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The role of 5´-nucleotidases in function of immune system cells

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Abstract

Disorder of purine metabolism, in particular the activity of 5'-nucleotidase, which is one of the key enzymes of the purine cycle, leads to functional inferiority of cells of the immune system. 5'-nucleotidase is considered a marker of the functional maturity of lymphocytes. In our opinion, research of activity of the enzyme at various immunodeficiency conditions can serve not only as additional prognostic criteria but also be essential help both at tactics choice and at an estimation of efficiency of therapy with purine analogs.

Keywords: Purine nucleotides; 5´-nucleotidase; Immune system; Lymphoproliferative diseases

1. Introduction

According to modern concepts, purine nucleotides, in particular ATP and its derivatives, are considered not only as of the main source of intracellular energy but also participate in the implementation of a variety of regulatory processes. It has been shown that disorder of purine metabolism, in particular the activity of 5'-nucleotidase, which is one of the key enzymes of the purine cycle, leads to functional inferiority of cells of the immune system. The 5′-nucleotidases (3.1.3.5) are a family of enzymes that catalyze the dephosphorylation of nucleoside monophosphates and regulate cellular nucleotide and nucleoside levels [1, 2].

The family of 5'-nucleotidases includes both membrane-bound and cytoplasmic and mitochondrial proteins. 5′-Nucleotidases are classified according to subcellular localization, nucleotidase specificity and their ability to hydrolyze deoxynucleoside monophosphate substrates, 4 four distinct groups of 5'-nucleotidases are distinguished: membrane-bound ecto-5'-nucleotidase (e5NT, also known as CD73), which predominantly hydrolyzes extracellular AMP with the formation of adenosine; soluble AMP-selective 5'-nucleotidase (cN-I) involved in the mechanism of adenosine formation during ATP cleavage; cytoplasmic 5'-nucleotidase/ cytosolic 5'-nucleotidase with "high Km" (cN-II), which has a high affinity for IMP and GMP; cytoplasmic pyrimidine-5'-nucleotidase with high affinity for pyrimidine nucleotides (cN-III or dNT-1). E5NT (CD73) is a glycosylated protein bound to the outer surface of the plasma membrane by a glycosylphosphatidylinositol anchor that regulates the concentration of adenosine on the cell surface by converting extracellular 5-AMP into adenosine, which is involved in the regulation of essential cellular functions. It was shown that in the membrane it is localized in the immediate vicinity of adenylate cyclase, providing a high local concentration of adenosine. Synergism with the activity of Mg-dependent ATPase was noted.

It has been established that Purinergic receptors are divided into two subfamilies known as P1 and P2 receptors. P1 receptors are differentiated by their ability to inhibit (A1) or activate (A2) adenylate cyclase: denosine receptors are coupled with different G proteins, modulating the activity of adenylate cyclase (AC) in a positive or negative manner, thus affecting cytoplasmic cAMP. Both A_1 and A_3 receptors couple with G_i protein, hence their activation suppresses AC with subsequent decrease in cAMP level.

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Also, adenosine receptors which are not associated with adenylate cyclase - A3 receptors are described. The P2 nucleotide receptor family is divided into tree subfamilies: P2x associated with ionotropic channels, P2y associated with the activation of G-proteins, and P2z - a family of non-selective pores.

CD73 is a cell adhesion molecule and is involved in cell-cell and cell-matrix interactions. Like other GPI anchored proteins, when interacting with antigens, CD73 transmits activation signals into the cell, causing phosphorylation and dephosphorylation of various proteins, thereby being involved in the mechanisms of signal transduction [3]. There is an opinion that activation of phosphoinositide 3-kinase prevents endocytic rearrangements of e5NT, thereby causing activation of cytoprotective mechanisms [4].

CD73 expression is induced by IL-1β, PGE2, TNF TN, and other factors. Thus, incubation of NK cells with PGE2 markedly reduced the cytotoxic activity of the latter, which correlated with a significant increase in e5NT activity.

CD73 is found on the surface of a variety of cell types, including endothelial cells, subtypes of lymphocytes, stromal cells and select types of tumor cells. Lymphocytes, in comparison with other cells, have high adenosine phosphorylating activity and are "traps" for adenosine and deoxyadenosine. This property of lymphocytes formed the basis for the differentiation of lymphoid and myeloid variants of blast crisis in chronic myeloid leukemia: enzyme activity was high in lymphoid variant, in myeloid crisis, the enzyme activity was sharply reduced or was not recorded at all [5].

E5NT is considered a marker of the functional maturity of lymphocytes. The e5NT activity in B-lymphoblasts is 5-6 times lower than in B-lymphocytes. In the process of differentiation of B cells, an increase in e5NT activity is observed. It was found that B-lymphocytes with low e5NT activity are not capable of normal synthesis of immunoglobulin. A decrease in the expression of e5NT in mononuclear cells in patients with primary immunoglobulin deficiency has been shown. In this case, the enzyme deficiency led to inhibition of the proliferation of B-lymphocytes, blocking the S-phase of the cell cycle.

The e5NT activity in T lymphocytes is much lower than that in B lymphocytes, which is the enzymatic basis for distinguishing between T and B lymphocyte populations. This difference underlies their different sensitivity to adenosine. It has been shown that adenosine and its analogs are selective activators of T-lymphocytes. In particular, e5NT plays a crucial role in the activation of cytotoxic T lymphocytes. Studies have shown [6] that 60% of e5NT + T cells consist of a population of CD8 + cells, represented mainly by T-suppressors, while e5NT cells are mainly represented by a population of CD4 + T-lymphocytes, consisting of T-helpers. Insufficiency of e5NT in T-lymphocytes is manifested by a block in the G-phase of the cell cycle, suppression of the suppressor effect of T-lymphocytes).

It is assumed that the low activity of e5NT is a marker of malignant lymphoid cells [7]. Thus, in patients with multiple myeloma, a correlation was found between the expansion of suppressor T cells (CD8 +, OKM1 +, DR +) and severely expressed e5NT deficiency. A twofold decrease in e5NT activity in T cells was found in Hodgkin's lymphoma, and the decrease in activity was equally observed both in remission and at the prodromal period of the disease. A noticeable decrease in e5NT activity was noted in patients with B-cell chronic lymphocytic leukemia and it is mostly determined at stages III and IV (according to Rai) of the disease. At the same time, low values of e5NT remained for 24 months. It is believed that a wide range of variation of e5NT activity in lymphoid cells is due to the degree of differentiation of cells and their immunological subtype. An ultrastructural cytochemical study of the distribution of e5NT activity in various types of non-Hodgkin's lymphomas and lymphoid leukemias showed that the highest color intensity was observed in the common variant of acute lymphoblastic leukemia. In chronic lymphocytic leukemia, prolymphocytic leukemia, and hairy cell leukemia, the staining intensity was below normal. The staining intensity in non-Hodgkin's lymphomas varied. Analysis of the data showed that large lymphoid cells are characterized by very moderate enzyme activity, while small follicle-like cells, centroblasts, and centrocytes are characterized by moderately strong or strong e5NT activity. This study was another evidence in favor of the marker role of e5NT in assessing the degree of lymphocyte differentiation.

CN-II nucleotidase is specific for 6-hydroxypurine nucleotide monophosphates: IMP, dIMP, GMP, dGMP, and XMP. It also exhibits phosphotransferase activity, capable of transferring a phosphate group from nucleoside monophosphates to a nucleoside acceptor. The enzyme is allosterically activated by nucleotides (mainly ATP) and 2,3-DPG and is inhibited by Pi [8].

It has been shown that cN-II can dephosphorylate anti-HIV and antitumor nucleoside analogs: FdUMP, AZTMP, CdAMP, araCMP, BVdUMP, ddCMP, and phosphorylate dideoxyinosine, thiosofuran, acyclovir, and ribavirin [9].

It was established that the activity of cN-II correlates with the sensitivity of lymphocytes to therapy with purine analogs [10]. It has been shown that lymphocytes with low cN-II activity respond well to treatment with nucleosides modified at the purine ring (fludarabine, cytarabine, chlordeoxyadenosine). Thus, in the study of the effectiveness of the use of cytarabine in the treatment of patients with acute myeloid leukemia, it was indicated that lymphocytes with high expression of cN-II were less sensitive to the action of cytarabine [11]. CN-II prevented the accumulation of the triphosphoric derivative of cytarabine, which is necessary for the toxic effect. At the same time, researchers have established a correlation between the level of cN-II expression and clinical outcome in patients receiving cytarabine. Analysis of the data showed that patients with high levels of the enzyme in their blast cells had a short duration of remission compared to patients with low cN-II activity (11 months versus 17.5 months). At the same time, a high level of cN-II was a harbinger of a short overall life expectancy (15.7 months versus 39 months) in young patients (≤ 57 years). As a result of multivariate data analysis (taking into account age, karyotypic risk, and other factors), it was shown that the expression of cN-II is an independent prognostic factor for determining the duration of remission in acute myeloid leukemia; high cN-II values in blast cells are a poor prognostic sign in young patients, indicating a short overall survival.

A comparative study of cN-II activity in plasma cells of patients with multiple myeloma and monoclonal gammopathies of unknown origin (MGNH) showed that almost half of the patients with multiple myeloma had cN-II + cells, while patients with MGNH, the number of such cells was only about 15%. In only 1% of healthy people examined, cN-II + cells were present. As a result, the authors came to the conclusion that the study of cN-II activity can be used to detect malignant monoclonal gammopathies [12].

The third 5´-nucleotidase, cN-III, has a high affinity for 5´-, 2´, and 3´-phosphates of other ribo- and deoxyribonucleosides. The mitochondrial form of the last nucleotidase (dNT-2), which is specific to uracil and thymine nucleotides, was also found. It is believed that it protects mitochondrial DNA from excessive accumulation of dTTP [13].

cN-III plays an important role in the functioning of red blood cells. It has been shown that the expression of the enzyme increases during the maturation of the erythrocyte. Congenital deficiency of cN-III in erythrocytes leads to the development of non-spherocytic hemolytic anemia. It was found that in the case of cN-III deficiency in erythrocytes, the accumulation of pyriminide-5'-nucleotides is observed, which suppresses the activity of the pentose phosphate pathway, reducing the activity of glucose-6-phosphate dehydrogenase by almost 50% [14]. Acquired erythrocyte cN-III deficiency occurs in patients with β-thalassemias.

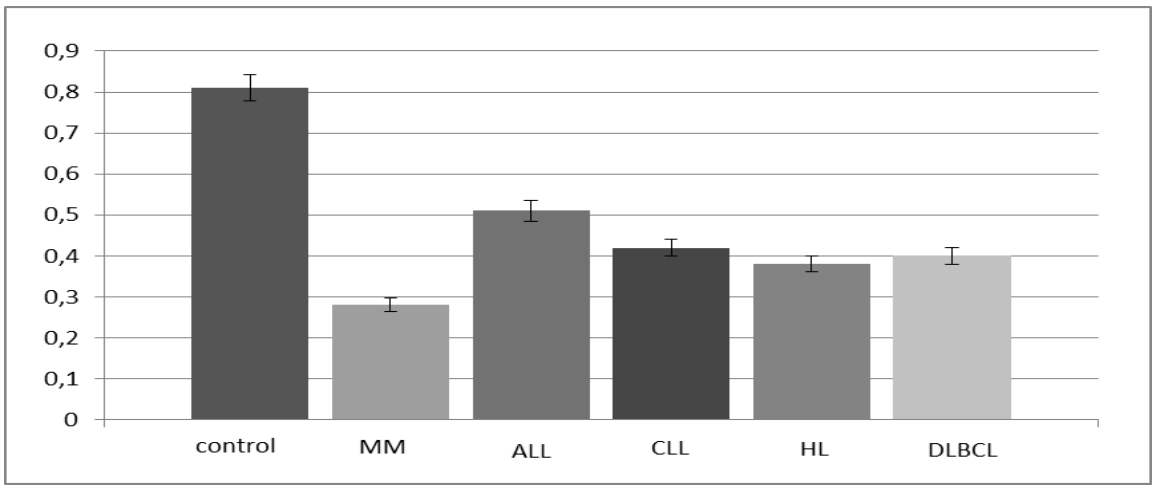

Figure 1 Changes in the activity of ecto-5' -nucleotidase in some lymphoproliferative diseases (in μmol P/g)

The activity of 5'-nucleotidase can be determined by both spectrophotometric methods and immunological (using monoclonal antibodies), cytochemical, radiochemical methods, PCR-method.

In the scientific department of the Hematology Center of Armenia, a spectrophotometric study of e5NT activity in various lymphoproliferative diseases was carried out: chronic lymphocytic leukemia, acute lymphoblastic leukemia, large B-cell non-Hodgkin's lymphoma, multiple myeloma, Hodgkin's lymphoma [15, 16]. A statistically significant decrease in enzyme activity was established in all studied nosology. At the same time, the most significant deviation from control was observed in patients with MM (threefold decrease). For other nosology, the activity of ecto-5'-

nucleotidase decreased as follows: in Hodgkin's lymphoma - by 2.21 times, in large B-cell non-Hodgkin's lymphoma - by 2 times, and in CLL - by 1.86 times.

Thus, we can conclude that the use of the 5 -nucleotidase test in hematology is quite justified. In our opinion, the determination of the activity of various types of 5 -nucleosidases can serve as a good help for the differentiation of T- and B-populations of lymphocytes, as a marker of the functional maturity of lymphocytes, for the detection of malignant clones of lymphoid cells, for the differentiation of lymphoid and myeloid variants of blast crisis, in as an additional criterion in the diagnosis of hemolytic anemias and β-thalassemias, as an independent prognosis factor in acute myeloid leukemia (in assessing the duration of remission and clinical outcome of the disease) in patients treated with purine analogs.

2. Conclusion

The analysis of the presented data allows revealing the definite correlation between functional fullness of immune cells and activity of 5´-nucleotidases. In our opinion, research of activity of the enzyme at various immunodeficiency conditions can serve not only as additional prognostic criteria but also be essential help both at tactics choice and at an estimation of efficiency of therapy with purine analogs.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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