ONCOLOGY

Changes in Bone Marrow and Peripheral Compartments of the Erythron under the Effect of Tumor Cells in Indolent Non-Hodgkin Lymphomas and Multiple Myeloma

E. E. Petrusenko, N. P. Domnikova, G. I. Nepomnyashchikh, and N. A. Mal'tseva

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Here we present the results of pathomorphological study of erythron cells from patients with indolent non-Hodgkin lymphomas and multiple myeloma. The revealed associations suggest that tumor cells can modulate the bone marrow and peripheral compartment of the erythron in lymphoproliferative diseases leading to quantitative and morphological changes in bone marrow erythrokaryocytes and peripheral blood erythrocytes. Clinical and pathomorphological markers of dyshemopoiesis associated with the presence of anemia were identified in indolent non-Hodgkin lymphomas. In multiple myeloma, a correlation between the parameters of bone marrow and peripheral compartments of the erythron and structural modifications of myeloma cells infiltrating the bone marrow was revealed.

Key Words: *non-Hodgkin lymphomas; multiple myeloma; erythron; dyserythropoiesis; anemia*

It is now established that anemia is a factor characterizing the course and prognosis of lymphoproliferative diseases. Structural and functional changes in the cell membrane, peculiarities of erythrocyte metabolism, iron metabolism, and hemoglobin synthesis in hemoblastoses are studied in detail [2,13]. The role of erythropoietin and other humoral factors in the regulation of proliferation, differentiation, and survival of erythron cells under normal conditions and in tumor diseases is now intensively studied [9,11,15]. Erythron is the total pool of erythroid cells of the organism, including nucleated bone marrow cells, bone marrow reticulocytes, blood reticulocytes, and mature erythrocytes [6].

Despite ample studies of the pathogenesis of anemia in hemoblastoses, morphological changes in cells of the erythron system and other hemopoietic stems are poorly studied [3,5,12]. Symptoms of dyserythropoiesis are usually studied as a component of secondary myelodysplastic syndrome developing against the background of chemotherapy and radiation therapy [8]. Pathomorphological changes in the erythron depending of the presence of bone marrow involvement and peculiarities of tumor cells infiltrating the bone marrow in lymphoproliferative diseases were never described.

Here we studied morphological changes in erythron cells in patients with indolent non-Hodgkin lymphomas and multiple myeloma depending on the pre-

Laboratory of Molecular, Cellular, and Immunomorphological Bases of Oncohematology, Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. E. E. Petrusenko

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sence of anemia syndrome and peculiarities of tumor involvement of the bone marrow.

MATERIALS AND METHODS

We examined 25 patients with indolent non-Hodgkin lymphomas (11 men and 14 women, mean age 58.42±2.44 years) and 28 patients with slowly progressing multiple myeloma (12 men and 16 women, mean age 65.96±2.00 years). The control group included 10 patients without anemia syndrome, in whom bone marrow biopsy specimens were taken for differential diagnosis of other diseases with hemoblastoses and hematological pathology was excluded after complex examination (6 men and 4 women, mean age 48.56±4.41 years).

The group of indolent lymphomas was presented by small-lymphocyte lymphoma and included also type I and type II follicular lymphomas, lymphoplasmacytic lymphoma, and extranodal MALT-lymphoma. Bone marrow involvement was found in 14 patients (56%) with non-Hodgkin lymphomas. Multiple myeloma ran a slowly progressing course (active myeloma) according to criteria proposed by E. I. Podol'tseva [4]. In all patients with multiple myeloma, the bone marrow was infiltrated with tumor plasma cells, their content in the myelogram surpassed 10%.

Lymphoma was diagnosed using REAL classification [10] and multiple myeloma was diagnosed according to criteria published elsewhere [14].

Differential blood cell count was studied using HEMOLUX 19 hematological analyzer. Pathomorphological study of the peripheral and bone marrow compartments of the erythron was performed using cytological methods. Smears of the peripheral blood and bone marrow aspirate stained by the method of Romanovskii–Giemsa served as the material for cytological analysis. The smears were analyzed for the presence of morphological dyserythropoiesis markers: anisocytosis and poikilocytosis of peripheral blood erythrocytes and abnormalities of bone marrow erythrokaryocytes. They included cytoplasmic and internuclear bridges, dissociation of nucleus and cytoplasm maturation, low hemoglobin content in the cytoplasm, megaloblastoid nuclei, bi- and multinuclear erythroid cells, nucleus fragmentation, basophilic punctuation, and Jolly bodies [1].

Smears of bone marrow aspirate were also used for detection of morphological signs of dysgranulocytopoiesis and dysmegakaryocytopoiesis. Signs of dysgranulocytopoiesis included hypersegmentation and pelgerization of neutrophil nuclei and hypergranulation of the cytoplasm. Signs of dysmegakaryocytopoiesis included changes in the number cell nuclei and cell size.

The data were processed statistically using SPSS 10.0 software. The variables were compared using Student *t* test and ANOVA.

RESULTS

In the control group, 60% patients had insignificant signs of dyserythropoiesis in bone marrow cells in the absence of anemia, morphological changes were detected only in the cytoplasm of few erythrokaryocytes (<3% cells). No nucleus abnormalities in erythroid cells were revealed. In the bone marrow, cells with signs of apoptosis constitute 0.60±0.22%. The number of megaloblasts in myelogram did not exceed 3% of the total number of erythroid cells (1.70±0.37%). In none patients of the control group, abnormalities of peripheral blood erythrocytes were revealed. It should be noted that signs of dyserythropoiesis (changes of the cytoplasm of bone marrow erythroid cells) can be found in the absence of hemoblastosis and anemic syndrome.

Changes in peripheral blood erythrocytes were found in 52% patients with non-Hodgkin lymphomas. Anisocytosis and poikilocytosis of erythrocytes were observed in 48 and 32% patients, respectively (Fig. 1, *a*).

Signs of dyserythropoiesis in the bone marrow were found in 86.4% patients of this group. Dissociation of nucleus and cytoplasm maturation, the presence of Jolly bodies in erythroid cells, low hemoglobin content in the cytoplasm, and intercellular bridges were most common findings in non-Hodgkin lymphomas (Table 1, Fig. 1, *b, c*). Morphological signs of nucleus pathology were detected in 35% and included the presence of multinuclear cells (Fig. 1, *d*), internuclear bridges, and nucleus fragmentation in normocytes. The mean number of megaloblasts considerably surpassed this parameter in the control group (p=0.039). It was found that the number of atypical lymphocytes in the bone marrow was higher in the presence of pathological changes in erythrokaryocyte nuclei (5.54±4.79%), than in the absence of morphological abnormalities of erythroid cell nuclei (0.46±0.23%, p=0.007). Pathology of erythrokaryocyte nuclei were in 100% cases associated with morphological abnormalities of peripheral blood erythrocytes. The number of erythroid cells with signs of apoptosis in non-Hodgkin lymphomas considerably surpassed the corresponding parameter in the control group (p=0.028). In 42.1% patients with non-Hodgkin lymphomas, dyserythropoiesis was associated with signs of dysgranulocytopoiesis, combined involvement of erythrocytic, granulocytic, and megakaryocytic bone marrow stems was found in 21.1% patients.

No differences in the incidence and severity of dyserythropoiesis were observed in patients with indolent lymphomas with and without bone marrow in-

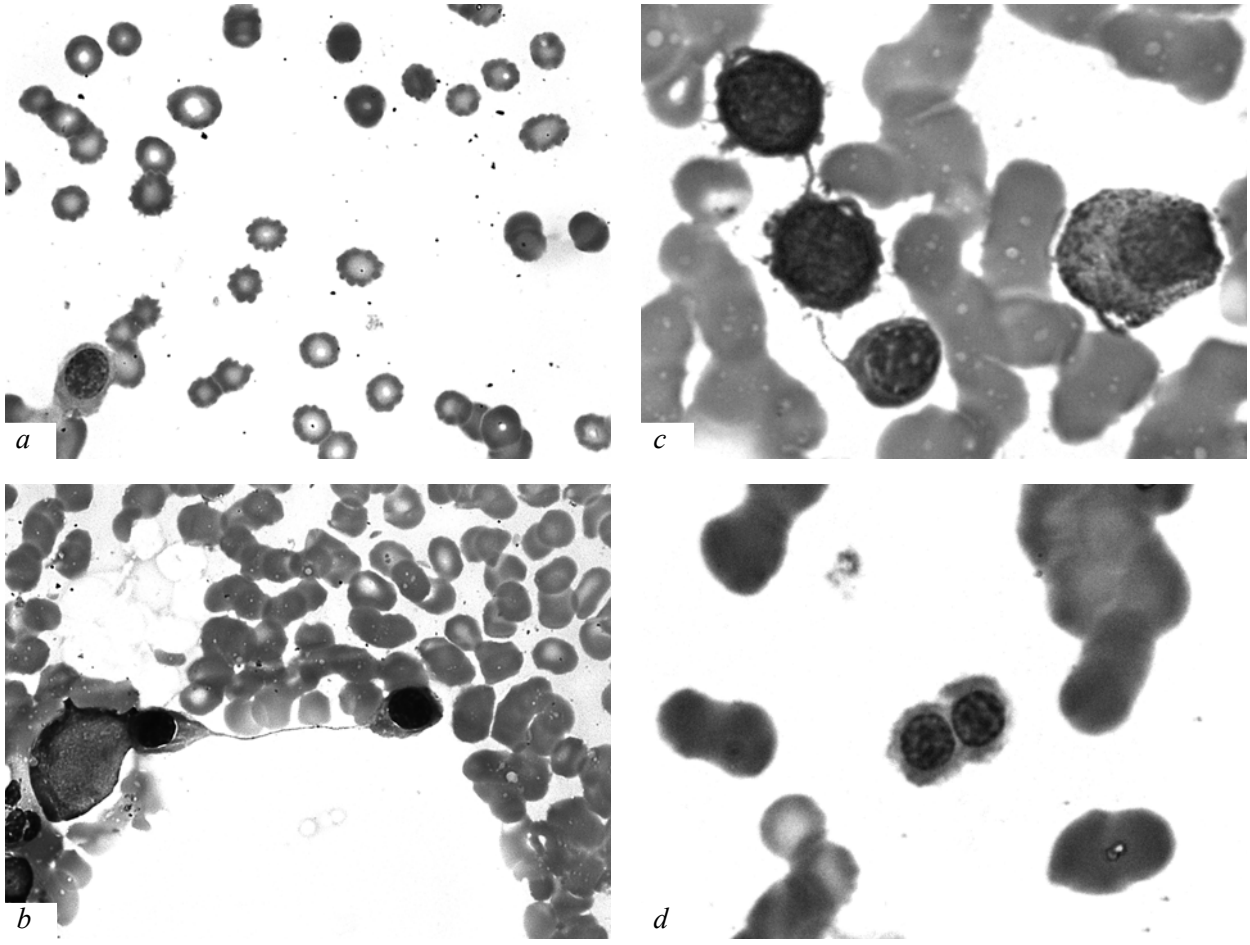

Fig. 1. Pathomorphological markers of dyserythropoiesis in indolent non-Hodgkin lymphomas. Smears of bone marrow aspirate. Romanovskii–Giemsa staining (×950). *a*) erythrocyte poikilocytosis. Basophilic megaloblast; *b*) cytoplasmic bridge between promegaloblast and basophilic normocyte; *c*) intercellular bridges between polychromatophilic normocytes with dissociation of nucleus and cytoplasm maturation and low hemoglobin content. Dysgranulocytopoiesis; *d*) binuclear polychromatophilic normocyte.

volvement. In the presence of bone marrow involvement, the degree of tumor infiltration also did not affect the incidence and severity of dyserythropoiesis.

Anemia (hemoglobin content <120 g/liter) against the background of dyserythropoiesis was diagnosed in 61% patients with non-Hodgkin lymphomas. Analysis of morphological changes in erythroid cells associated with the presence of anemia in this group showed that anemia more often developed in patients with combined morphological abnormalities of the nucleus and cytoplasm (in 71.4% cases), than in patients with morphological changes in the cytoplasm alone (46.2%). Moreover, anemia was associated with the presence of >3% meganoblasts in myelogram (OR=6.22, 95% CI 0.94-41.38; p=0.047) and was observed in 100% patients with dysplasia of the three hemopoietic stems (OR=1.60, 95% CI 0.94-2.74; p=0.034). Signs of dyserythropoiesis in the bone marrow were observed in 100% patients with non-Hodgkin lymphomas and anemia and in 70% patients with normal hemoglobin content (OR=2.57, 95% CI 1.44-4.58; p=0.050).

In patients with multiple myeloma, morphological changes in peripheral blood erythrocytes were found in 100% cases. Moderate anisocytosis of erythrocytes was most often seen; in 62.5% cases it was associated with poikilocytosis. In 50% patients, erythrocyte rouleau formation in peripheral blood smears was observed.

Morphological signs of dyserythropoiesis in the bone marrow were also detected in all patients with multiple myeloma. In 84.6% of them, dyserythropoiesis was associated with signs of dysgranulocytopoiesis, combined involvement of erythrocytic, granulocytic, and megakaryocytic bone marrow stems was observed in 50% patients. Jolly bodies in erythroid cells were found in 100% cases, dissociation of nucleus and cytoplasm maturation were noted in the majority of patients (Fig. 2, *a*). Low hemoglobin content in the cytoplasm (Fig. 2, *b*) and intercellular (cytoplasmic) bridges were often observed. Morphological signs of nucleus pathology were found in 42.3% patients (Fig. 2, *c, d*). The mean number of megaloblasts was

TABLE 1. Morphological Signs of Dyserythropoiesis in Indolent Non-Hodgkin Lymphomas (NHL) and Multiple Myeloma (MM)

Signs	Control group (*N*=10), %	Patients with NHL (*N*=25), %	Patients with MM (*N*=28), %
Abnormalities of peripheral blood erythrocytes	0	52	100
Signs of dyserythropoiesis in myelogram	60	86.4	100
Jolly bodies	60	81.8	100
Dissociation of nucleus and cytoplasm maturation	60	81.8	96.2
Intercellular bridges	60	42.9	65.4
Low hemoglobin content in the cytoplasm	40	69.2	68
Basophilic punctuation of the cytoplasm	0	23.1	13
Abnormalities of erythrokaryocyte nuclei	0	35	42.3
Bone marrow megaloblasts	1.70±0.37	12.43±5.31	7.82±1.81
Number of erythroid cells with signs of apoptosis in the bone marrow	0.60±0.22	7.04±2.60	3.55±1.17

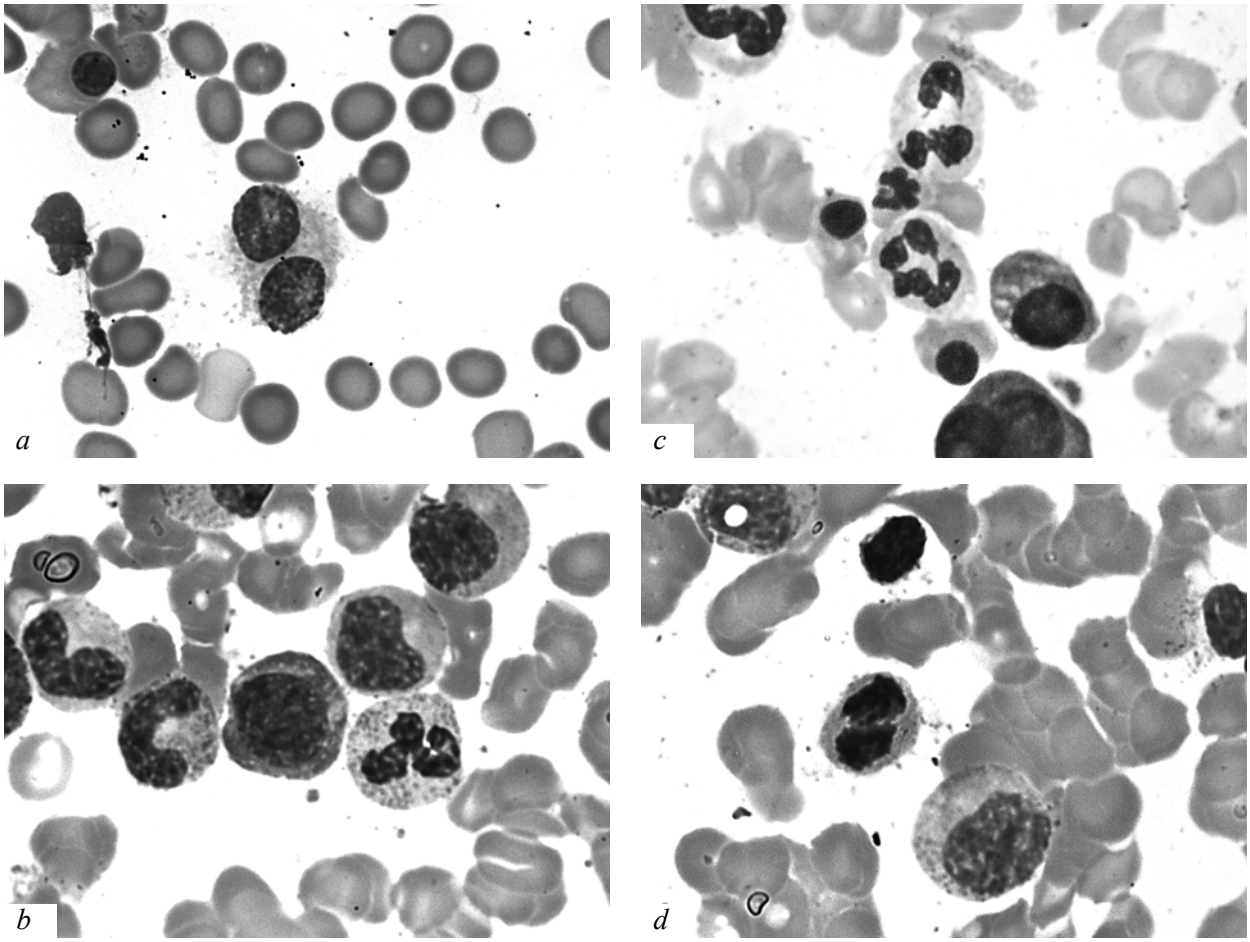

Fig. 2. Pathomorphological markers of dyserythropoiesis in multiple myeloma. Smears of bone marrow aspirates. Romanovskii–Giemsa staining (×950). *a*) binuclear plasma cell, polychromatophilic normocyte with dissociation of nucleus and cytoplasm maturation, Jolly bodies in erythrocytes; *b*) basophilic normocyte with low hemoglobin content in the cytoplasm; *c*) plasma cell with crystalline inclusions in the cytoplasm, normocyte with fragmented nucleus, dysgranulocytopoiesis; *d*) formation of binuclear normocyte at the stage of telophase, erythrocyte agglutination.

TABLE 2. Peripheral Erythron Parameters Depending on Morphology of Tumor Cells in Multiple Myeloma (*M*±*m*)

Morphological peculiarities of tumor plasma cells	Hemoglobin, g/liter	*p*	Erythrocytes, $\times 10^{12}$/liter	*p*
Medium size, giant forms				
absent	112.0±9.0	0.005	3.71±0.42	0.009
present	78.75±3.63		2.61±0.13	
Multinuclear forms				
absent	97.40±7.93	0.016	3.23±0.26	0.022
present	75.78±3.79		2.51±0.15	
Cells with signs of clasmatosis				
absent	103.67±10.68	0.021	3.37±0.41	0.042
present	78.27±3.94		2.60±0.14	
Accumulation of plasma cells in the bone marrow				
absent	97.00±7.00	0.004	3.25±0.24	0.003
present	73.38±2.70		2.41±0.89	

7.82±1.87% of the total number of erythroid cells in the bone marrow, which surpassed the corresponding parameters in the control group (p=0.046). The number of erythrokaryocytes with signs of apoptosis in the bone marrow increased compared to the control group (p=0.022; Table 1).

In multiple myeloma, the degree of morphological changes in erythroid cells of the bone marrow did not depend on peripheral erythron parameters (hemoglobin content and erythrocyte count).

A correlation between morphological type of tumor plasma cells and parameters of bone marrow and peripheral erythron compartments was found in patients of this group. For instance, a tendency towards more pronounced reduction of the erythroid bone marrow stem was detected in patients with predominance of immature plasma cells compared to patients in whom the tumor clone consisted primarily of mature plasmacytes (12.55±2.76 and 20.48±3.49% erythroid cells in myelogram, respectively, but the differences were insignificant, p=0.131). A dependence of the peripheral erythron parameters (hemoglobin content and erythrocyte count) on morphological peculiarities of tumor plasma cells was revealed. The mean hemoglobin content and erythrocyte count in the peripheral blood were significantly lower in patients with predominance of medium-size plasma cells with giant forms in the myelogram, in the presence of multinuclear forms, tumor cells with signs of clasmatosis, and accumulations of plasmacyte lineage cells in the bone marrow (Table 2).

The predominance medium-size tumor cells and the presence of giant forms in the myelogram of patients with multiple myeloma were associated with low hemoglobin content (<100 g/liter, OR=12.0, 95% CI 1.84-78.37; p=0.003). Clasmatosis of bone marrow plasma cells was associated with hemoglobin content below 80 g/liter (p=0.026). Moreover, pathology of erythrokaryocyte nuclei was more often seen in the presence of plasma cell clasmatosis (p=0.028).

Thus, the revealed associations suggest that tumor cells can modulate the bone marrow and peripheral compartment of the erythron in lymphoproliferative diseases leading to quantitative and morphological changes in bone marrow erythrokaryocytes and peripheral blood erythrocytes. Morphological abnormalities can appear in erythrokaryocyte cytoplasm alone, or can involve erythroid cell nuclei. In non-Hodgkin lymphomas, morphological changes in the nuclei of erythroid cells were associated with increased number of atypical lymphocytes in the bone marrow and increased risk of anemia development.

It is known that morphology of tumor cells in multiple myeloma reflects the degree of malignancy and affects the prognosis of the disease [7]. Here we showed that morphological peculiarities of the tumor clone can be a marker associated with peripheral erythron changes and severity of anemia in multiple myeloma. In both groups, the number of erythroid cells with signs of apoptosis in the bone marrow significantly increased compared to the control group, which can be regarded as death of morphologically changed and, probably, functionally incompetent cells of the erythron and manifestation of inefficient erythropoiesis. Dyserythropoiesis in both groups was often associated with morphological signs of dysgranulo- and

dysmegakaryocytopoiesis. More pronounced signs of dyserythropoiesis in patients with multiple myeloma were more often associated with abnormalities of other bone marrow stems. This attests to possible interrelation of hemopoietic stems under conditions of lymphoproliferative disease.

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