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A CONTRIBUTION TO THE INCIDENCE OF NUCLEOLI IN NORMAL HUMAN BLOOD MONOCYTES

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ABSTRACT

Background and Objective. Normal blood monocytes were studied in blood donors to provide more information on the presence of nucleoli and nucleolar types in these cells.

Methods. Nucleoli in monocytes were visualized using cytochemical procedures that detect RNA and characteristic nucleolar proteins, i.e. nucleophosmin, nucleolin, fibrillarin and AgNOR proteins, in peripheral blood smears.

Results. Nucleoli were detected in all blood monocytes. The nucleolar coefficient (number of nucleoli per cell) was 2.6 and no differences were found between men and women. Concerning the incidence of nucleolar types, monocytes from both male and female blood donors possessed mainly only inactive micronucleoli characteristic of mature or advanced maturation stages of blood cells; however, 16-20% of monocytes also contained functionally dominant ring-shaped nucleoli, which reflect a reversible decrease of rRNA transcription and in blood cells are intermediate stages between actively transcribing large nucleoli in highly immature cells and inactive micronucleoli in terminal nucleolated maturation stages. Monocytes containing large nucleoli with a relatively uniform distribution of RNA characteristic of immature or stimulated blood cells were rare (< 2%).

Interpretation and Conclusions. Nucleoli are present in all normal blood monocytes. The incidence of the main nucleolar types represents a very convenient complementary marker for evaluating the maturation and possibly the stimulated state of these cells.

Key words: hematopoiesis, monocytes, nucleolus

As is generally known, the main functions of nucleoli are represented by the rRNA transcription and processing morphologically expressed by a characteristic nucleolar size, structural organization of main nucleolar components and, especially, by the distribution of nucleolar RNA-containing structures.[1-4] The differentiation and maturation of blood cells is accompanied by a decrease in the nucleolar size and conversion of large nucleoli with more or less distinct nucleolonemas (under the light microscope they show a relatively uniform distribution of RNA) to ring-shaped nucleoli and finally to micronucleoli, which reflect the decrease and cessation of nucleolar biosynthetic activities.[3-5]

In contrast to the extensive data collected on the maturing and mature nucleated cells of the erythroid, granulocytic and lymphocytic cell lineages, information about nucleoli in circulating peripheral blood monocytes is very limited. In addition, according to the literature only some mature blood monocytes contain nucleoli or nucleoli are absent in these cells.[6-9] On the other hand, other studies demonstrated that circulating monocytes after special staining contain several small nucleoli, some of which are ring shaped.[3,10-14] The presence of nucleoli in some monocytes was also noted at electron microscopy[3,8] but the incidence of the main nucleolar types with respect to RNA transcription, such as active nucleoli with a relatively uniform distribution of RNA, resting ring-shaped nucleoli and inactive micronucleoli,[3,5] in these cells has not been reported. Therefore the present study was undertaken to provide the missing information on the incidence of the above mentioned main nucleolar types in monocytes in the peripheral blood of blood donors. This information might be useful for complementary studies on the biology of these cells, including nucleolar function and cell maturation, resting or proliferative state.

Materials and Methods

Nucleoli in monocytes were investigated on venous peripheral blood smears from 20 healthy blood donors (10 men and 10 women) whose ages ranges from 23 to 35 years. Nucleoli in monocytes were identified by means of light microscope cytochemical procedures for the visualization of their characteristic components such as RNA and the main nucleolar proteins, i.e. nucleophosmin, nucleolin, fibrillarin and AgNOR proteins.

In order to detect RNA-containing structures, smears were

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stained with buffered methylene blue without previous fixation at pH 5.1 for 10 min.[15] The density of nucleoli and cytoplasm in monocytes and lymphocytes stained for RNA was compared using the CCD camera (COHU 4915) equipped with a frame store (Colorado Video 440), LG-3 frame grabber (Scion corp.) and NIH image software written by Wayne Rasband (US National Institutes of Health, available from the Internet by anonymous ftp from *zippy.nimh.nih.gov*; see ref. #16).

Silver stained proteins from nucleolus organizer regions (AgNOR proteins, see ref. #2) were stained using a previously described one-step procedure.[17] Other characteristic nucleolar proteins investigated, i.e. fibrillarin,[18] nucleophosmin (protein B23, refs. #16 and 19), nucleolin (protein C23, ref. #19) were stained by means of indirect immunofluorescence using mouse monoclonal antibodies prepared in the Department of Pharmacology, Baylor College of Medicine (courtesy of R.K. Busch, P.K. Chan, L. Perlaky) and FITC-conjugated goat anti-mouse IgG. Smears were fixed in 2% formaldehyde for 20 min and washed in PBS (phosphate buffered saline, pH 7.1); then cells were permeabilized with acetone at –20°C for 4 min, incubated in specific antibodies at 37°C for 40 min, washed in PBS, incubated with FITC-conjugated anti-mouse IgG at 37°C for 40 min, washed in PBS and mounted in glycerol containing n-propyl-gallate to reduce the bleaching of the fluorescence.[20] The fluorescence of monocytic nucleoli was captured by the CCD camera and the signal was magnified using the NIH image analyzing software program (see above).

Main nucleolar types were classified according to size and in particular according to the RNA distribution[3,5] in smears stained for the demonstration of this nucleic acid (see above). Three main classes of nucleoli: (a) compact nucleoli with relatively uniform RNA distribution, representing nucleoli with more or less distinct nucleolonemas; (b) ring-shaped nucleoli containing RNA only in their peripheral part, and (c) micronucleoli were evaluated at least in 30-40 monocytes on each smear. Peripheral blood monocytes were also classified according to the presence of functionally dominant nucleoli.[3,5] The first group of cells was characterized by the presence of *active* large nucleoli with relatively uniform RNA distribution but, in addition, these cells might also contain some of the other above mentioned nucleoli. The second group consisted of cells with functionally dominant, *resting,* ring-shaped nucleoli which might also include some *inactive* micronucleoli. The third group of monocytes contained only *inactive* micronucleoli. The nucleolar coefficient was also determined by evaluating the number of nucleoli in the monocytes from the same specimens which were used for the determination of the presence of main nucleolar types in these cells. Then the values of the nucleolar coefficient were calculated by dividing the number of nucleoli by the number of monocytes in which they were counted.[10]

Results

Visualization and identification of nucleoli in circulating peripheral monocytes

Nucleoli were present in all monocytes irrespective of the procedure used for their visualization, and appeared mostly as small but distinct and intensively stained bodies (Figures 1-4). They exhibited similar positivity for RNA and characteristic nucleolar proteins, such as nucleophosmin (protein B23), nucleolin (protein C23), fibrillarin and AgNOR proteins, as the nucleoli in lymphocytes in the same smear preparations (Table 1). In addition, after staining for RNA the density of monocytic nucleoli was compared with that of known nucleoli in lymphocytes, and the values of arbitrary units found for nucleoli in both monocytes and lymphocytes were practically the same (Table 2). When the density of structures stained for RNA was decreased

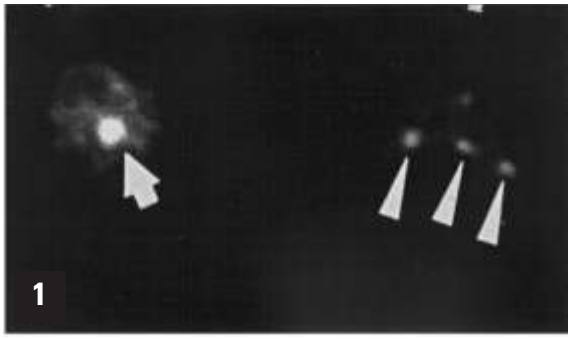

Figure 1. Immunofluorescence of nucleophosmin (protein B23) in several nucleoli from a monocyte (pointers) and one nucleolus from a lymphocyte (arrow). × approx. 1,800.

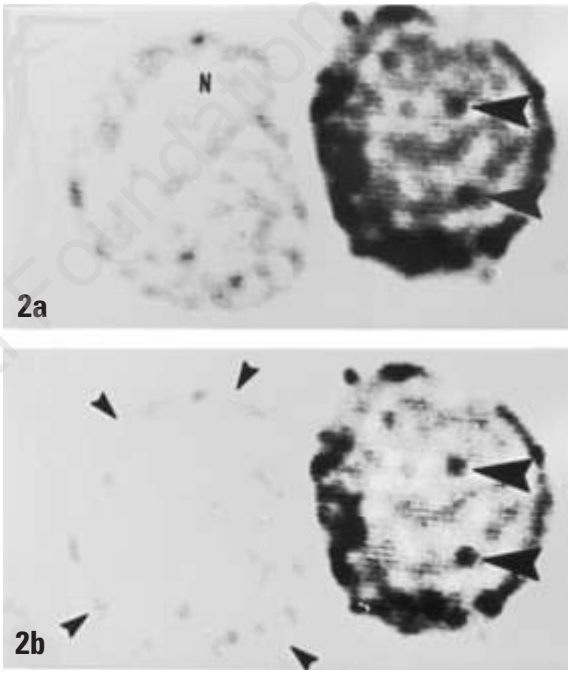

Figure 2. A) micronucleoli (large pointers) in a monocyte after staining for RNA. Note the presence of an adjacent granulocyte without nucleoli in the nucleus (N). When the density of stained structures was reduced using the image analyzer, nucleoli in the monocyte were more distict (B) and the adjacent originally less stained granulocyte (small pointers) became almost invisible. × approx. 2,800.

Table 1. RNA and proteins in nucleoli of monocytes.

	RNA	*Fibrillarin*	*B23*	*C23 proteins*	*AgNOR*
Monocytes	+	+	+	+	+
Lymphocytes	+	+	+	+	+
Granulocytes	–	–	–	–	–

B23: Nucleophosmin; C23: Nucleolin. + All cells exhibit the presence of positive nucleoli. – Most cells do not exhibit positivity for nuclear structures representing characteristic nucleoli.

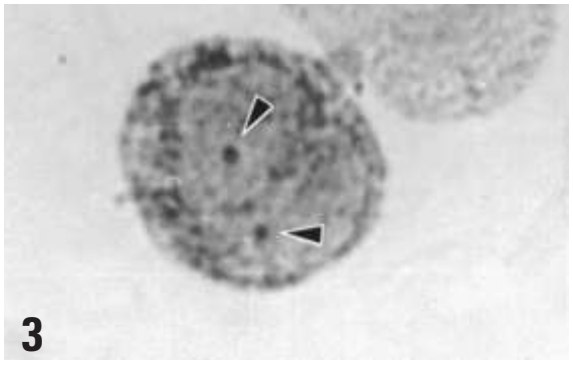

Figure 3.Micronucleoli (pointers) in a monocyte stained for RNA. × approx. 2,200.

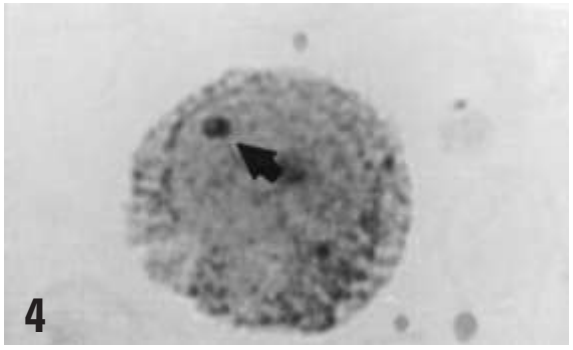

Figure 4. A ring-shaped nucleolus (arrow) in a monocyte stained for RNA. × approx. 2,500.

through image processing, micronucleoli in monocytes were even more apparent and the visibility of adjacent granulocytes without nucleoli[3] substantially decreased because of their small RNA content (Figure 2a, 2b).

The incidence of main nucleolar types in circulating peripheral monocytes

Circulating monocytes in the peripheral blood usually contained only micronucleoli and these nucleoli represented the most frequent nucleolar type present in these cells. No substantially different values were found between men and women (Tables 3, 4).

Ring-shaped nucleoli were noted less frequently in peripheral blood monocytes (Table 3); nevertheless, the percentage of monocytes containing such nucleoli still represented a significant proportion of these cells in the peripheral blood of investigated subjects (Table 4). Differences between the values found for men and women were neither substantial nor statistically significant (Tables 3, 4).

Large nucleoli with relatively uniform distribution of RNA-containing structures, i.e. compact nucleoli in circulating monocytes, were very rare and exceptional (Table 3). The percentage of cells with such nucleoli was very limited (Table 4) and, again, no substantial or statistical differences were noted between men and women (Tables 3, 4).

Table 2. The nucleolar density in monocytes and lymphocytes stained for RNA.

Cells	*Nucleoli*	*Cytoplasmic RNA*	*N**
Monocytes	186.1 (3.2)°	182.0 (2.2)	30
Lymphocytes	183.5 (4.6)	184.6 (4.2)	30

**Number of cells investigated in one smear; °arbitrary units, mean and standard error. The complete gray scale is from 0 to 255 units.*

Table 3. The incidence of main nucleolar types and values of the nucleolar coefficient in monocytes.

Nucleoli	*compact#*	*ring-shaped*	*micronucleoli*	*No coeff.°*
Men	0.5 (0.2)+	7.3 (1.1)	92.1 (1.2)	2.62 (0.08)
Women	0.5 (0.2)	11.5 (2.9)*	87.8 (3.0)*	2.64 (0.09)

*+Mean and standard error of mean; *statistically non significant difference using Mann-Whitney test; #compact nucleoli, i.e. large nucleoli with a relatively uniform distribution of RNA; °nucleolar coefficient (number of nucleoli per cell).*

Table 4. The percentage of circulating monocytes classified according to the incidence of main nucleolar types.

Monocytes with	*compact nucleoli#*	*ring-shaped nucleoli*	*micronucleoli*
Men	0.8 (0.3)+	16.0 (1.8)	83.2 (2.4)
Women	1.5 (0.6)*	20.9 (2.5)*	77.5 (2.5)*

*+Mean and standard error of mean; *statistically non significant difference using Mann-Whitney test; #compact nucleoli, i.e. large nucleoli with a relatively uniform distribution of RNA.*

The number of nucleoli in circulating peripheral monocytes

In blood smears stained for RNA, the number of nucleoli ranged between 1 and 5, with the mean value of the nucleolar coefficient (number of nucleoli per cell) being 2.6 in both men and women (Table 3). This was similar to the AgNOR score (2.8, S.E. 0.2) determined in five male blood donors. Such a similarity apparently indicates that the micronucleoli were practically identical to the solitary silver stained dots representing AgNORs in blood cells.[21,22]

Discussion

The present study confirmed previous data on the presence and number of nucleoli in monocytes[12] and provided some missing information on the incidence of the main nucleolar types in circulating monocytes in the peripheral blood of healthy adults.

The present study shows that peripheral monocytes contain mostly micronucleoli and less frequently ring-shaped nucleoli. Large nucleoli with

more or less distinct nucleolonemas, i.e. large compact nucleoli, were rare in these cells. Thus the majority of circulating monocytes in human peripheral blood seem to be in the mature stage since micronucleoli are the only nucleolar type present. In the cell nucleus such nucleoli are characteristic markers for mature or terminal nucleolated stages of blood cells, as has been demonstrated for orthochromatophilic erythroblasts, mature nucleated erythrocytes, metamyelocytes and thymocytes.[3-5,23] In these cells micronucleoli apparently reflect a cessation or minimal activity of nucleolar RNA transcription. Their formation from other nucleolar types has also been induced in a great variety of cells, including blood cells, under experimental conditions through severe inhibition of nucleolar RNA transcription.[4,23-26] The smaller AgNOR score in peripheral monocytes observed in the present study as compared to the larger values in bone marrow monocytes[22] also favors the above suppositions. Bone marrow contains immature precursors of these cells which possess a larger number of silver stained dots organized mainly in clusters that represent AgNORs, similarly to other highly immature blood cells.[10,22]

The presence of small nucleoli in mature monocytes has already been mentioned in classical hematological literature.[10,27] Descriptions of the absence of nucleoli or their presence only in a limited number of monocytes in various hematological publications were based primarily on visualization of these cells by electron microscopy and light microscope panoptic staining or phase contrast procedures which did not facilitate the selective visualization and classification of nucleoli and especially micronucleoli.[6-9,22] In addition, as is generally known, the possibility of seeing nucleoli in ultrathin sections of monocytes or other cells with the electron microscope is also limited, and the absence of a cell structural component in ultrathin sections is not evidence that the cell does not possess that structure.[28]

The presence of micronucleoli in peripheral circulating monocytes does not contrast with our present knowledge of the function of these cells either. Under normal conditions, peripheral blood monocytes are mostly nondividing mature cells which migrate to various tissues where they become macrophages and may transform into multinucleated giant cells.[6,7,29,30]

The presence of ring-shaped nucleoli as functionally dominant nucleoli in a relatively large proportion of monocytes (about 16-20%) suggests that these cells are less mature and/or are in a resting state which may be stimulated to *blast* transformation. This hypothesis is supported by the following observations. In resting lymphocytes and in maturing erythroid or granulocytic precursors, ring-shaped nucleoli are known to represent intermediate nucleolar types between fully active large nucleoli with more or less distinct nucleolonemas and inactive micronucleoli with respect to nucleolar RNA transcription.[3,4] As demonstrated by experiments on peripheral lymphocytes, resting ring-shaped nucleoli are characterized by a reversible decrease in nucleolar RNA transcription, and after proper stimulation they can transform back to fully active large nucleoli with more or less distinct nucleolonemas characteristic of immature blast cells.[3,4] According to recent observations, isolated monocytes with ring-shaped nucleoli, after stimulation with interferon-γ, transform into activated cells with characteristic large nucleoli with more or less distinct nucleolonemas that are transcribing nucleolar RNA.[14] However, these transformed monocytes apparently did not proliferate *in vitro*.[14] On the other hand, the possibility that proliferation might occur *in vivo* cannot be ruled out.[30] Proliferation might also be reserved in target tissues for a few circulating monocytes that contain large nucleoli with a relatively uniform distribution of RNA (present results), since such nucleoli are usually present in immature and proliferating blood cells.[3] A small percentage of monocytes have been observed to incorporate labeled thymidine.[30,31] Moreover, some studies have also demonstrated that a small number of thymidine-incorporating macrophages in various tissues may possibly originate from circulating monoblasts and/or promonocytes.[31,32]

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