Short Communication

The RNA Content of Nucleolar Bodies Is Related to Their Size – a Cytochemical Study on Human Monocytes and Lymphocytes in Blood Smears and Blood Cytospins

(nucleolar bodies / size and RNA density / human monocytes)

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Abstract. The present study was undertaken to provide more information on the relationship between the nucleolar size and RNA density. Mature monocytes circulating in human peripheral blood appeared to be very convenient for such study because they contain multiple nucleoli of various sizes in one and the same nucleus. In addition, nucleoli without perinucleolar chromatin represented by nucleolar bodies are easy to be visualized by a simple cytochemical procedure for RNA demonstration. The diameter and density of NoBs in specimens stained for RNA were determined by computer-assisted measurements of individual cells. According to the results, the nucleolar RNA content was apparently related to the nucleolar size because the RNA density of small and large NoBs was practically the same. In addition, the diameter measurements also indicated that one or two of several NoBs in one nucleus were dominant – larger – than the others. It should also be mentioned that the diameter and RNA density of NoBs were studied in monocytes in peripheral blood smears regardless of their limited number. Additional study on lymphocytes indicated that the preparation procedure of smears modified both diameter and density of the measured parameters less than the preparation of cytospins as demonstrated by lower variability of the resulting values. Thus, the observed differences between smears and cytospins also indicated that the specimen preparation procedures should always be considered during evaluation of the nucleolar size or staining intensity of nucleoli or cytoplasm due to the presence of RNA.

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Abbreviation: NoB – nucleolar body.

Introduction

Recent cytochemical studies of our laboratory suggested that the amount of RNA in the nucleolar body was rather related to the nucleolar size than to the concentration of this nucleic acid. In leukaemia granulocytic progenitors the RNA density appeared to be the same in both large and small nucleoli (Smetana et al., 2006). In stimulated lymphocytes during blastic transformation, the nucleolar size markedly increased in comparison with the nucleolar RNA density (Smetana et al., 2008a). The present study was thus undertaken to provide more information on the relationship between the nucleolar size and RNA density. Mature monocytes circulating in human peripheral blood appeared to be very convenient for such study because they contain multiple nucleoli of various sizes in one and the same nucleus (Smetana et al., 1997). In addition, nucleoli without perinucleolar chromatin represented by nucleolar bodies (NoBs) are easy to be visualized by a simple cytochemical procedure for RNA demonstration. The size and density reflecting RNA concentration in specimens stained for RNA were determined by computer-assisted measurements of individual cells. On the other hand, the number of monocytes in the peripheral blood of healthy blood donors in smear preparations is very limited (Undritz, 1972). Therefore, in an additional study, the diameter and RNA density of NoBs were measured not only in peripheral blood smears, but also in cytospins. Cytospins are known to facilitate study of a larger number of nucleated blood cells than smears. Lymphocytes appeared to be more convenient for such study because they are more frequent in peripheral blood than monocytes.

The results clearly demonstrated that the diameter and RNA density of NoBs in smears and cytospins differed and thus depended on the specimen preparation procedure. It should be added that the preparation of smears modified both nucleolar diameter and RNA density less, as demonstrated by lower variability. Therefore, NoBs of monocytes were studied only in blood smears regardless of the limited number of these cells. According to the results, the nucleolar RNA con-

tent appeared to be mainly related to the nucleolar size because the RNA density of small and large NoBs was practically the same. In addition, the diameter measurements also indicated that one or two of several NoBs in one monocyte were dominant – larger – than the others. It should also be mentioned that the cytoplasmic RNA density of monocytes was lower than in lymphocytes.

Material and Methods

Monocytes were studied in venous peripheral blood smears of six healthy blood donors. NoBs were visualized by a simple cytochemical procedure for demonstration of RNA using acidified methylene blue at pH 5.3 as described previously (Smetana et al., 1969; Ochs, 1998). The diameter and RNA density of NoBs in both monocytes and lymphocytes were measured in thin monolayer films of blood smears selected for optimal morphological evaluation according to Undritz (1972). For preparation of cytospins, human lymphocytes were isolated from the peripheral blood of healthy volunteers using a discontinuous density gradient of Histopaque (Sigma Chemical Co., St. Louis, MO) according to Böyum (1968). Isolated cells (1 x 10^6/ml), suspended in RPMI 1640 medium and supplemented with 10% heat-inactivated foetal bovine serum (FBS, BioWhittaker, Walkersville, MD), were prepared for light microscopic observations using a Shandon Cytospin 2 cytocentrifuge (Shandon Southern Products, Chesire, UK) at 800 rpm for 10 min (Kalousek and Krizkova, 2000).

Micrographs were captured with a Camedia digital photo camera C.4040 ZOOM (Olympus, Tokyo, Japan) placed on a Jenalumar microscope (Carl Zeiss Microimaging, GmbH, Göttingen, Germany) equipped with a double adapter to provide larger magnification of resulting images on the computer screen. The images were then processed and the diameter of NoBs (Figs. 1, 2) was measured with Quick Photoprogram (Olympus).

The density of NoBs and cytoplasm in specimens stained for RNA was measured after image conversion to grey scale using the NIH Image Program – Scion for Windows (Scion Corp., Frederick, MD, see Figs. 1, 2). In contrast to earlier studies, the RNA density was expressed in relative arbitrary units calculated by subtracting maximal measured density of NoBs or cytoplasm from the minimal background density surrounding the measured cell. Such calculation and standardization of arbitrary density units facilitated the comparison of results in various portions of smears and especially in cytospins, which occasionally exhibited different artificial densities due to both preparation and staining techniques. This approach decreased artificial measurements and thus provided better results than the background adjusted to zero, which depended on the investigator.

Results and Discussion

Comparison of the nucleolar diameter and density of lymphocytes in smears and cytospins (for quantitative data see Table 1)

Nucleoli in circulating blood lymphocytes were mostly ring-shaped (Busch and Smetana, 1970). As was expected, their diameter in cytospins was apparently smaller than in smears. In contrast, the nucleolar RNA density (NoDn) of lymphocytes in cytospin preparations appeared to be larger. However, the difference was not significant, probably because of the large variability. Similarly, the mean cytoplasmic RNA density (CyDn) also appeared larger in cytospins than in smears, but the difference was marginally significant. On the other hand, the nucleolar to cytoplasmic RNA density ratio in lymphocytes was practically the same in both smears and cytospins. It should also be mentioned that the measured values of both nucleolar and cytoplasmic RNA density in cytospins were characterized by large variability coefficients such as 30 and 24 % (see also standard deviations) because of the cell distribution and occasional aggregation even at the periphery. In contrast, in the optimal region of smears for the morphological cell evaluation (see Undritz, 1972) the variability of measured values of the nucleolar and cytoplasmic RNA density in smears was low, 7 and 5 %, as was also demonstrated by low standard deviations. The nucleolar RNA density was larger than that of the cytoplasm (Fig. 1) and the

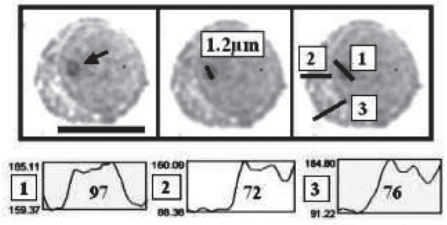

Fig. 1. A lymphocyte in the blood smear stained for RNA. Black lines represent measured diameter and RNA density of a nucleolar body (1) or cytoplasm (2,3). The value of the diameter is in the rectangle and values of calculated arbitrary units within densitographs. The thick black line represents 5 µm.

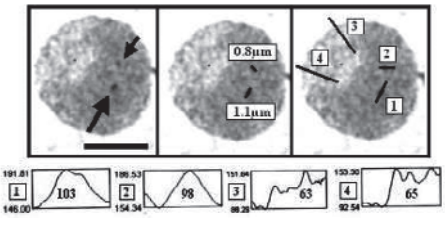

Fig. 2. A monocyte in the blood smear stained for RNA. Black lines represent measured diameters of large and small nucleoli with values in rectangles. Values of calculated RNA density units of a measured large (1) and small (2) nucleolar body or cytoplasm (3, 4) are within densitographs. The thick black line represents 5 µm.

Table 1. Nucleolar diameter, nucleolar, cytoplasmic RNA density in monocytes† and lymphocytes‡

Cells	NoD	NoDn	CyDn	No/Cy Dn
Monocytes (SMEARS) NoBs < 1 μm	**0.87** ± 0.01[a]	**87.0** ± 6.2	**63.1** ± 9.1[**]	1.37
> 1 μm	**1.03** ± 0.03[*]	**91.5** ± 5.4		1.45
Lymphocytes (SMEARS)	**1.26** ± 0.07	**95.2** ± 7.7	**84.9** ± 5.5	1.12
(CYTOSPINS)	**1.02** ± 0.03[#]	**105.4** ± 32.3	**93.1** ± 23.4[##]	1.13

Legend
† – based on values of six blood donors
‡ – based on 160 measurements of one healthy volunteer
[*] – significantly different from micronucleoli (< 1 μm) using t-test (P < 0.001)
[**] – significantly different from NoDn using *t*-test (P < 0.000); measured in monocytes regardless of the nucleolar diameter; significantly different from CyDn of lymphocytes measured in smears
[#] – significantly different from smears using *t*-test (P < 0.001)
[##] – marginally different from smears using *t*-test (P = 0.05)
[a] – **mean** and standard deviation
NoD – diameter of NoBs in μm
NoDn – nucleolar RNA density in arbitrary units – see Material and Methods
CyDn – cytoplasmic RNA density in arbitrary units – see Material and Methods

ratio of the nucleolar to cytoplasmic RNA density was about 1.1.

Nucleolar diameter, nucleolar RNA density and cytoplasmic density of monocytes (for quantitative data see Table 1)

Similarly as previously published, monocytes mostly possessed several micronucleoli (Smetana et al., 1997) and the mean diameter of all NoBs per one cell was below 1 μm (0.90 ± 0.02 μm). However, the diameter of one or two NoBs in each cell was larger, i.e. slightly above, and the rest of them below that value. The measured RNA density of NoBs was always significantly larger than that of the cytoplasm, regardless of the nucleolar diameter. The ratio of the nucleolar to cytoplasmic density was about 1.4. The RNA density of large and small NoBs was very similar (Fig. 2) and the measured values were not significantly different.

From the methodological point of view it seems to be clear that the diameter of NoBs of lymphocytes in cytospins is smaller than in smears. Thus, the resulting staining of NoBs for RNA in cytospins appeared to be more intense and was reflected by higher values of RNA density. Such difference, however, was not markedly significant because of a large variability of values measured in cytospins. On the other hand, the observed differences indicated that the specimen preparation procedures such as smears or cytospins should always be considered during evaluation of the nucleolar size or staining intensity of nucleoli or cytoplasm due to the presence of RNA.

Concerning the diameter and RNA density of NoB bodies, the computer-assisted measurements demonstrated that the nucleolar RNA content was apparently related to the nucleolar size. The diameter differences between "small" and "large" nucleoli were significant in contrast to nucleolar RNA density values, which were very similar. Such findings were in harmony with previous studies on leukaemic myeloblasts or stimulated T lymphocytes, which also suggested that the nucleolar RNA content might be related to the nucleolar size (Smetana et al., 2006; 2008a,b). On this occasion it should also be noted that NoBs in mature monocytes were mostly small (Smetana et al., 1997), i.e. smaller than 1 μm, and only few nucleoli were larger and more prominent.

In contrast to lymphocytes, the RNA density of the cytoplasm of mature monocytes was markedly lower regardless of the nucleolar diameter. Thus, the ratio of the nucleolar to RNA cytoplasmic density was higher. Since a similar phenomenon was noted in apoptotic myeloblasts (Smetana et al., 2008a), it seems to be likely that mature blood monocytes are in the state of terminal differentiation. This speculation is also supported by previous observations according to which micronucleoli, mostly present in monocytes (Smetana et al., 1997), are characteristic for pre-apoptotic cells (Biggiogera et al., 2004). Moreover, it must be added that stimulation of mature monocytes to the cycling state, i.e. to proliferation, was not successful (Schedle et al., 1992; Schwarzacher and Wachtler, 1993).

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