THE CELL BODY SPACE OCCUPIED BY THE NUCLEUS DURING THE CELL DIFFERENTIATION IN HUMAN LYPHOCYTIC, GRANULOCYTIC AND ERYTHROID CELL LINEAGES

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Summary. **The present nuclear and cell body diameter measurements demonstrated size differences of the approximate cell space estimate occupied by the cell nucleus during the cell differentiation in lymphocytic, granulocytic and erythroid cell lineages. These lineages were used as convenient models because all differentiation steps were easily identified and accessible in diagnostic peripheral blood or bone marrow smears of blood donors (BDs), patients suffering from chronic lymphocytic leukemia (CLL), patients with chronic myeloid leukemia (CML) and refractory anemia (RA) of the myelodysplastic syndrome (MDS). The cell space occupied by the nucleus was constant and did not change during the cell differentiation in the lymphocytic cell lineages of BDs and CLL patients despite the decreased cell size. In contrary, the cell space occupied by the nucleus markedly decreased in differentiating cells of granulocytic and erythroid lineages of patients suffering from CML. In the erythroid cell lineage in patients with RA of MDS the small reduction of the cell space occupied by the nucleus during the differentiation was not significant. The measurements also indicated that in progenitor cells of all studied cell lineages nuclei occupied more than 70 percent of the cell space. Thus, the nucleus-cytoplasmic morphological and functional equilibrium appeared to be characteristic for each differentiation step and each specific cell lineage.**

Key Words: Nucleus, cell space, cell differentiation

Introduction

The morphological relationship of the nucleus and cytoplasm was a subject of a great interest since the end of the 19th and onset of the last century **(Hertwig R 1903, Sharp 1921)**. This relationship was expressed by the "Kern-Plasma-Relation", i.e. nuclear-cytoplasmic index **(Hertwig R 1903, Hertwig G 1929, Ries and Gersch 1953)**. The changing equilibrium of both these main cell compartments was considered to precede the cell division. Then, the changing proportion of the nucleus in the cell body was apparent in the course of the cell differentiation and maturation or aging **(Sharp 1921, Hertwig G 1929, Bessis 1973)**. Concerning the calculation of the nuclear - cytoplasmic ratio or index there are several calculation formulas depending on the specimen preparations and field of interest. In basic research or classical cytology, the nuclear-cytoplasmic index was calculated by dividing the nuclear volume by the cytoplasmic volume subtracted from the nuclear one (Robertis de and Robertis de jun 1987). In the clinical and hematological cytology, the nucleus to cell ratio was calculated dividing the nuclear size estimate by the cell size. Such simple calculation appeared to be very useful in both light and electron microscopy if the number of measured cells was adequate to reduce the variability **(Ochiai and Eguchi 1987, Doughty 2012).** It should be added that in the clinical cytology or histology the simple nuclear to cytoplasmic ratio appeared to be a very important tool to estimate the cell proliferation potential and cell malignancy **(Cardozo 1954)**. In addition, the nuclear size related to the cell body might be also useful to detect further cell abnormalities or pathology in various cell lineages including hematological disorders. At his occasion it should be also mentioned that the nucleus to cell body ratio multiplied by 100 just reflected the rough cell space estimate occupied by the nucleus.

Since it was generally believed that the cell differentiation and maturation is accompanied by the reduction of the nuclear size in the cell space, the present study is dealing with the nucleus to cell body ratio (Nu/CBR) in differentiation steps of human lymphocytic, granulocytic and erythroid cell lineages. These cell lineages appeared to be very convenient models because differentiation steps of non-leukemic or leukemic lymphocytic lineages were easily recognizable and accessible in diagnostic peripheral blood smears of blood donors (BDs) and patients with chronic lymphocytic leukemia (CLL). The differentiation steps were also easily identified and accessible in diagnostic bone marrow smears of selected patients with chronic myeloid leukemia

(CML) and refractory anemia (RA) of the myelodysplastic syndrome (MDS). The morphology of differentiation steps of lymphocytes in BDs and CLL patients was similar and mature or terminally differentiated lymphocytes of T or B lymphocytic lineages possessed the same types of nucleoli (**Smetana *et al* 1980, 2020**). Similarly, the morphology of differentiation steps of neutrophils with characteristic nucleoli of CML patients exhibited a remarkable similarity to those in non-leukemic persons **(Cline 1975, Smetana *et al.* 2019).** In addition, the bone marrow of CML patients also possessed the non-leukemic erythroid lineage with all differentiation steps. The bone marrow of patients suffering from RA of MDS contained all differentiation steps of the erythroid lineage including the abnormal ones **(Bessis 1973, Cazzola and Malcovati 2005)**. It should be mentioned that all erythroid nucleated differentiation steps also possessed characteristic nucleolar bodies **(Smetana *et al.* 1975)**. The results based on nuclear and cell body diameter measurements in single cells clearly demonstrated that the cell space occupied by the nucleus accompanying the cell differentiation differed in studied cell lineages bur appeared to be characteristic for each differentiation step.

Material and methods

Nu/CBR **(Ochiai and Eguchi 1987)** during the cell differentiation was studied in lymphocytic, granulocytic and erythroid cell lineages of 4 blood donors, 4 patients suffering from CLL, 4 patients with CML and 4 patients with RA of MDS. Lymphocytic cell lineages were studied in peripheral blood smears, granulocytic (neutrophilic) and erythroid cell lineages were studied in bone marrow smears. All peripheral blood and bone marrow samples were originally taken for diagnostic purposes with the approval of the Institute authorities. Since blood smears of blood donors did not contain lymphocytic progenitors or a satisfactory number of immature lymphocytes for measurements, these cells were replaced by stimulated and de-differentiated T-lymphocytes with phytohemagglutinin *in vitro* **(Kalousek and Křížková 2000, Smetana *et al.* 2020).** To get such cells, isolated lymphocytes of the peripheral blood of BDs were cultured for 48 hrs. in RPMI 1640 medium with inactivated 10% FBS and phytohemagglutinin (10μg/mL-Sigma) at 37°C in 5% atmosphere with CO_2. For microscopy, cytospins of these cells were prepared using the cytocentrifuge Shandon Cytospin 2 (Shandon Southern Products UK).

The largest diameter of nuclear and cell body outlines was measured in micrograph of specimens stained for RNA **(Smetana et *al.* 1969, Ochs 1998).** Such procedure facilitated to

visualize nuclear outlines together with the classification of nucleoli (Fig, 1). The nucleolar classification was helpful to distinguish advanced and terminal differentiation steps of studied cell lineages **(Smetana 2002, Smetana *et al.* 2019)**. The largest nuclear and cell body diameter was measured in each differentiation step of studied cell lineages because such simple measurements appeared to provide satisfactory and useful results **(Tseleni et *al*. 1997, Monge et *al.* 1999, Politi et *al.* 2003, Hong *et al.* 2012).** Digitized micrographs captured with a Camedia digital camera C4040 ZOOM (Olympus, Japan) placed on Jenalumar microscope (Zeiss, Germany) were magnified and further processed using Quick Computer Photoprogram (Olympus, Japan). Nucleus to Cell Body Ratio (Nu/CBR) was calculated by dividing the largest nuclear by the largest cell body diameter for each measured cell. Nu/CBR multiplied by 100 estimated the approximate size – proportion – of the cell body space occupied by the nucleus. The results of all measurements and calculations at the single cell level such as mean and standard deviation followed by the t-test were evaluated using Primer of Biostatistic Program, version 1 developed by S.A. Glantz (McGraw-Hill, Canada, 1968).

Results

Lymphocytic lineages (Graph 1)

Blood donors. Lymphocytic progenitors originated by the de-differentiation of T lymphocytes of blood donors **(Smetana *et al.* 2020)** were characterized by a large nucleus that occupied about 70 per cent of the cell space (Tab. 1). Differentiated mature lymphocytes with ring shaped nucleoli still possessed large nuclei, which also occupied more than 70 per cent of the cell space similarly as progenitors (Tab. 1). However, the cell size of these lymphocytes was markedly smaller (Tab. 1). The cell size of terminally differentiated lymphocytes with micronucleoli was also small but more than 70 per cent of the cell space was still occupied by the cell nucleus similarly as in previous developmental steps. (Tab. 1). At this occasion it should be mentioned that most of lymphocytes in the peripheral blood of blood donors are represented by the T cell lymphocytic lineage.

CLL patients. More than 70 percent of the cell space in all differentiation steps of lymphocytes was occupied by the cell nucleus. On the other hand, the cell size of mature lymphocytes with ring shaped and terminal lymphocytes with micronucleoli was significantly smaller in

comparison with lymphocytic progenitors - lymphoblasts (Tab. 1). At this occasion it should be also noted that lymphocytes of CLL patients mostly belong to the B cell lymphocytic lineage.

Granulocytic (neutrophilic) and erythroid cell lineages (Graph 2, 3)

The granulocytic lineage in CML patients. Granulocytic progenitors, i.e., myeloblasts were characterized by a large nucleus that occupied more than 70 percent of the cell space (Tab. 1). The further differentiation steps – promyelocytes were characterized by a slightly smaller nuclear size in the less enlarged cell space (Tab. 1). The last step of the proliferating pool of differentiating granulocytic – neutrophilic cells such as myelocytes possessed a significantly smaller nucleus that occupied the cell space. (Tab. 1).

The erythroid cell lineage in CML patients. Erythroid progenitors, i.e., proerythroblasts possessed a large nucleus that occupied more than 70 per cent of the cell space (Tab. 1). Nuclei in further differentiation steps comprised significantly smaller portion of the cell space. However, such change was not significant due to the large variation coefficient (about 11 per cent). The largest nuclear size reduction in the cell space was noted in advanced and terminally differentiated nuclear stages of smallest erythroblasts (Table 1).

The erythroid cell lineages in patients suffering from RA of MDS. The size and morphology of erythroid progenitors and nucleated precursors exhibited signs of normoblastic and macroblastic or megaloblastic hematopoiesis **(Bessis 1973, Cazzola and Malcovati 2005)**. Such variable size reflected a large variation coefficient of early and late erythroblasts that reached almost 20 per cent. Nevertheless, nuclei in progenitors, i.e., proerythroblasts or promegaloblasts occupied more than 70 per cent of the cell space (Table 1). However, the reduction of the cell space occupied by the nucleus was less apparent. The cell size reduction was significant only in intermediate and terminally differentiated cells (Table 1).

Discussion

Since the onset of the last century, it is generally accepted that nuclear size is generally proportional to that of the cytoplasm and reflects the equilibrium of these both main cell constituents. The breakdown of such equilibrium results in a variety of events including the mitotic division or terminal differentiation (**Sharp 1921, Hertwig G. 1929, Bessis 1973,**

Robertis de and Robertis de jun. 1987). According to the present note, this equilibrium of the cell nucleus and cytoplasm apparently differed during the cell differentiation of various studied cell lineages but was characteristic for each differentiation step. On the other hand, regardless of studied cell lineages, progenitor cells of were characterized by the large cell nucleus which occupied about 70 per cent of the cell space. Such large and predominant nuclear size is not surprising because the narrow rim of the cytoplasm does not possess specific structural components characteristic for further differentiation steps (Bessis 1973). In addition, progenitor cells possibly represent early differentiating unipotent stem cells recognizable by current visualization procedures and asymmetric cell division **(Gomez-Lopez *et al.* 2014)**. The asymmetric division of granulocytic and erythroid progenitors has been also reported previously (Likovský and Smetana 1995).

The similarity of Nu/CBR in lymphocytic progenitors and "mature" differentiated lymphocytes might indicate a surprising equilibrium between the nucleus and cytoplasm in both T (BDs) and B (CLL patients) cell lineages despite the cell body size reduction. At this occasion it should be mentioned that mature cells of both these lineages possessed ring shaped nucleolus **(Smetana *et al.* 1980, 2020)** that reflected the sleeping state with the de-differentiation potential (**Astaldi and Lisiewicz 1971, Greaves *et al.* 1974, Smetana 2002, Smetana *et al.* 2020).** The large nuclear to cytoplasmic ratio in various cells related to the cell division potential was noted already at the onset of the last century **(Hertwig R. 1903, Hertwig G. 1929, Sharp 1921, Ries and Gersch 1953).** On the other hand, it was surprising that the terminal steps of lymphocytes in BDs as well as CLL patients also exhibited a large Nu/CBR. However, micronucleoli in these cells indicated the irreversible cessation of the nucleolar RNA transcription characteristic for the terminal differentiation **(Smetana 2002, Smetana *et al.* 2020**).

In the granulocytic cell lineage represented by neutrophils of CML patients, the cell space occupied by the cell nucleus was reflected by the decreasing Nu/CBR. The terminal step of the granulocytic proliferation compartment and cell differentiation (**Cline 1975**) was characterized by a significantly smallest cell space occupied by the nucleus. It should be added that this step was preceding the cell maturation, i.e., terminal differentiation and possessed micronucleoli similarly as terminal differentiation steps of the lymphocytic lineages **(Smetana *et al.* 2019, 2020)**.

In the non-leukemic erythroid lineage of patients with CML there was a fluent reduction of the Nu/CBR reflecting the decreasing cell space occupied by the nucleus. Sub-terminal and terminal steps of this lineage were characterized by the small cell space occupied by the nucleus that possessed only micronucleoli as the only one nucleolar type **(Smetana *et al.* 1975**). It was not surprising that in RA of MDS the reduction of the cell space containing the nucleus was less apparent. and statistically not significant. The ineffective erythropoiesis in this blood disorder possessed not only normoblastic cell lineage but also macro- and megaloblastoid erythroblasts **(Bessis 1973, Cazzola and Malcovati 2005)**. In late megaloblastoid erythroblasts the nucleus still occupied a large space of the cell body, such as 69.5±9.4 per cent (additional data), that was not substantially different from erythroid progenitors (Table 1).

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Competing interests

The authors declared that no competing interests exist.

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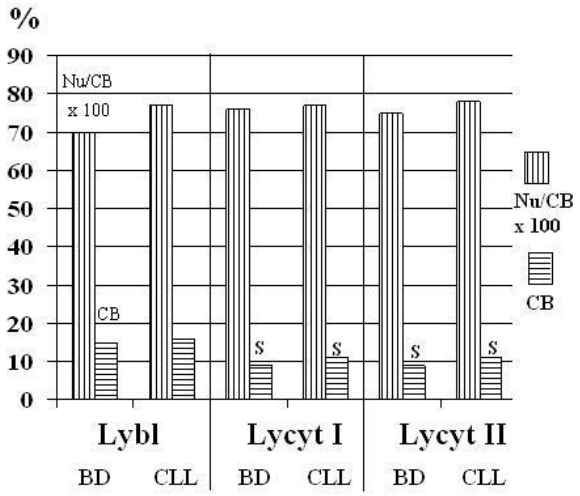
Table 1. The approximate cell space estimate occupied by the cell nucleus (expressed by the nucleus to cell body maximal diameter ratio multiplied by 100) during the differentiation of human lymphocytes, neutrophils and erythroblasts*

Cells	**LNu/LCB**	**Cell LDm**	**Nucleoli**	**Persons**
Dm x 100	(μm)	(μm)	(μm)	Dg
Lybl (stim)#	70.0±7.2	15.3±1.2	>1.2	BD
Lybl	***77,1±1.5***	***16.2±1.0***	***>1.2***	***CLL***
Lycyt I	76.9±1.0	9.2±1.0§	<1.2	BD
Lycyt I	***77.9±0.6 (101,0%)***	***11.7±1.3§ (72,2%)***	***<1.2***	***CLL***
Lycyt II	75.4±3,8§	9.7±0.8§	<1.2	BD
Lycyt II	***78.3±1,1§ (101.5%)***	***11.0±0.7§ (67.9%)***	***<1.2***	***CLL***
Mybl	***77.6±2.0***	***16.7±1,7***	***>1.2***	***CML***
Promyelo	***71.8±8.0 (92.5%)***	***17.5±1.5 (104.7%)***	***>1.2***	
Myelo	***61.6±3.9§(79.3%)***	***15.8±1.4 (94.6%)***	***<1.2***	
Proery	78.1±5.9	13.6±2.0	>1.2	
Baso	67.7±2.2§ (86.6%)	14.3±1,8 (105.1%)	>1.2	
Early+Late Ebl	59.1±1.5§ (75.6%)	10,0±0.1§ (73.5%)	<1.2	
Proery	72.6±7.5	17.1±0.1	>1.2	RA MDS
Baso Ebl	71.8±2.0 (98.8%)	15.7±3.1 (84.7%)	>1.2	
Early+Late Ebl	66.8±1.2 (92.0%)	13.5±1,2§(73.0%)	<1.2	

Legend to the Table 1

* - Mean and standard deviation of measurement results in each group of 4 blood donors (BD), 4 patients suffering from chronic lymphocytic leukemia (***CLL***), 4 patients with chronic myeloid leukemia (***CML***) and 4 patients with refractory anemia of the myelodysplastic syndrome (RA MDS) based on at least 100 measurements in each differentiation stage of studied cell lineages except the smaller number of measurements in progenitors (lymphoblasts, myeloblasts, proerythroblasts) the incidence of which was less frequent.. The lymphocyte lineages were studied in the peripheral blood smears, granulocyte (neutrophils) and erythroid cell lineages were studied in bone marrow smears. # - Lymphoblasts stimulated with PHA from the peripheral blood of blood donors were studied in the intermediate layer of cytospins. § - Significant difference in comparison with progenitor cells such as lymphoblasts, myeloblasts and proerythroblasts t-test ((2α=0.05). Bold lettering - leukemic cells. Lymphocytes I - mature differentiated lymphocytes with ring shaped nucleoli indicating the reversible decrease of the RNA transcription. Lymphocytes II - terminally differentiated lymphocytes with micronucleoli characterized by the cessation of the RNA transcription. Early+Late Ebl - differentiated + terminally differentiated erythroblasts such as polychromatic and orthochromatic erythroblasts. Numbers in brackets represent percentage of Nu/CBR x 100, i.e. approximate percentage of the cell space occupied by the nucleus in comparison with progenitor cells.

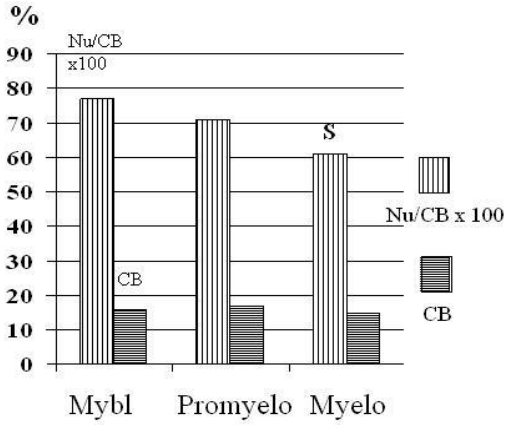
Graph 1



Legend to the Graph 1

Graph 1. Cell body space occupied by the nucleus and largest diameter of the cell body in lymphocytic lineages of blood donors and patients suffering from chronic lymphocytic leukemia. Lybl – progenitor cell, Lycyt I – mature lymphocyte with ring shaped nucleolus, Lycyt II – terminal lymphocyte with micronucleoli. BD – blood donors, CLL – patients with chronic lymphocytic leukemia, CB – largest cell body diameter in μm, S – significant change in comparison with progenitors, % - percentage of the cell body occupied by the nucleus based on. NuCBR x 100. For other legend, mean values and standard deviation see Table 1.

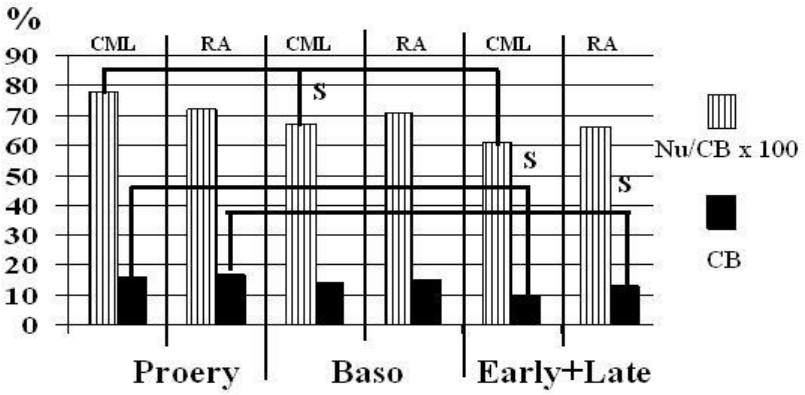
Graph 2. The granulocyte (neutrophil) lineage



Legend to the Graph 2

Graph 2. Cell body space occupied by the nucleus and largest cell body diameter in the granulocytic (neutrophil) lineage. Mybl – progenitor cell myeloblast, Promyelo – promyelocyte, i.e. further differentiation step, Myelo – myelocyte, i.e. last differentiation step of the granulocytic proliferation compartment. For other legend, mean values and standard deviations see Graph 1 and Table 1.

Table 3. The erythroid cell lineage



Legend to the Graph 3

Graph 3. Cell body space occupied by the nucleus and largest cell body diameter in erythroid lineages. CML – chronic myeloid leukemia, RA – refractory anemia of MDS, Proery – erythroid progenitor cell proerythroblast, Baso – further differentiation step basophilic eythroblast, Early+Late – preterminal and terminal differentiation steps of erythroblasts. For other legend, mean values and standard deviations see Graph 1 and Table 1.

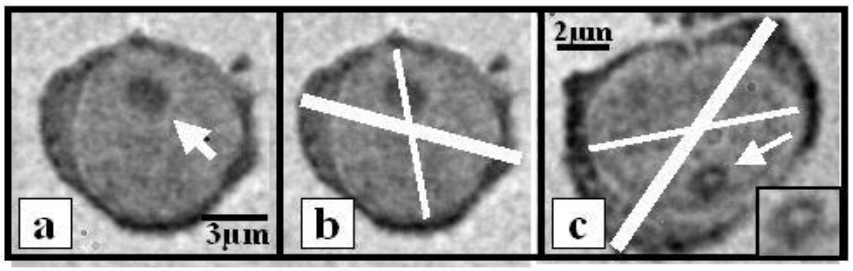


Fig.1. a, b - Leukemic lymphoblast - lymphocytic progenitor cell - with a large nucleolus (arrow) and c - mature differentiated lymphocyte with a ring shaped nucleolus (arrow and insert). Bold (cell body) and thin (nuclear) lines indicate largest diameter measurements. In the lymhocytic progenitor: Nu/CBR x 100 = 78.6 % of the cell space. In the mature lymhocyte with the ring shaped nucleolus: NuCBR x 100 = 77.3 % of the cell space.