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DYNAMIC PORTRAIT OF ALVEOLAR MACROPHAGES IN EXPERIMENTAL DIABETES MELLITUS

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SUMMARY. Diabetes mellitus (DM) is a globally prevalent metabolic disorder with profound implications for systemic organs, yet its intricate relationship with the respiratory system, particularly the alveolar macrophages (AM), remains underexplored.

The aim – to comprehensively investigate the pathological alterations in AM during the progression of experimental diabetes mellitus.

Material and Methods. An experimental investigation was conducted involving 88 male Wistar rats, categorized into intact (Group 1), control (Group 2), and experimental (Group 3) groups. Experimental diabetes was induced using streptozotocin (Sigma, USA), diluted in 0.1 M citrate buffer with a pH of 4.5, at a dose of 60 mg/kg of body weight. Tissue samples were collected at intervals of 14, 28, 42, and 70 days. Electron microscopy analysis was employed to examine lung tissue fragments.

Results. On the 14th day, increased functional activity of AMs was observed, marked by distinct nuclear contours and shallow nucleolemic intussusceptions. Subsequent days revealed alterations in mitochondrial morphology, Golgi apparatus, and endoplasmic reticulum, culminating in dystrophic changes by the 70th day. The study period exhibited a dynamic temporal progression, showcasing the multifaceted responses of AMs to diabetic conditions. These responses include heightened functional activity, structural modifications in organelles, and a subsequent shift towards dystrophic changes, providing a comprehensive view of the evolving dynamics of AMs during the course of experimental diabetes mellitus.

Conclusions. The study reveals a dynamic temporal progression in alveolar macrophages during the course of experimental diabetes mellitus. Early heightened functional activity transitions into dystrophic changes, providing insights into the evolving functional deficiency of macrophages as diabetes advances, contributing to the understanding of diabetes-related respiratory pathology.

KEY WORDS: diabetes mellitus; lungs; experiment; alveolar macrophages; pathophysiology.

Introduction. Diabetes mellitus (DM) stands as a pervasive and complex metabolic disorder characterized by aberrations in glucose homeostasis, affecting millions worldwide [1]. Its impact extends beyond its recognized influence on systemic organs, delving into intricate interactions with specific cellular elements of diverse physiological systems. While the well-documented consequences of diabetes on cardiovascular, renal, and nervous systems have garnered considerable attention, the intricate relationship between diabetes and the respiratory system remains a subject of burgeoning interest [2].

The respiratory system, tasked with vital functions of gas exchange and maintenance of physiological equilibrium, becomes a focal point for investigation in the context of diabetes mellitus. Mounting evidence suggests that diabetes-induced systemic alterations may extend their influence to the respiratory apparatus, potentially disrupting its delicate balance [3]. The intricate cellular milieu of the respiratory system, particularly the alveolar macrophages (AM), emerges as a key player in deciphering the nuanced interplay between diabetes and pulmonary function.

Alveolar macrophages, strategically positioned within the alveoli of the lungs, serve as guardians of pulmonary homeostasis, participating in immune surveillance and maintaining tissue integrity [4]. Their role in defending against inhaled pathogens and regulating inflammatory responses underscores their significance in respiratory health. However, the impact of diabetes mellitus on these sentinel cells remains inadequately explored, necessitating a comprehensive investigation into the potential pathological alterations that may compromise pulmonary well-being.

The aim of the study – to enhance our comprehension of how diabetes mellitus impacts the respiratory system by examining the pathological alterations in alveolar macrophages in an experimental diabetes mellitus setting.

Material and Methods. In this experimental investigation, 88 male Wistar rats with a weight range of 170–210 g were involved. The rats were divided into three groups: Group 1 (n=10) consisted of intact rats; Group 2 (n=40) served as the control group, and Group 3 (n=38) constituted the experimental group. Experimental diabetes was induced by intraperitoneally administering streptozotocin (Sigma, USA), diluted in 0.1 M citrate buffer with a pH of 4.5, at a dose of 60 mg/kg of body weight. The control group received an equivalent volume of 0.1 M citrate buffer solution with a pH of 4.5 through intraperitoneal injection. All procedures were performed under sodium thiopental anesthesia at a dose of 60 mg/kg of body weight. Tissue samples were collected at time intervals of 14, 28, 42, and 70 days post streptozotocin injection.

For electron microscopy analysis, lung tissue fragments were fixed in a 2.5 % glutaraldehyde solution, followed by immersion in a 1 % osmium tetroxide solution for further fixation. After dehydration, the specimens were embedded in Epon Araldite. Sections, obtained using a "Tesla VS-490" ultramicrotome, were scrutinized with a "PEM-125K" electron microscope.

Results and Discussion. On the 14th day of the study, a notable increase in the functional activity of alveolar macrophages (AM) was identified within the alveoli. The nuclei of these macrophage elements exhibited distinct contours and shallow nucleolemic intussusceptions. A prominent feature in their submicroscopic structure was the presence of a well-defined lysosomal apparatus. The cytoplasm revealed small-sized mitochondria, slightly expanded cisterns and tubules of the Golgi apparatus (GA), and granular endoplasmic reticulum (GER). Ribosomes were clearly visible on the outer surface of the latter. Phagosomes containing surfactant fragments and destroyed cells were also observed in the middle of the cell.

Submicroscopic examinations of the alveoli in the respiratory department of the lungs 28 days into the experiment revealed nuclei of individual AMs with a matrix of low electron-optical density (Fig. 1). Chromatin granules were often positioned along the inner surface of the nucleolem. Some mitochondria displayed an increased volume with a lightened matrix and occasional reduced cristae. The GA consisted of moderately expanded cisterns and small bubbles, while GER tubules were hypertrophied with a reduced number of ribosomes on their membranes. Lysosomes and phagosomes with polymorphic osmiophilic material were evident in the AM cytoplasm. At this stage, certain macrophage elements exhibited signs of heightened functional activity. The nuclei of such AMs had a nucleoplasm of moderate electron-optical density, with chromatin granules evenly distributed throughout the nucleus. Mitochondria varied in size and shape. GA showed no significant structural changes. GER featured moderately expanded tubules with well-defined ribosomes on their outer surface. The cytoplasm contained numerous lysosomes and phagosomes.

By the 42nd day of the study, AMs with dystrophic-destructive changes were observed in the alveolar lumen. The nuclei of these cells had fine-grained nucleoplasm of low electron-optical density, with granular components concentrated along the inner surface of the nuclear membrane or grouped into separate clumps. The perinuclear space was expanded. Mitochondria exhibited a lightened matrix, shortened and disoriented cristae. GA and GER cisterns and tubules were hypertrophied and disorganized, with fine granular content. The number of ribosomes on the outer membrane of GER was reduced. Lysosomes were represented by individual granules, and isolated phagosomes with polymorphic osmiophilic material were identified in the cytoplasm. The progression of organelle changes coincided with a decrease in the electron-optical density of the cytoplasmic matrix.

The analysis conducted on the 70th day after the start of the experiment revealed nuclei of many AMs with an enlightened matrix (Fig. 2). Chromatin granules were positioned along the inner surface of the nucleolem, and the perinuclear space was expanded. Mitochondria displayed an enlightened matrix and disoriented cristae, with some undergoing vacuolar transformation. In the perinuclear area, a GA consisting of vesicularly expanded cisterns and a small number of vesicles was observed. GER tubules were vacuolated and fragmented, with a reduced

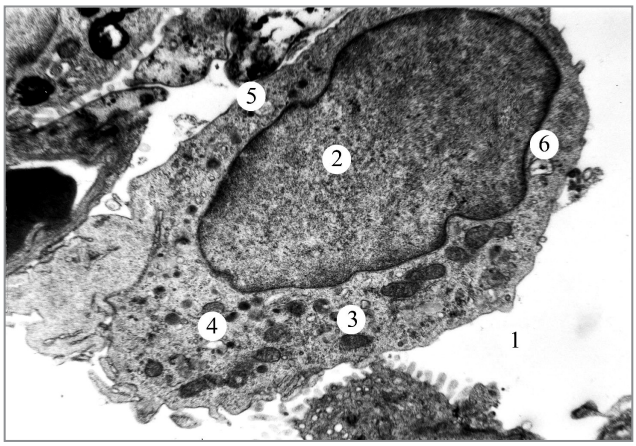

Figure 1. Submicroscopic changes of the alveolar macrophage 28 days after the start of the experiment. Electron micrograph. Magnification: 6400.

Description: 1 – alveolar lumen; 2 – core; 3 – mitochondria; 4 – granular endoplasmic reticulum; 5 – lysosome; 6 – phagosome.

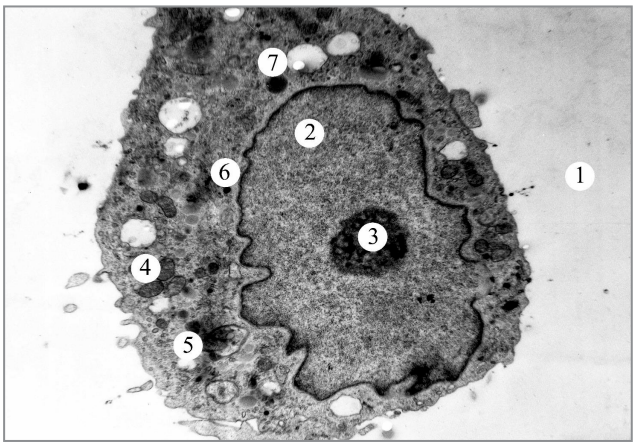

Figure 2. Submicroscopic changes in the alveolar macrophage 70 days after the start of the experiment. Electron micrograph. Magnification: 4800.

Description: 1 – alveolar lumen; 2 – core; 3 – nucleolus; 4 – mitochondria; 5 – granular endoplasmic reticulum; 6 – lysosome; 7 – phagosome.

number of ribosomes. The fragmentation of GER membranes was noted in individual AMs. Macrophage elements included cells with a small number of lysosomes and phagosomes.

The study's results indicated that during the early stages of diabetes mellitus development (14–28 days), there is an increase in the number and functional activity of AMs, representing the primary response of macrophage elements to lung tissue damage [5, 6]. As the study duration extended (42–70 days), a progressive disruption of the structural organization of the respiratory department of the lungs was observed. Among macrophage elements, cells with a low number of lysosomes but a high number of phagosomes, containing polymorphic osmiophilic material, were identified, indicating a functional deficiency of macrophages [7].

Conclusions. Through a meticulous examination conducted over 70 days, we observed a temporal progression in alveolar macrophages dynamics. The initial heightened functional activity transitioned into dystrophic changes, indicating a functional deficiency in macrophages as diabetes advanced.

Prospects for further research. Delving deeper into the molecular mechanisms underlying the observed changes in AM dynamics could offer a more detailed understanding of the interplay between diabetes mellitus and respiratory health. Investigating signaling pathways and gene expressions associated with AM alterations may reveal novel therapeutic targets. Moreover, establishing correlations between the observed structural alterations in AM and functional consequences on immune surveillance and inflammatory responses is essential. Functional assays and immune profiling could provide insights into how these structural changes impact the overall respiratory health.

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ДИНАМІЧНИЙ ПОРТРЕТ АЛЬВЕОЛЯРНИХ МАКРОФАГІВ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ ЦУКРОВОМУ ДІАБЕТІ

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РЕЗЮМЕ. Цукровий діабет (ЦД) є глобально поширеним метаболічним розладом із серйозними наслідками для системних органів, проте його складний зв'язок із дихальною системою, зокрема альвеолярними макрофагами (АМ), залишається вивченим недостатньо.

Метою даного дослідження є всебічне дослідження патологічних змін АМ під час прогресування експериментального цукрового діабету.

Матеріал і методи. Нами було проведено експериментальне дослідження за участю 88 самців щурів лінії Вістар, поділених на інтактну (група 1), контрольну (група 2) та експериментальну (група 3) групи. Експериментальний цукровий діабет індукували стрептозотоцином (Sigma, США), розведеним у 0,1 М цитратному буфері з pH 4,5, у дозі 60 мг/кг маси тіла. Зразки тканин збирали з інтервалами в 14, 28, 42 і 70 днів. Для дослідження фрагментів легеневої тканини використовували електронну мікроскопію.

Результати. На 14-ту добу спостерігали підвищення функціональної активності АМ з чіткими ядерними контурами та неглибокими нуклеомічними інвагінаціями. Наступні дні виявили зміни в морфології мітохондрій, апараті Гольджі та ендоплазматичному ретикулумі, що завершилися дистрофічними змінами до 70-го дня. Період дослідження продемонстрував динамічний прогрес у часі, демонструючи багатогранну реакцію АМ на діабетичні стани. Ці реакції включають підвищену функціональну активність, структурні модифікації в органелах і подальший зсув до дистрофічних змін, що забезпечує комплексне уявлення про динаміку розвитку АМ під час експериментального цукрового діабету.

Висновки. У дослідженні виявлено динамічне прогресування змін в альвеолярних макрофагах при перебігу експериментального цукрового діабету. Рання підвищена функціональна активність переходить у дистрофічні зміни, що дає змогу зрозуміти розвиток функціональної недостатності макрофагів у міру розвитку діабету, сприяючи розумінню респіраторної патології, пов'язаної з діабетом.

КЛЮЧОВІ СЛОВА: цукровий діабет; легені; експеримент; альвеолярні макрофаги; патофізіологія.

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