET.

In conclusion, the conducted study confirms the important role of APRIL and BAFF in dysregulation of megakaryopoiesis in ET patients. Moreover, APRIL acts as a growth factor in the process of megakaryopoiesis, and blocking its activity may reduce platelet production in the course of ET.