Appearance of iron-labeled blood mononuclear cells in electron microscopy

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ABSTRACT: Mononuclear cells from rabbit bone marrow were cultured for 14 days in cell-free medium for hematopoietic cells together with iron oxid nanoparticles, and then they were processed by technique for free cells for TEM (transmission electron microscopy). Staining with turnbull blue was used for the detection of iron using a light microscope. It was shown that iron nanoparticles were incorporated into the cytoplasm of mononuclear cells during 14 days cultivation. Here they were localized within different sized vacuoles with distinct membranes.

Keywords: ultrastructure; iron nanoparticles; cultivation; animal model

It is necessary to note that although there is a considerable number (several thousands) of publications regarding the use and role (presupposed) of bone marrow mononuclear cells in the therapy of different diseases (Futterman and Lemberg, 2004; Uzan, 2004) and reparation of posttraumatic lesions, we have been unable find information on their morphology. References such as "identification of morphology, size and shape is difficult" were mentioned in some papers (Dalrup-Link et al., 2003). Therefore the description of the morphology of this cell type is the aim of our study. These cells will be put to morphometric measurement, and their distribution whithin different regions of experimental myocardial infarction in the next phase of the experiment.

MATERIAL AND METHODS

Animal model

Rabbits (Hy-Plus, weight 2.5 to 3.5 kg) were used as an experimental animal model. Experimental protocol was approved by the Ethics Committee of University of Veterinary Medicine, Brno.

Bone marrow collection

Mononuclear cells were harvested from rabbit bone marrow on day –14, before the ligation of the coronary artery. Bone marrow was obtained by multiple aspiration from iliac bone into heparin flushed syringes under general anesthesia. Samples were collected into tubes containing heparin and 0.9%NaCl (1:1) under sterile conditions. A sufficient amount of bone marrow was collected in order to obtain the required numbers of enriched cells (approximately 5–10 ml).

Selection of mononuclear cells

Mononuclear cells were enriched from bone marrow by density gradient centrifugation. Bone marrow was diluted with Hanks/4% albumin solution (1:1) and overlaid on a sterile density medium

(Histopaque, density 1077). Mononuclear fraction was obtained by 20 minutes centrifugation at 1 200 g; cells were washed and resuspended.

Mononuclear cells labeling

The mononuclear cells were cultured for 14 days in cell-free medium for hematopoietic cells (H3000, Stem Cell Technologies, Canada) together with iron oxide nanoparticle suspension (Resovist, Schering, D). During the culture, the nanoparticles were incorporated into mononuclear cells by endocytosis. This allowed the specific detection of labeled cells by electron microscopy or by Fe staining in light microscopy. At the day of implantation, cells were collected from the medium, washed and resuspended in fresh Hanks/4% Albumin solution. Prior to coronary artery infusion, the cells were filtered through the nylon mesh filter, diameter 70 μm. The samples were then taken for cell counting.

TEM

Mononuclear cells from bone marrow were separate by centrifugation at 800 rpm/min for 5 minutes, fixed in 400 mmol/l glutaraldehyde in cacodylate buffer pH 7.4. The following postfixation was done by two baths of 40 mmol/l OsO_4 in cacodylate buffer pH 7.4. Dehydration, immersion and embedding into Durcupan ACM were carried out using standard procedures. Ultrathin sections were made on LKB Nova ultramicrotome and stained with lead citrate or with uranyl acetate and lead citrate. The sections were viewed and photographed in a Morgagni 268D electron microscope.

RESULTS AND DISCUSSION

Mononuclear cells from bone marrow were oval-shaped and their size was from 9 to 12 μm in diameter. The round nucleus was 5 to 6 μm in diameter, and several karyosomes were localized near the nuclear envelope. Nucleoli of reticular type, usually 1 to 2, were found in the sections through the nucluei. Segregation of nucleolar components (probably the result of cultivation) was rarely observed. Isolated cisternae of rough endoplasmic reticulum (GER) and numerous free ribosomes and polysomes were difused in the light cytoplasm. Mitochondria were usually gathered in one region

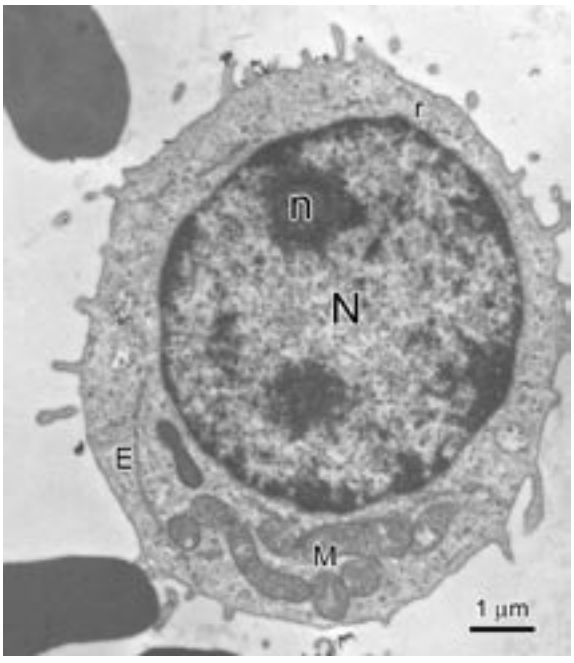

Figure 1. Mononuclear cell from bone marrow. Cultivation for 14 days in medium without iron oxide. Nucleus (N), nucleolus (n), mitochondria (M), granular endoplasmic reticulum (E)

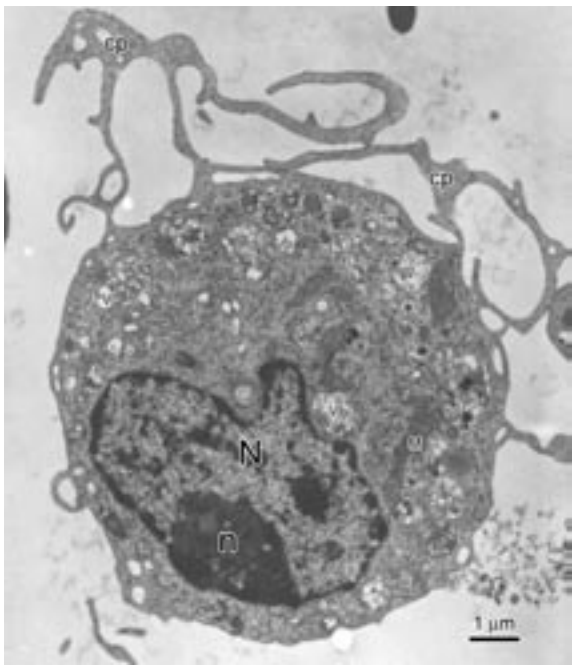

Figure 2. Mononuclear cell from bone marrow cultivated for 14 days with iron nanoparticles. Nucleus (N), nucleolus (n), mitochondria (M), large vacuoles (V) containing small empty vesicles or vesicles with dark granular material

of the cytoplasm and most of them were elongated, with their inner membrane forming distinct cristae. Some mitochondria contained a regionaly cleared matrix, probably the manifestation of changed osmotic conditions; the matrix of the other mitochondria was found to be dark. Several vacuoles, sized approximately 0.8 μm, and containing finely granulated material, were present in most of the followed cells (Figure 1).

Some mononuclear cells from bone marrow changed their ultrastructural appearance after 14 days cultivation in medium with Fe nanoparticles. Deep invaginations of nuclear envelope, enlarged nucleoli and increased number of vacuoles were observed in these cells. The vacuoles were approximately 0.5 μm sized. Excerpt from them, numerous large vacuoles having up to 1.5 μm in diameter were found in the cytoplasm near the cell membrane. They were filled with granular material of different density, or with numerous smaller vesicles containing electron-dense or light material. Mitochondria were diffused together with cisternae of GER among the vacuoles. The plasma membrane of some cells projected as the long branched cytoplasmic processes on some parts of the cell surface (Figure 2).

An increased number of GER cisternae was observed sporadically in some bone marrow mononuclear cells. Cisternae were localized in the peripheral regions of the cytoplasm, and they were arranged in parallel with one another and with the cell surface. Mitochondria of average size were round or elongated and situated along the cisterna, or irregularly difused in the cytoplasm. Large dark vacuoles of oval shape and up to 2 × 1.5 μm in size were distinct parts of the cytoplasm. These vacuoles contained particles of granular material of the same appearance as Fe nanoparticles that occurred in the space around the cells (Figure 3).

Some mononuclear cells from the bone marrow (and they represented the majority of the cells cultured for 14 days with nanoparticles of iron oxide) incorporated particles of iron in a higher quantity. Several vacuoles are surrounded with a membrane. Vacuoles from 0.5 to 1.0 μm were identified in cytoplasm of this cells. They contained granular material of either middle, or high electronoptic density (Figure 4).

Iron nanoparticles stored by some mononuclear cells of the bone marrow reached an excessive step, and so their cytoplasms were practically filled up with large electron-dense vacuoles (Figure 5).

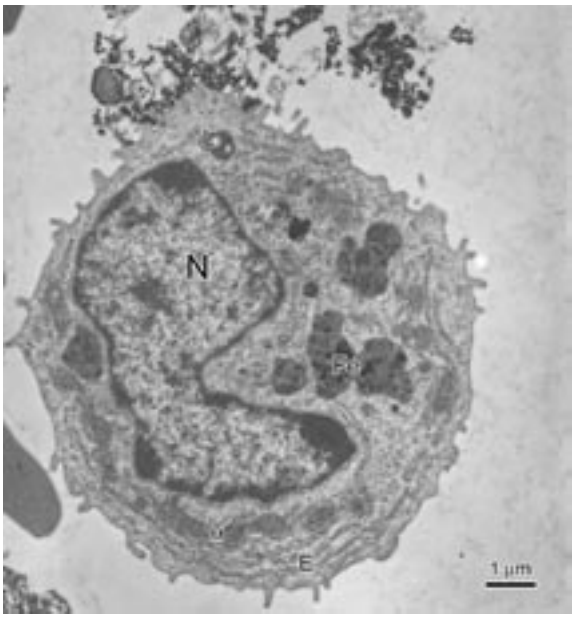

Figure 3. Mononuclear cell of the bone marrow after 14 days cultivation with iron nanoparticules. Nucleus (N) with deep invagination nuclear envelope, mitochondria (M) along cisternae of granular endoplasmic reticulum (E), lysosome (L), large vacuoles with granular material of moderate density containing iron nanoparticules (Fe)

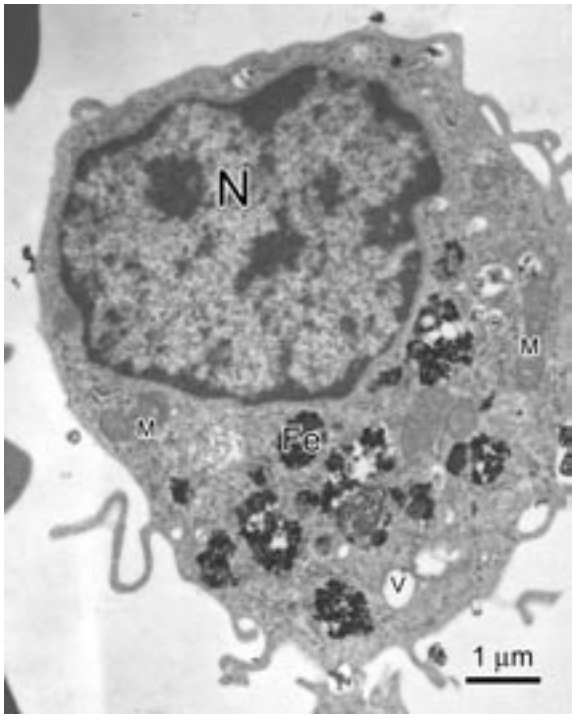

Figure 4. Mononuclear cell from the bone marrow cultivated 14 days with nanoparticles of iron oxide. Nucleus (N), mitochondria (M), empty vacuoles (V), large vacuoles with clusters of iron nanoparticles (Fe)

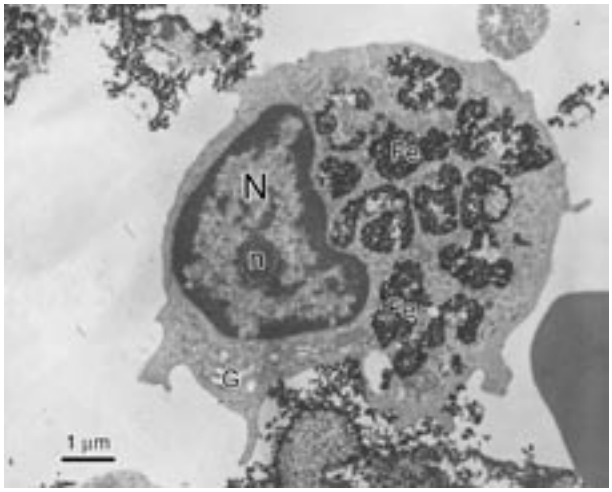

Figure 5. Mononuclear cell from bone marrow after 14 days cultivation with iron nanoparticles. Nucleus (N), nucleolus (n), Golgi complex (G), cytoplasm containing a large number of vacuoles with dark granular material

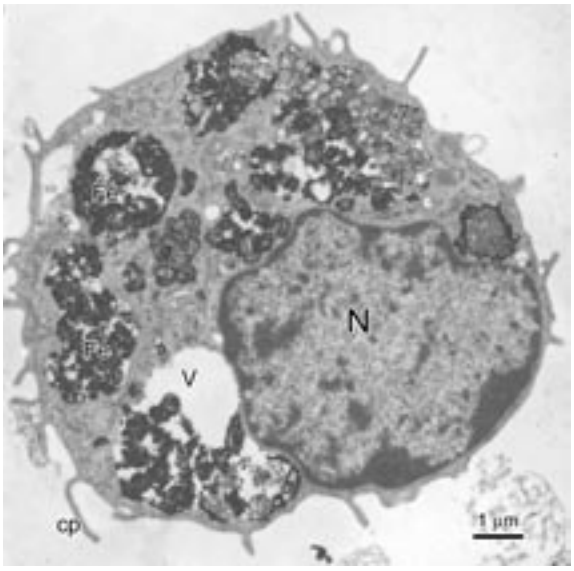

Figure 6. Mononuclear cell from the bone marrow after 14 days cultivation with Fe nanoparticules. Nucleus (N), cytoplasm is filled up of large vacuoles containing dark material (Fe), rarely part of largest vacuoles are empty

Rarely, some mononuclears ultrastructure showed the picture of phagocytosis of Fe nanoparticles released from adjacent degraded (degenerating) cells. Extremely large vacuoles sized 3–3.5 µm in diameter probably arisen from the fusion of smaller vacuoles with Fe nanoparticles. These large vacuoles completely filled up the cytoplasm of the cells and sometimes formed electronopticaly empty (clean) regions (Figure 6).

Regarding the ultrastructural appearance of nucleoli and mitochondria, the cell cultured in medium containing an oxide of Fe did not affect the viability of the mononuclear cells. Schoepf et al. (1998) and Lewin et al. (2000) reached similar conclusions concerning the viability of blood cells labeled with iron.

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Received: 2006–01–29
Accepted: 2006–03–06

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