Ultrastructural features of blood cells in atherosclerosis

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Abstract
Atherosclerosis is a complex process that affects specific tissues of the vasculature, susceptible to developing atherosclerosis. From 10 patients with atherosclerosis, between 50–70 years, peripheral blood was prelevated and after the technical procedures the blood cells were studied using the electron microscopy. The most characteristic morphological modifications were observed in agranulocytes and platelets. The granulocytes polymorphs showed insignificant ultrastructural changes, and the erythrocytes presented weak anzocytosis and poikilocytosis.

Keywords: atherosclerosis, blood cells, electron microscopy.

Introduction
Atherosclerosis is the major source of morbidity and mortality in the modern world [1], with significantly progress in the understanding of the basic mechanisms involved in atherotic plaques and acute cardiovascular events showed the important role of inflammation at all stages of disease [2, 3].

Recently, a consensus established that atherosclerosis represents a state of heightened oxidative characterized by lipid and protein oxidation in the vascular wall [4].

This include the production of reactive oxygen and nitrogen species by vascular cells as well as oxidative modifications contributing to important clinic manifestation of atherosclerotic disease, such as endothelial dysfunction and plaque disruption. Both classic and actual studies bring pathologic dates that note the vascular lesions of the disease [5, 6].

These observations were completed by recently immunohistochemical researches [7, 8]. Suggest too, that immune response activation may promote atherosclerosis, either inducing or then perpetuating arterial inflammation, or by selective suppression of proatherogenic immune responses activating certain immune functions responsible for inhibiting atherosclerosis and arterial inflammation [9].

Innate and adaptive immunity influence the development of atherosclerosis and the detection of macrophage and dendrite cell receptors, especially the scavenger receptors [10] and the Toll-like receptors [11], considered most important in the inflammatory and toxic responses to the invading microorganisms [12].

Experimental animal studies, especially on rabbits, brought news to histopathology and histoenzymology of atherosclerotic vessels [13–16].

Researches concerning morpho-physiologic modifications of blood cells in the atherosclerotic disease are rare.

The aim of the present study is to observe, using the electron microscope, ultrastructural features of blood cells and their alterations in atherosclerosis.

Materials and methods
Four groups of 10 patients with ages between 50–70 years (strokes with and without complications, cardiac ischemic disease, high blood pressure), were analyzed.

Leucograma and plateletograma were counted by automatic analysis.

From two patients randomly selected from each group, peripheral blood was studied using the electron microscope by classic fixation, inclusion, sectioning and observation (Table 1).

Table 1 – Polynuclear leukocytes (neutrophiles, eosinophiles, and basophiles) had normal values

<table>
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<tr>
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<th>Vascular strokes</th>
<th>Cardiac ischemic disease</th>
<th>High blood pressure</th>
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<td></td>
<td>+ Sequence</td>
<td>- Sequence</td>
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<tr>
<td>Monocytes [%]</td>
<td>5–20 6–10</td>
<td>8–9 7–10</td>
<td>5–8 6–8</td>
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F – females; M – males
Results

The electron microscope characteristics of monocytes separate them in immature cells, mature cells and pre-macrophages.

**The immature cells** have irregular nuclei, with predominant euchromatin. The cytoplasm is slightly basophile with free ribosome, isolated, and frequent mitochondria and lysosomes. Golgi’s apparatus lies near the nucleus and the endoplasmic reticule is weakly developed. The immature cells are 15–20% monocots.

**The mature cells** are more: 20–75%. The nucleus is kidney-shaped-like, with equal amounts of euchromatin and heterochromatin in the majority of cells (Figure 1).

Frequently one nucleolus can be observed in the nucleus. The basophile cytoplasm contains frequent cellular organists, especially very well developed rough endoplasmic reticule (RER), mitochondria, lysosomes and Golgi’s apparatus. The cell membrane has different lengths and thickness prolongations (Figure 2).

**The pre-macrophages** are 0–5% of the cells and they present ultra structural characteristics close to extra vascular macrophages. The euchromatic nucleus with nucleolus and heterochromatin granules is kidney-shaped-like. The plentiful cytoplasm contains cellular organists, lissome being very numerous. The cells membrane has pseudopods.

**The lymphocytes**, due to their size, are present in the circulatory blood in the following proportion: 80–85%, small, 10–28%, medium, 1–5% large.

The small lymphocytes have a large heterochromatic nucleus with one nucleolus. There is reduced cytoplasm, slightly basophilic and with few cellular organist (Figure 3).

The medium lymphocyte has more cytoplasm with many mitochondria, lysosomes, rough endoplasmic reticule (RER), and Golgi’s apparatus well developed (Figure 3).

The large lymphocyte is organized like lymphoblast characteristics: euchromatic nucleus with large nucleolus, plentiful cytoplasm with many cellular organists, especially those implicated in protein synthesis (lysosomes in different degrees of activity – primary, secondary, tertiary) (Figure 4).

**The platelets** are seen with many different shapes and electron intensity granules. Many large size platelets have well developed chronomer and hyalomer. Rare small platelets, with few central granules and a narrow hyalomeric zone can be seen.

Generally, **polynuclear leukocytes** show a normal ultrastructure. The granulocytes, especially neutrophiles, appear preapoptotic.

Discussions

The count for a granulocytes and platelets showed characteristic aspect, as these types of cells being actively implicated in the atherosclerosis process.

The count varies from one individual to the other, from one disease to the other, less between sexes. Blood cells variability in atherosclerosis is present [17] in blood vessels walls depending on the histological structure and function of that vascular area. The inflammatory response of arteries [18] has regional differences due to homodynamic parameters (turbulence), leading to selective areas of atherosclerosis.

Monocytes appear in normal limits, except for the patients with stroke, especially women, which have high number. There are high levels of small lymphocytes, especially at patients with cerebral atherosclerosis, with no significant differences between sexes, versus ischemic cardiac disease, where the number tend to stay at minimum.

The platelets present obvious numeric variation between patients and sexes, but generally, women having small counts.

The polynuclear leukocytes have no significant aspects.

Electron microscope observations bring useful new data concerning the leukocyte pattern in atherosclerosis. These data are important and relevant especially for monocytes lymphocytes and platelets. Generally, for these three types of blood cells the presence of immature cell stages is common, which can indicate medullar stimulation by intense activity in studied pathology, each of them with a characteristic role:

1) The high level of macrophages at blood vessels’ atherosclerotic walls [19], where the inflammatory response and the pathogenesis of atherosclerosis are strongly involved [20].

2) The T-lymphocytes, highly represented by immature pro-T cells, with specific receptors (CD4+ and CD117+), and adult T-cells (CD4+ and CD8+) regulate local vascular inflammatory processes and immunoglobulin in atherosclerosis by association with MHC molecules and cytokines secretion (interferon and chemokines) [21]. Ultrastructurally, more developed platelets lead to positive aspects, on one hand: rapid response in vascular hemorrhages and spontaneous in atherom deposits disruption, and, on the other hand, a negative one: frequent formation of vascular thrombi.

Their role is played by activating/inhibiting molecules located in granulomer and hyalomer via pro-thrombosis/anti-thrombosis factors [22].

The polynuclear leukocytes, with normal aspects, are situated between rare neutrophiles, which present preapoptotic status at nuclear level, which might suppose apoptotic structural modifications started before their diapedesis and passage to the extra vascular medium.

At the present time, some studies revealed the activity of the immune system in different stages of the atherosclerotic disease, and more recent observations suggested the activation of an immune response which might accelerate atherosclerosis inducing consequently arterial inflammation.

For this reason, there are some efforts to control the immune response using immunosuppressive medication or immunization with vaccines [23]. Immunization can reduce development of atherosclerosis with 50–60% [9], and might be adopted in addition to the well-known statine treatment and prevention of risk factors.
Figure 1 – Electron micrograph of an active monocyte–premacrophage. In the cytoplasm, numerous ribosomes, several mitochondria and lysosomes are present (×21 000)

Figure 2 – High magnificence from a segment of monocyte. The cytoplasm contains many lysosomes in different stages of their activity. The cell membrane presents pseudopodes (×43 200)

Figure 3 – Electron micrograph of the medium-size lymphocytes; between them a premacrophage (×15 000)

Figure 4 – Electron micrograph of a mature circulating lymphocytes (×21 000)
Conclusions

The electron microscope observations show that peripheral blood cells, especially monocytes, lymphocytes, and platelets, present ultrastructural modifications in the atherosclerotic disease. The alterations are at the nuclear and cytoplasm level, with changes in the quality and quantity of nuclear organs.

Well-developed granulomer platelets are the main cellular type involved in atherosclerotic processes, showing an increase by megakaryocytic stimulation.

The existence of a high amount of immature cells and the presence of premacrophages might suggest an accelerated cell cycle, and an active granulocyte formation, both stimulated by the atherosclerotic process.

This fact stands for polynuclear leukocytes too. Even if they do not seem ultrastructurally modified certain circulating neutrophiles present preapoptotic nuclear aspects.

References


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