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Senescent Changes of the Rat Aortic Endothelium Studied *en face*

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In order to study the senescent changes of the rat aortic endothelium, this study examines on *en face* preparations the common morphology of the endothelial cells and the distribution of the mononuclear cells, adherent to the endothelium, in four age groups. The endothelium of the young, puberty and adult animals was composed of an even layer of elongated endothelial cells with one oval nucleus and small number of mononuclear cells. The most prominent findings in old endothelium were the presence of giant multinucleated endothelial cells and the larger number of the mononuclear cells adherent to the endothelium. The observations found and their literature explanations are discussed.

Key words: senescence, aortic endothelium, rat.

Introduction

There are number of studies examining the *en face* morphology of the endothelial cells of the rat aorta in experimental models of different pathologic conditions [4, 5, 10]. However, the information about the normal senescent of the rat endothelium is scarce. Because the *en face* method of studying the endothelium allows to observe a large amount of cells, we used it to examine the common morphology of the rat aortic endothelium in different age groups and to describe the characteristics of the aortic endothelium in old animals.

Materials and Methods

Male Wistar rats, bred in our laboratory, 1, 3, 6 and 18-month-old, were used for this study. Under deep pentobarbital anesthesia, a perfusion fixation with 10% phosphate buffered formalin was provided. The aortas were carefully dissected, opened longitudinally, then removed, pinned out flat on polyethylene strips and stored in formalin. Impregnation of the cell borders and haematoxylin staining of the nuclei, as described by J o n e s et al. [3] visualized the endothelial cells. The *en face* preparations were made according to the method of J o r i s et al. [5].

Results

The endothelium of the aortas of the animals from different ages showed differences of endothelial cell morphology and arrangement. The presence of the mononuclear cells, adherent to the endothelium, also differed between age groups.

The endothelium of the young animals (1-month-old) (Fig. 1) was composed of an even layer of small spindle-shaped endothelial cells with their long axes aligned parallel to the direction of the blood flow. The cells had one oval nucleus. Only few mononuclear cells throughout the endothelium were observed.

The endothelium of the puberty (3-month-old) (Fig. 2) and adult animals (6-month-old) (Fig. 3) was presented by an even layer of larger than in young animals cells with one oval nucleus. The number of the mononuclear cells, adherent to the endothelium, was greater than in young animals.

The endothelium of the old animals (18-month-old) showed areas of similar in size endothelial cells and also areas of differently sized endothelial cells (Figs. 4, 5). Most of the cells showed one oval nucleus, but also a small number of giant multinucleated (with two or more nuclei) endothelial cells was observed (Fig. 4). The number of the mononuclear cells, adherent to the endothelium, was larger than in adult group. In some areas these cells were detected either scattered throughout the endothelium or in small groups (Fig. 5).

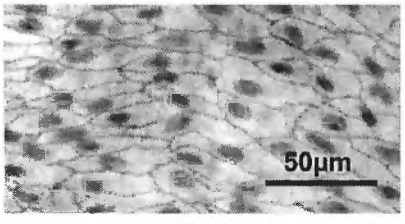

Fig. 1. *En face* preparation of the aorta of 1-month-old rat

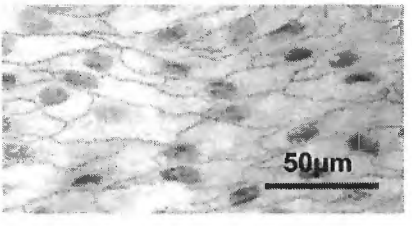

Fig. 2. *En face* preparation of the aorta of 3-month-old rat

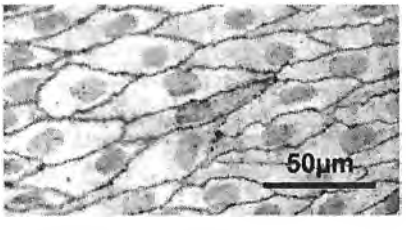

Fig. 3. *En face* preparation of the aorta of 6-month-old rat

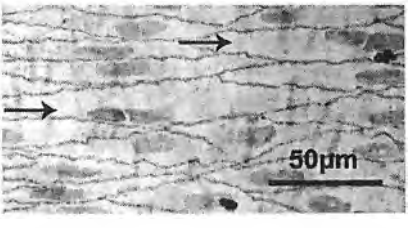

Fig. 4. *En face* preparation of the aorta of 18-month-old. Arrows indicate the giant multinucleated endothelial cells

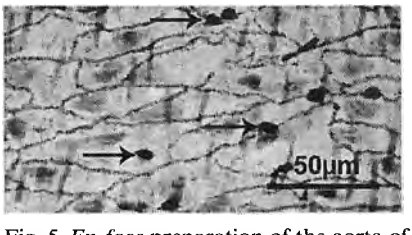

Fig. 5. *En face* preparation of the aorta of 18-month-old. Arrows indicate the mononuclear cells adherent to the endothelium

Discussion

The differences in the morphology of the endothelium among the rats from different ages represent the normal senescent process of the endothelial cells. In young animals the endothelium is composed of cells of nearly equal size that gradually become larger in puberty and adult animals. The differences in the endothelium composition of the old rats are due to the increased mean generation time of the endothelial cells [9]. Such long generation times diminish the ability to replace desquamated cells resulting in gradual loss of endothelial cells and presence of giant endothelial cells. In our study, in young, puberty and adult animals, the endothelial cells have only one nucleus, while in old animals, additionally to the usual cells with one nucleus, a small number of giant multinucleated cells is present. According to some authors [2] the multinucleated endothelial cells are prominent in newborn and adult animals (up to 10% of the cell population) and the increasing number of these cells is a characteristic of the aging mammalian and human endothelium [6]. The multinucleated endothelial cells are also known to be prevalent in atherosclerotic lesions of human [1, 7] and present in the rabbit aorta in experimental hypercholesterolemia [8].

Interesting findings presented in our study and most prominent in advanced age group were the mononuclear cells adherent to the endothelium. These mononuclear cells are variously reported as lymphocytes, macrophages and monocytes [4]. They have been observed singly or in small clusters on the endothelium of "normal" laboratory animals and suggested a minimal pathologic event [4]. Compared to the young animals, the endothelium of the old animals showed an increased number of the mononuclear cells.

Even comprising only four age groups, this *en face* study depicts the main changes of the rat aortic endothelium during the postnatal period. To present a more detailed information on this topic, a future study is necessary.

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