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PECULIARITIES OF BLOOD FORMATION FROM BONE MARROW IN SECONDARY CHRONIC INFLAMMATION ON THE BACKGROUND OF MESENCHYMAL STEM CELLS

O. Shevchenko, N. Petryk

Kharkiv National Medical University, Ukraine

Author: **Shevchenko Oleksandr**, doctor of medical sciences, professor, Kharkiv National Medical University, professor of the Department of Pathological Physiology honorary named after professor D. O. Alpern, +380978299581, shevchenkoalexandr9@gmail.com, ORCID: 0000- 0001-6008-0362

Corresponding Author: **Nataliia Petryk,** Kharkiv National Medical University, MD, PhD student of the Department of Pathological Physiology honorary named after professor D. O. Alpern, +380955077724, perfectgynecologist@gmail.com, ORCID: 0000-0001-6427-0155

Abstract

The use of MSCs reduces the chronicity of inflammation due to greater activation of hematopoiesis, and, consequently, the entry of leukocytes into the blood and the focus in the initial stages of inflammation. The study of bone marrow hematopoiesis in the dynamics of secondary chronic inflammation shows that the use of MSCs reduces the chronicity of the process. This proves the possibility of using MSCs to prevent chronic inflammation.

Thus, inflammation on the background of the application of MSCs compared with the natural course of the process in the early stages of inflammation in the cell emigrates more leukocytes, compared with more distant terms, as they are less. In the early stages, there is more release of cells from the bone marrow into the blood, hematopoiesis is more significantly stimulated, and in the later stages, corresponding to the period of chronic inflammation - less activation of hematopoiesis.

This is probably because the increased migration of leukocytes into the site of inflammation in the early stages of the process provides a more effective fight against inflammation and, consequently, less likely to chronicle the process.

The use of MSCs leads to a decrease in the chronicity of inflammation due to greater activation of hematopoiesis, and, consequently, the entry of leukocytes into the blood and hearth in the initial stages of inflammation.

The results of our studies of bone marrow hematopoiesis in the dynamics of secondary chronic inflammation show that the use of MSCs reduces the chronicity of the process.

The prospect of further research is related to the improvement of pathogenetic therapy and the prevention of chronic inflammation. Further additional research should be conducted to study not only the local effect of MSCs on the course of inflammation but also the systemic administration of MSCs.

Key words: secondary chronic inflammation; bone marrow; hematopoiesis; mesenchymal stem cells.

Background

Chronic inflammation is the basis of most human diseases. It is a central problem of medicine throughout its history – no less common and acute inflammatory processes, which can become life-threatening. Simultaneously, the number of primary chronic inflammatory diseases is growing [1-3]. This is probably due to the external and internal environment factors, the problems of immunological reactivity [4-6].

Inflammation in evolutionary terms is a protective-adaptive reaction in the form of pathology, an emergency way of protecting the whole organism at the cost of damaging its part [7-10]. Chronic inflammation is characterized by the loss of protective and adaptive value of this reaction and the transformation into an isolated damaging factor [11-14]. At the same time, the general pathology and prevention of chronic inflammation are insufficiently studied.

It is known from the literature that there is a general concept according to which chronic inflammation can be the leading cause of cancer and severe aging. Moreover, many studies show that chronic inflammation can play a significant role in various age-related diseases, including diabetes, cardiovascular, and autoimmune diseases [15]. Mesenchymal stem cells (MSCs) are valuable but not well-founded means of improving chronic inflammation regulation. MSCs are universal progenitor cells found in most tissues of the body where there is a vascular component. It is known that these cells can differentiate. MSC is characterized by immunomodulatory and trophic activity [16].

Initially met with great skepticism, mesenchymal stem cells' immunomodulatory ability was then proven and well reproduced in experiments [17]. This opened the possibility for the use of mesenchymal stem cells for tissue replacement-regeneration and the treatment of immune-mediated and inflammatory diseases [18]. It turned out that the use of mesenchymal stem cells in inflammatory diseases had the most significant effect.

Even though there are many works on the regenerative qualities of mesenchymal stem cells [19], but very few studies on the pathogenetic effects of mesenchymal stem cells on the processes of chronic inflammation.

Therefore, the study of mesenchymal stem cells' influence in preventing chronic inflammation in the experiment is an important area of modern pathophysiology.

Materials and methods

The experiment was performed on 132 adult male laboratory rats (weighing 200-220 g, aged 4-5 months) in Kharkiv National Medical University's vivarium. Animals were randomly divided into 22 groups of 6 animals in each group, the usual number of animals, and the minimum sufficient to achieve the study's goals and objectives, the number of experimental groups.

MSCs were introduced into the area of carrageenan inflammation in rats at a dose of 0.5 ml (1 - 2 million cells) in isotonic sodium chloride solution once a day with λ -carrageenan.

The control of the usual course of inflammation was intact rats - 6 animals, and rats injected with MSCs without causing inflammation (6 animals).

Animals were killed by inhalation of high concentrations of carbon dioxide (CO2) followed by decapitation at 6 o'clock, 1st, 2nd, 3rd, 5th, 7th, 10th, 14th, 21st on the 28th day of inflammation. Laboratory animals were kept in separate cages, six rats in each cage at room temperature $21 \pm 2^{\circ}$ C, humidity 45 - 50%, cyclic mode of daylight/darkness 12 hours / 12 hours with access to food water for 24 hours. The Ethics Committee of Kharkiv National Medical University approved our study.

The rat femur's bone marrow canal was washed with 3% acetic acid solution in an amount of 1 ml. The total number of megakaryocytes was counted in Goryaev's chamber. Bone marrow smears were fixed in ethanol and stained with azure-eosin by the method of

Romanovsky-Gimse. The relative number of blast cells, mature and immature neutrophils, eosinophils, monocytes, lymphocytes, and erythroid cells was counted [20].

The obtained results were processed using the Student's t-test.

Results and discussion

In the natural course of inflammation, there were wavy changes in the total number of megakaryocytes (Fig. 1).



Fig. 1. The total number of megakaryocytes in the bone marrow in the dynamics of carrageenan secondary chronic inflammation in rats during its natural course and on the background of mesenchymal stem cells

There are several waves of significant increase in the number of megakaryocytes on the 1st day, 14th, and 28th day (respectively two times, p <0,05; 2,3 times, p <0,05 and 2,9 times, p <0,05). One wave coincides with the activation of hematopoiesis due to a pronounced neutrophil reaction. The other two waves of increased megakaryocyte counts indicate further activation of hematopoiesis due to chronic inflammation.

Besides, in the natural course of inflammation, the total number of megakaryocytes significantly dropped on the 1st day compared with the control by 1,9 times, p <0,05. This is probably due to the provoked release of leukocytes into the inflammation site, even though a monocytic-macrophage reaction with bone marrow hyperplasia was observed.

In inflammation caused by the use of mesenchymal stem cells, compared with the natural course of the inflammatory process, the total number of megakaryocytes is significantly higher on the 1st day 1.8 times, p <0,01 and significantly lower on the 14th day (2,6 times, p <0,001, p <0,05).

Thus, in inflammation caused by the use of mesenchymal stem cells, the total number of megakaryocytes in the early stages is slightly higher, and in the later stages - significantly lower than in the natural course of inflammation.

The number of blast cells in the natural course of inflammation was significantly reduced by 6 hours compared with the control by 6,3 times, p < 0,05. At other times the study did not differ statistically from that in control. (Fig. 2)



Fig. 2. The total number of blast cells in the bone marrow in the dynamics of carrageenan secondary chronic inflammation in rats during its natural course and on the background of mesenchymal stem cells

There is also a tendency to increase the number of blast cells on the 2nd - third day and 10th day.

At an inflammation against application of MSK, in comparison with a natural current of an inflammation, the considerable excess of number of blast cells on the 10th day in 3,9 times, p <0,05, is significantly lower on the 7th day in 1,6 times is registered, p <0.05 and there was a decrease in the number of blast cells to the 14th day.

The number of neutrophils in the natural course of inflammation is significantly increased by 6 hours; seventh and 28th days (respectively 1.3 times, p < 0.05; 4.0 times, p < 0.01 and 5.4 times, p < 0.05) (Fig. 3)



Fig. 3. The total number of neutrophils in the bone marrow in the dynamics of carrageenan secondary chronic inflammation in rats during its natural course and on the background of mesenchymal stem cells

With inflammation on the background of MSCs, compared with the natural course of inflammation, there is a significant increase in the number of neutrophils at 6 hours and ten days (respectively, 1.2 times, p <0,05 and 5,3 times, p <0.05). Attention is drawn to the marked decrease in immature neutrophils on the 7th and 21st day (respectively 2.38 times, p <0.05 and 2.9 times, p <0.01). There is a shift of the peak from the 21st day to the 6th hour, which, as well as about blast cells, reflects the earlier activation of hematopoiesis.

The content of eosinophils in the bone marrow in the natural course of inflammation also has a wavy tendency to change: there was a significant decrease in the number of eosinophils on the 1st, 7th and 14th days (respectively 3.89 times, p <0.05; in 2, 65 times, p <0.05 and 7.6 times, p <0.01) and then on the 21st and 28th day of recovery of their number (Fig. 4).



Fig. 4. The total number of eosinophils in the bone marrow in the dynamics of carrageenan secondary chronic inflammation in rats during its natural course and on the background of mesenchymal stem cells

Noteworthy is eosinophils' process of leaving the depot in the periphery, which predominates in their products, especially on the 1st, 7th, and 14th days. Increased release of eosinophils into the blood during this period of inflammation reflects their emigration to the cell in the acute period of inflammation, and on the 21st and 28th day - chronic inflammation, probably because eosinophils are found in large numbers in the blood and foci of granulomatous inflammation [21].

When inflammation on the background of the use of MSCs in comparison with the natural course of inflammation, the content of eosinophils is significantly lower on the 7th and 21st day (respectively 1.3 times, p <0.05 and 1.4 times, p <0.001), but significantly higher on the 14th day by 9.1 times, p <0.05. More eosinophils are released into the blood on the 7th and 21st day, greater activation of hematopoiesis on the 14th day due to bone marrow hyperplasia than in the natural course of the process. The decrease in the content of eosinophils in the bone marrow on the 21st-28th day is associated with less chronic inflammation during the period of chronic inflammation.

The number of monocytes in the bone marrow in the natural course of inflammation was significantly reduced on the 7th and 21st day (respectively 4.3 times, p <0.05 and 6.4 times, p <0.01) and increased by 5- in and on the 28th day (respectively 4.8 times, p <0.05 and 6.8 times, p <0.05), which is characteristic of chronic inflammation (Fig. 5).



Fig. 5. The total number of monocytes in the bone marrow in the dynamics of carrageenan secondary chronic inflammation in rats during its natural course and on the background of mesenchymal stem cells

The decrease in monocytes' content on the 7th and 21st day is associated with their increased output from the bone marrow into the peripheral blood and subsequently into the site of inflammation.

With inflammation on the background of the application of MSCs compared with its natural course, the monocytes' content is significantly higher on the 10th day and less on the 5th day (respectively 4.4 times, p <0.001 and 2.1 times, p <0.001).

Thus, at an inflammation against MSKs application compared with a natural current of an inflammation, more expressed activation of a monocytopoiesis in early terms compared with late is noted. This indicates a decrease in the chronicity of inflammation.

Conclusions

Thus, in inflammation on the background of the application of MSCs compared with the natural course of the process in the early stages of inflammation in the cell emigrates more leukocytes, compared with more distant terms, as they are less. In the early stages, there is more output of cells from the bone marrow into the blood, hematopoiesis is more significantly stimulated, and in the later stages, corresponding to the period of chronic inflammation - less activation of hematopoiesis [22, 23].

This is probably because the increased migration of leukocytes into the inflammation site in the early stages of the process provides a more effective fight against inflammation and, consequently, less likely to chronize the process. The use of MSCs leads to a decrease in the chronicity of inflammation due to greater activation of hematopoiesis and, consequently, the entry of leukocytes into the blood and hearth in the initial inflammation stages.

The results of our studies of bone marrow hematopoiesis in the dynamics of secondary chronic inflammation show that the use of MSCs reduces the chronicity of the process. This proves the feasibility of using MSCs to prevent chronic inflammation.

Further research related to the improvement of pathogenetic therapy and chronic inflammation prevention is in high demand.

Further additional research should be conducted to study the local effect of MSCs on the course of inflammation and the systemic administration of MSCs.

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