Structural And Functional Features Of Immunocompetent Breast Cells Glands During Pregnancy And Lactation In Chronic Hepatitis

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ABSTRACT

We investigated the structural and functional features of immunocompetent breast cells in the dynamics of pregnancy and lactation and against the background of chronic hepatitis. The work was carried out on sexually mature female white outbred rats, the right inguinal mammary glands of non-pregnant women were examined on days 5, 14, 17, 21 of pregnancy and on days 1, 3, 7, 15, and 21 after birth in normal conditions and chronic gelnotrine hepatitis. The samples were examined using morphological and electron microscopic research methods. It was found that in the dynamics of pregnancy and lactation, there is a gradual infiltration of the perialveolar connective tissue and terminal secretory sections of the mammary gland by immunocompetent cells, reaching their peak on the 3rd day of lactation. In immunocompetent cells of the mammary gland, ultrastructural changes occur, associated with certain relationships with epithelial cells, which determine the transfer of adoptive immunity to the newborn. In experimental chronic hepatitis in late pregnancy and in the dynamics of lactation, a decrease in the infiltration of the mammary gland by immunocompetent cells, an increase in the content of heterophagosomes of macrophages and lysosomes in lymphocytes, indicating violations of the immuno-epithelial relationships in the mammary gland, was established.

Keywords: mammary glands, pregnancy, lactation, chronic hepatitis, immunocompetent cells.

Article Received: 18 October 2020, Revised: 3 November 2020, Accepted: 24 December 2020

INTRODUCTION

It is known that postnatal development and formation of internal organs is a genetically determined natural process, an integral part of a complex, not fully deciphered process of organogenesis, adaptation, formation and establishment of interorgan relationships [2, 3]. Integrative relations in each organ, between them, between maternal and child organisms before and after birth, as well as the influence of unfavorable factors on their development and formation, have repeatedly become the object of research. The frequency of various extragenital pathologies in women of childbearing age is increasing. First of all, the frequency of chronic lesions of the hepatobiliary system increases as a consequence of the transferred viral or toxic hepatitis, which, under the influence of unfavorable environmental conditions, often take on a chronic, protracted form [8, 14].

In recent years, women suffering from hepatitis during pregnancy and after childbirth have become the object of close attention from obstetricians, gynecologists, infectious disease specialists, pediatricians, since specialists are well aware of the possible dire consequences of this disease for the fetus and offspring. In addition, it is well known that the mammary glands after childbirth are the only organ that connects the body of the mother and the newborn, which has been found to be immature in many mammalian species, including humans.

Therefore, the importance of breastfeeding for the full growth, development and formation of the internal organs of the offspring during early postnatal ontogenesis is decisive, both for providing it with the necessary nutrients, as well as for the transfer of adaptive immunity. In this regard, the impact of various extragenital diseases on the body of pregnant and breastfeeding women remains one of the most pressing problems. In recent years, significant data have been collected on the effect of diseases of the hepatobiliary system in pregnant women on mother and child [8, 15].

An adverse effect on the immune, digestive and other systems of children born to mothers who had chronic hepatitis before and during pregnancy and subsequent lactation was revealed [2]. At the same time, in the literature available to us, we were unable to find data on the effect of maternal hepatitis on the structural and functional features of lactation processes. Whereas, the secretory process of the mammary glands is described in detail in the literature [4, 6], however, as for the immunocompetent cells (ICC) of the mammary gland, in particular, the study of their dynamics during pregnancy and lactation, as well as their ultrastructural features, are still not completely studied. The results of scientific research on the ultrastructural features of immunocompetent cells are sometimes even contradictory: some authors interpret immunocompetent cells in the mammary gland as large lymphocytes, while others call them dendritic cells similar to Langerhans cells [16, 17, 19]. In this regard, the purpose of our study was to study the structural and functional properties of immune cells of the mammary glands during pregnancy and lactation in intact female rats and patients with chronic hepatitis.

MATERIALS AND RESEARCH METHODS. In the experiment, 150 sexually mature female white outbred rats weighing 150-170g were used. The animals were kept in a vivarium on a regular diet with free access to water, in accordance with the recommendations of international ethical committees on humane treatment of laboratory animals (EU Council directive of November 24, 1986 86 / 609EEC and the provisions of the Declaration of Helsinki of the World Medical Association, 1996). The rats were divided into 2 main groups. The first (intact) group of rats (60 rats) were mated with male rats, and their pregnancy was monitored using vaginal swabs. In this group, 100% of female rats were pregnant. In the second group (90 rats) - chronic heliotrinic hepatitis was induced according to the method recommended by N.Kh. Abdullaev and H.Y. Karimov [1]. On the 60th day of chronic hepatitis, female rats mated with males.

The study of vaginal swabs showed that only 84% of animals in this group were pregnant. Animals of both intact and experimental groups were killed by decapitation under ether anesthesia on days 5, 14, 17, 21 and 1, 3, 7, 15 and 21 of lactation. The comparison group used healthy and completely unfertilized females, as well as female rats on the 60th day of heliotrinic hepatitis of the same weight. Samples of the right inguinal mammary gland were used for the study, using morphological and electron microscopic research methods. Ultrathin sections were prepared on an LKB-4800 ultratome and examined under a JEM-100S electron microscope.

On semi-thin sections stained with pyronine G and methylene blue, the number of ICCl was calculated per 1000 alveolar lactocytes, and in the perialveolar stroma of the mammary gland - per 1x106 mm2 of the section area, the digital data are expressed in%. The obtained digital data were processed by parametric methods of variational statistics using the Fisher-Student criteria and computer programs. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION. The mammary gland of non-pregnant sexually mature rats is represented mainly by numerous glandular ducts surrounded by connective tissue, which are located in adipose tissue in the form of large compact groups. The glandular tissue is represented by interlobular and intralobular ducts, as well as end secretory sections in the form of terminal and alveolar kidneys. Studies have shown that the terminal secretory sections in non-pregnant sexually mature rats are formed by lactocytes and myoepithelial cells, and not related to epithelial cells, the so-called "light" cells. During cytometric studies, it was found that the "light" cells (2.2 ± 0.12), located among the lactocytes of the alveoli in non-giving birth, non-pregnant rats, are represented mainly by lymphocytes (1.4 ± 0.11) and monocytes (0.9 ± 0.12). The predominant ICCl of the perialveolar connective tissue of the mammary gland of the control group of animals are monocyte-like cells (transitional form of cells from monocytes to macrophages) and small lymphocytes.

Significant changes in the amount of ICCl are found on the 14th day of pregnancy. During this period, there was a tendency towards the beginning infiltration of the alveoli of the mammary gland with ICCl, the number of which increased by 1.2 times, mainly due to large lymphocytes (1.56 ± 0.96) and monocyte-like cells (1.14 ± 0.11). Perialveolar connective tissue at this time is loosened and contains a large number of cellular elements. The number of ICCl in the connective tissue also increases and exceeds the control values by 1.8 times (it increases by 22.6 ± 1.4 with control 12.5 ± 0.8). This is mainly due to the appearance of a significant number of large lymphocytes (2.9 ± 0.24), an increase in the number of medium lymphocytes (4.8 ± 0.2) and macrophages (2.5 ± 0.14). For the first time during this period, plasmocytes were detected among the ICCl of the perialveolar stroma.

On the 21st day of pregnancy, the number of ICKl reaches its maximum, more than 3 times higher than that in the previous gestational age (8.3 ± 0.5). During this period, intraepithelial macrophages (0.9 ± 0.09), small lymphocytes (1.3 ± 0.22) and plasma cells (0.1 ± 0.09), appear in the alveoli of the mammary gland. In the connective tissue stroma, the number of ICCl decreases slightly (15.0 ± 0.8), which is probably due to their migration into the epithelium. Attention is drawn to the high content of plasma cells (2.4 ± 1.4). A peculiar dynamics of quantitative changes in ICCL is observed on the 1st day after childbirth. During this period, the number of intraepithelially located ICCs decreases by 1.4 times (0.6 ± 0.06), compared with that on the 21st day of pregnancy, which occurs mainly due to lymphocytes. At the same time, the number of ICCl in the perialveolar connective tissue increases due to its infiltration by monocyto-like, plasma cells and small lymphocytes.

On the 3rd day of lactation, the number of intraepithelial ICCs in the alveoli reaches its maximum values (12.7 ± 1.1), mainly due to an increase in the number of small lymphocytes (3.0 ± 0.26), plasmocytes (0, 62 ± 0.06) and monocyte-like cells. The amount of ICCl in the perialveolar connective tissue also increases and reaches its maximum (31.8 ± 2.9). At the same time, a significant increase in the number of monocyte-like cells (11.2 ± 0.49) was found, with a moderate decrease in the number of small lymphocytes (1.5 ± 0.14) and plasmocytes (9.6 ± 1.1). Further, on the 15-21st day of lactation in the mammary gland against the background of a decrease in the secretory activity of lactocytes, the number of ICCl gradually decreases both in the alveoli (3.6 ± 0.19) and in the perialveolar stroma of the organ (10.8 ± 2.1).

Analysis of the ultrastructure of these cells showed that there are several types. Among the "light" cells, the most common are cells that resemble monocytes in their submicroscopic structure. Their nuclei are irregularly bean-shaped and contain dense clumps of heterochromatin. The cells have a light cytoplasm, and the plasmolemma forms numerous outgrowths resembling pseudopodia. These cells are characterized by the presence of a membrane of 5-11 electron-dense granules in the cytoplasm.

Another cell type is distinguished by the presence of a high nuclear-cytoplasmic ratio and a relatively thin rim of the surrounding cytoplasm. Their nuclei have an irregular shape due to deep invaginations. Individual lysosomal granules can be seen in some cells. These cell types are similar in their submicroscopic properties to medium-sized lymphocytes.

Finally, the structure of the third type of cells found in the mammary glands is closely related to the structure of lymphoblasts and prolymphocytes. They have a large nucleus containing mainly euchromatin. The thin cytoplasm contains a large number of polysomes, while other organelles are less developed. It should be noted that all these ICCLs are in close contact with epithelial cells, using cytoplasmic micro-outgrowths. Macrophages and lymphocytes are also common in the perialveolar stroma of the mammary glands of non-pregnant rats. By their submicroscopic properties, they are almost indistinguishable from similar cells in other organs. At the same time, it should be noted that cells similar to the above-mentioned intraepithelial ICCs are not found in the stroma.

It is known that pregnancy is the strongest factor in the development of the mammary glands and their readiness for lactation. Pregnancy also leads to submicroscopic changes in the ICC of the mammary glands. During pregnancy, most ICCLs resemble secondary lymphocytes and monocytes in their submicroscopic properties. At the same time, during pregnancy, cells transitional forms of monocytes into macrophages. In addition, lactocytes include cells that are at least in the process of converting lymphocytes into plasma cells. The formation of cells at different stages of the transformation of monocytes into macrophages, lymphocytes into plasma cells can be explained by some manifestations of local immunogenesis in the mammary glands. The formation of these processes ultimately ensures the synthesis and secretion of immunoglobulins in milk.

After the birth of the fetus, that is, during lactation, the mammary glands begin to function actively. During this period, the submicroscopic structure of immunocompetent cells found among the secretory epithelium can be divided into monocytes, granulocytes, macrophages and plasma cells. Intraepithelial macrophages are characterized by the presence of a large number of primary and secondary lysosomes in the cytoplasm. These cells are irregular in shape and have numerous cavities, invaginations, processes and pseudopodia. Some macrophages contain a group of lipid granules concentrated at one pole of the cytoplasm. Similar granules are found in the cytoplasm of some active lymphocytes. Submicroscopic changes close to the above were also observed in immunocompetent cells located in the perialveolar stroma during pregnancy and lactation. In non-pregnant animals, these cells are mainly monocytes and small lymphocytes. Towards the end of pregnancy, they are in the process of converting monocytes to macrophages and lymphocytes to plasma cells. The early stages of lactation are characterized by the transformation of monocytes into typical macrophages, the formation of a large number of plasma cells and granular lymphocytes with high activity.

In chronic toxic hepatitis in the mammary gland in the dynamics of pregnancy and lactation, there is some lag in the development of the glandular tree relative to control animals. It was cytometrically established that starting from the 17th day of pregnancy, a 1.2-fold decrease in the number of intraepithelially located ICCs (2.9 ± 0.1) was established, mainly due to large lymphocytes (0.36 ± 0.08) and macrophages (0.31 ± 0.02), against the background of a 1.4-fold increase in the number of small lymphocytes, relative to control animals. This trend continued on the 21st day of pregnancy, where the number of intraepithelial cells decreases (6.56 ± 0.21), against the general background of a decrease in all types of ICC, an increase of 1.5 times in the number of small lymphocytes is noted. With the onset of the lactation period in the mammary gland of female rats, a decrease in the number of ICCl of the terminal secretory divisions continues until the end of the lactation period. In the study of the perialveolar connective tissue of the mammary gland of hepatitis rats starting from the 14th day of pregnancy against the background of a decrease in the total number of ICCL, an increase in the infiltration of the gland stroma by medium and small lymphocytes is noted, which lasts up to 3 days of lactation.

A study of ultrastructural features in the setting of chronic hepatitis showed that pregnancy led to the activation of intraepithelial immune cells in the mammary glands. As in a healthy organism, an increase in typical lysosomes in the cytoplasm of macrophages and the formation of large granulo-containing lymphocytes were found. Pregnancy caused by hepatitis has also led to the activation of immune cells in the connective tissue stroma. The highest activity of macrophages, granular lymphocytes and plasma cells in animals with chronic hepatitis was observed during lactation.

It should be noted that in conditions of chronic hepatitis, immune cells in the mammary glands have a specific submicroscopic character. In particular, unlike healthy animals, the cytoplasm of macrophages has a large number of large heterophagosomes, and lymphocytes have a significant increase in the number of lysosomes. By the end of the lactation period, different stages of the process of phagocytosis of destroyed secretory cells by macrophages were observed.

The results presented above show that the submicroscopic structure of intraepithelial and stromal cells has unique characteristics before pregnancy, during pregnancy and lactation. In non-pregnant animals, they mainly consist of less differentiated cells such as lymphoblasts. The submicroscopic structure of cells does not change much during pregnancy. An increase in the number of organelles and lysosomes was found only in cells with a monocyte structure and an increase in the number of lysosomal granules in lymphocytes. The strongest changes in the submicroscopic structure of cells are observed during lactation. During this time, lysosomes are formed in the cytoplasm of monocytes, as well as lipid droplets, which become typical macrophages. An increase in the number of organelles was observed in all types of cells, which indicates an increase in their activity (Scheme 1).

Pregnancy also changes the immune cells around the alveoli. At the end of pregnancy, intermediate cells such as macrophages and plasma cells were identified. In the early stages of lactation, a large number of macrophages and plasma cells with a typical structure were found in the connective tissue around the alveoli (Scheme 2).

Thus, pregnancy and lactation in healthy rats were accompanied by constant activation of various immune cells located in the mammary gland. There is ample evidence that pregnancy and subsequent lactation lead to profound immune endocrine changes in the body [16]. Submicroscopic changes in immune cells of the mammary gland can be interpreted as a manifestation of the body's adaptive processes. The activation of immune cells during pregnancy and especially during lactation is also associated with the transfer of adaptive immunity to the baby through breast milk. Since breast milk contains a certain amount of immunoglobulins, and other protective factors that protect the child's body from antigens in the early stages of his birth [3,7].

Chronic heliotrinic hepatitis leads to some delay in the development of glandular tissue and to a decrease in the number of ICCl both in the end secretory sections and in the perialveolar connective tissue stroma of the mammary gland during pregnancy and lactation. In contrast to the control animals, a higher activity of macrophages is noted, in the cytoplasm of which the formation of a large number of heterophagosomes is noted. Along with this, granules of lysosomal structure were identified in most lymphocytes, and an increase in the number of disintegrating plasma cells in the perialveolar stroma of the mammary gland was observed.

It is shown that during pregnancy and lactation in the mammary gland there is an enhanced development of the glandular epithelium due to the regression of adipose tissue, caused by hormonal shifts in the mother's body. After implantation of the fetus before delivery, the formation of a large amount of progesterone and estrogen is observed primarily in the ovary, and then in the placenta. If estrogens contribute to the development of the duct system, then progesterone activates the formation of alveoli [9, 19].

In hepatitis, profound changes in metabolic processes occur, including a violation of protein metabolism, which, naturally, affects the hormonal balance, and, consequently, the development of the placenta and mammary gland [12]. This is probably the first possible reason for the lag in breast development. Changes in the hormonal background in the mother's body after childbirth is the reason for the activation of the immune system. In the initial period of lactation, a relative increase in connective tissue is noted in the mammary gland. There is an increase in its permeability due to swelling and formation of a capillary network in the form of basket-like plexuses. These changes, apparently, are associated with the transfer of adoptive immunity to the infant. The second reason for some lag in the development of the gland is most likely associated with disturbances in the tissue relationships of the organ during this period, which also affects the qualitative composition of colostrum [5,13]. If we also take into account the immunodeficiency state of the mother with hepatitis, it becomes clear what is the reason for the lag in the development of the digestive and immune systems of the offspring established by many researchers [10,11,18].

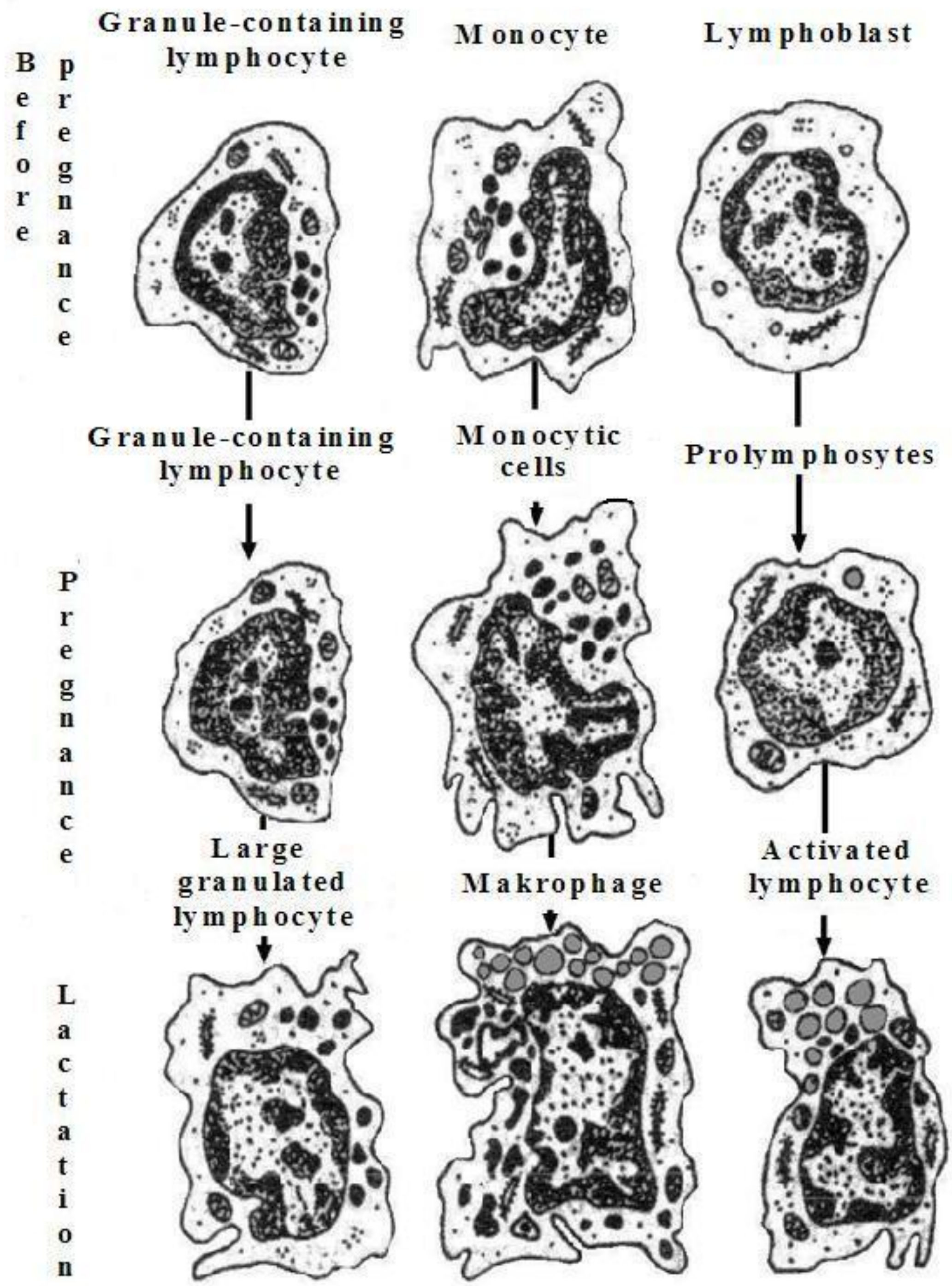
In addition, it has been established that the process of chronic heliotrin hepatitis is accompanied by profound morphological changes in the body's immune system. These changes lead to an imbalance between the T and B systems of the immune system and the development of an autoimmune process [14]. The increased activity of macrophages and granular lymphocytes in the mammary gland can be explained by the manifestation of immune disorders observed in heliotrinic hepatitis.

CONCLUSION

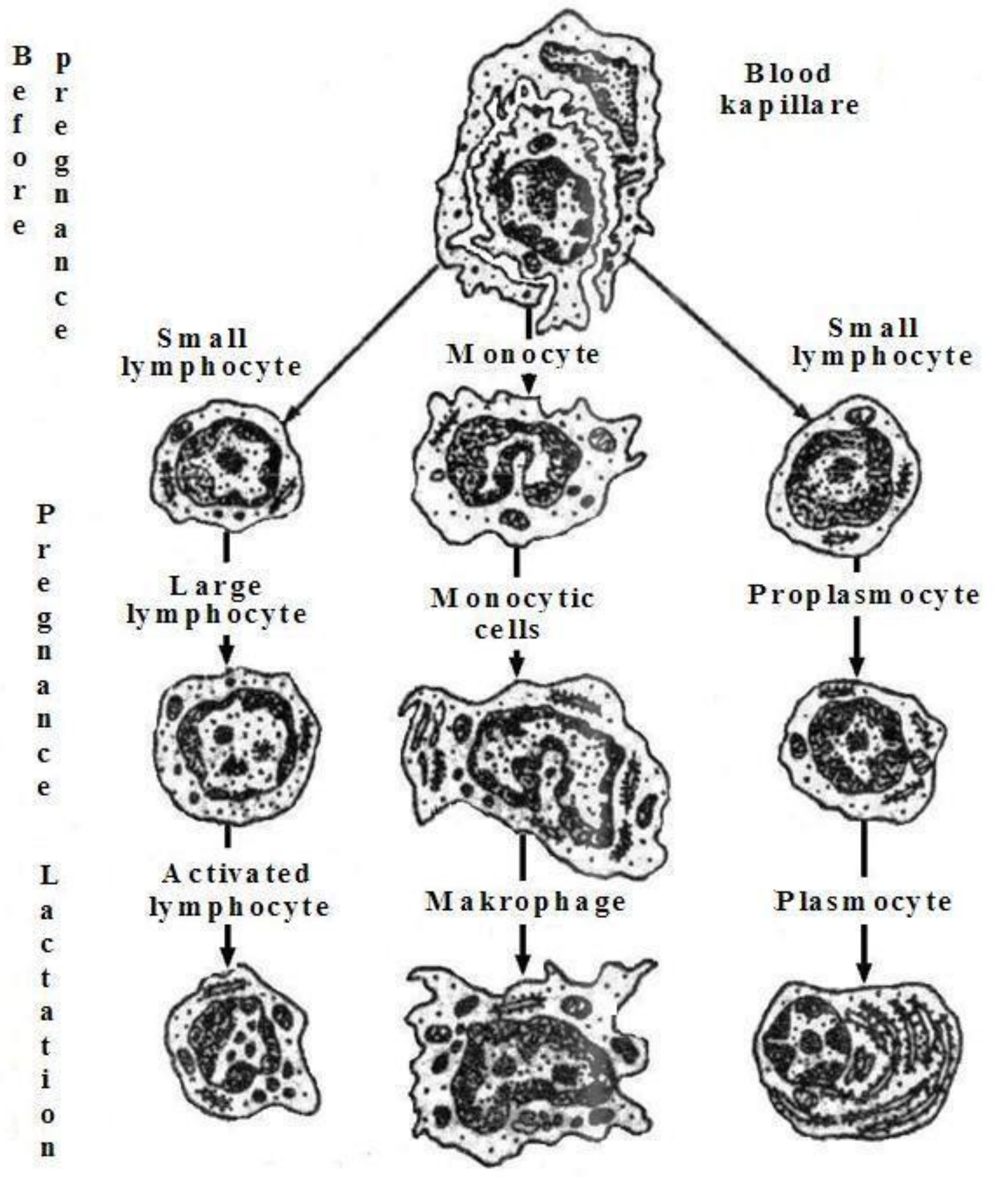
1. In the dynamics of pregnancy and especially during lactation, the breast tissue is infiltrated by immunocompetent cells, the submicroscopic structure of immune cells located in the mammary glands changes radically, and these changes are associated with the provision of adaptive immunity through breast milk.

2. A decrease in the number of interepielial ICCs and infiltration of the perialveolar stroma, a high level of hyperplasia of macrophages and granular organelles of lymphocytes in the mammary glands and an increase in the number of destructive plasma cells of the perialveolar stroma in the dynamics of pregnancy and lactation in chronic hepatitis indicates a violation of the immune-glandular epithelial relationship.

Scheme 1. Ulttrastural organization of intraepithelial immunocompetent cells of mammary gland during pregnanacy and lactation



Scheme 2. Ulttrastural rearrangements of immunocompetent cells of per alveolar connective tissue of mammary gland during pregnancy and lactation



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