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REGENERATIVE AND HEPATOSPECIFIC ACTIVITY OF TOTAL RNA FROM XENOGENIC BONE MARROW CELLS

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Objective: to study the peculiarities of the induction effect of total RNA (tRNA) from xenogenic bone marrow cells (BMCs) on regeneration processes in the recipient's native liver with extensive liver resection using an adoptive transfer model. **Materials and methods.** The study was carried out on an adoptive transfer model using male Wistar rats (n = 20) and guinea pigs (n = 17). The donors were rats (n = 10). 12 hours after extensive liver resection (70-75%), tRNA was isolated from BMCs and injected into intact (non-operated) recipients intraperitoneally at a dose of 30 μg/100 g of weight. The induction effect of the tRNA on operated rats was studied in 3 groups of recipients: Group 1 (control, n = 5) – administration of saline to guinea pigs; Group 2 (control, n = 10) – administration of tRNA from a donor rat to a recipient rat (allogeneic transfer); Group 3 (experiment, n = 12) – administration of tRNA from a donor rat to a recipient guinea pig (xenogeneic transfer). In histological preparations of recipient livers, after 48, 72 hours and 7 days, we studied the mitotic activity of hepatocytes and the features of the microscopic picture of the liver. The significance of differences in the compared groups was assessed using the parametric Student's t-test. **Results.** The ability of BMC tRNA to tissue-specifically activate regenerative and immune responses in the liver after extensive resection was found to depend on the donor and recipient species identity. Introduction of allogeneic donor tRNA in the recipient's liver resulted in predominant enhancement in hepatocyte mitotic activity ($p < 0.05$). The use of xenogeneic donor tRNA leads to enhanced activity of only immuno-inflammatory reactions in the recipient's liver, such as sinusoidal cell activation, lymphocytic infiltration into sinusoids, and portal tract infiltration by inflammatory cells. **Conclusion.** To induce regenerative processes in the liver, tRNA obtained from allogeneic BMCs should be used.

Keywords: bone marrow cells, total RNA, xenogeneity, adoptive transfer, liver, resection, regeneration.

The therapeutic potential of RNA specimens from animal parenchymatous organs, which were used to activate regenerative processes in homologous damaged organs, was the subject of in-depth studies at the end of the last century [1–3]. Currently, owing to the development of the theory of stem cells and the improvement in application of cell technologies in medicine, research on regulation of regenerative processes in damaged organs has focused on studying the prospects of using total RNA (tRNA) from lymphoid bone marrow cells (peripheral blood lymphocytes, thymus, spleen and bone marrow cells). It has been shown that tRNA isolated from these cells, similarly to immune system cells, is able to actively participate in the regulation of physiological and repair regenerative processes in organs and tissues of various histotypes [4–7], and therefore can be used as a universal means of regenerative therapy. When injected into a recipient's body, various tissue RNAs and, moreover, RNAs of immune system cells, which include bone marrow cells, do not only regulate repair morphogenesis processes in damaged organs. They are also capable of inducing immune responses that can weaken or even distort the degree of regenerative processes. For example, when using xenogenic donor material, which, in terms of economy and availability, is one of the most preferred sources for obtaining tRNA specimens for medicine [8].

The aim of this study is to study the effect of tRNA derived from rat bone marrow cells after extensive liver resection on induction of regenerative processes in a guinea pig liver, using an adoptive transfer model.

MATERIALS AND METHODS

The work was performed on male Wistar rats weighing 250–300 g (n = 20) and guinea pigs weighing 350–450 g (n = 17). The adoptive transfer model was used to study the features of the effect of xenogeneic tRNA on regenerative processes in the liver [9]. Earlier, using this model, we proved that tRNA from bone marrow cells (BMCs) of an allogeneic donor effectively performs targeted delivery of regenerative signals to the damaged liver of an allogeneic recipient [7]. To prove the ability of tRNA from xenogeneic BMCs in liver damage to

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transfer regenerative information, we used an experimental model of extensive liver resection (70–75%), which is known to be accompanied by activation of hypertrophic regeneration mechanisms with pronounced mitotic activity in the remaining part of the organ [10]. Rats with partial hepatectomy constituted the donor group (n = 10). Bone marrow was taken from donor rats 12 hours after liver resection (this interval is required for the appearance of morphogenetically active cells in the bone marrow) and mononuclear (hematopoietic) BMCs fraction was isolated from it, which was then used to obtain tRNA. Total RNA from the mononuclear fraction of BMCs was isolated by the method developed by biotechnology company Evrogen (Russia) using the ExtractRNA reagent, which made it possible to obtain from 105.5 to 127.7 μg of total RNA from each 3.5×10^7. The ability to accumulate and transfer regenerative signals specifically to the liver when using tRNA from the mononuclear fraction of BMCs was assessed in rats based on severity of induction of the proliferative activity of liver hepatocytes in intact recipients 48 hours, 72 hours, and 7 days after they were injected with donor material (tRNA from rats with liver resection) at a dose of 30 μg/100 g of animal weight.

The recipients were divided into 3 groups: group 1 consisted of control injection of saline to guinea pig (n = 5); group 2 included administration of tRNA from a donor rat to a recipient rat (n = 10); group 3 was made up of administration of tRNA from donor rat to recipient guinea pig (n = 12). At the indicated time after tRNA administration, liver pieces were taken from recipients, histological specimens were prepared from them, followed by hematoxylin and eosin staining. The number of mitotically dividing cells was determined in 30 fields of view (Leica DMLS microscope, Germany) with subsequent calculation of the mitotic index (MI) in ppm (‰). The significance of differences in the mitotic activity of hepatocytes in the compared groups was assessed using the parametric Student's t-test ($p < 0.05$).

RESULTS AND DISCUSSION

It was found that in control group 1, where the recipients (guinea pigs) were injected with saline, mitotic activity of hepatocytes did not significantly differ from the original values at all the studied periods (48 hours, 72 hours, and 7 days). MI values did not exceed 0.02 ± 0.01‰ (0–2 mitosis per 30 fields of view), Fig. 1. There were no signs of cellular infiltration in the liver tissue of guinea pigs in this group at all the follow-up periods.

However, in control group 2, where activated tRNA from BMCs of rats was injected not into guinea pigs but into healthy allogeneic recipient rats intraperitoneally, a significant increase in mitotic activity of hepatocytes was noted at 48 and 72 hours after adoptive transfer. MI values at these periods were 0.7 ± 0.2 respectively ($p < 0.05$). Mitoses were detected in 5–7 out of the 30 studied fields of view compared to the original level (0–2 mitosis per 30 fields of view), Fig. 2.

By day 7, the mitotic activity of hepatocytes in this group had returned to the original values. It is important to note that in control group 2, at 48 and 72 hours, not only was there an increase in mitotic activity in the liver tissue of recipient rats, but also a weakly marked increase in cellular infiltration, indicating the appearance of hepatospecific (tissue-specific) immune signals in a healthy allogeneic recipient.

The results obtained in control group 2 showed that tRNA from BMCs is a carrier of both regenerative (proliferative) and immune signals induced by extensive liver resection in the donor's body. A study of the effect of adoptive transfer in experimental group 3, where activated

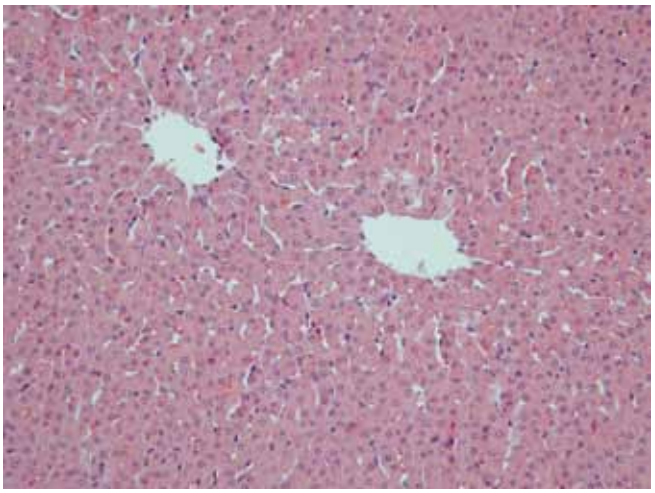

Fig. 1. Histological picture of the liver of a healthy guinea pig after administration of saline (control). No signs of hepatocyte proliferative activity and sinusoidal cell activation. H&E stain. 200× magnification

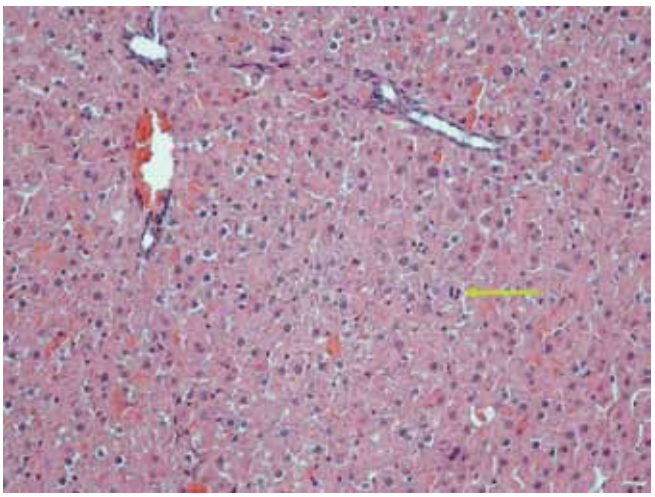

Fig. 2. Histological picture of the liver of a healthy rat 48 hours after administration of tRNA obtained from a rat with extensive liver resection (allogeneic adoptive transfer). Signs of hepatocyte proliferative activity (mitosis indicated by arrow). H&E stain. 200× magnification

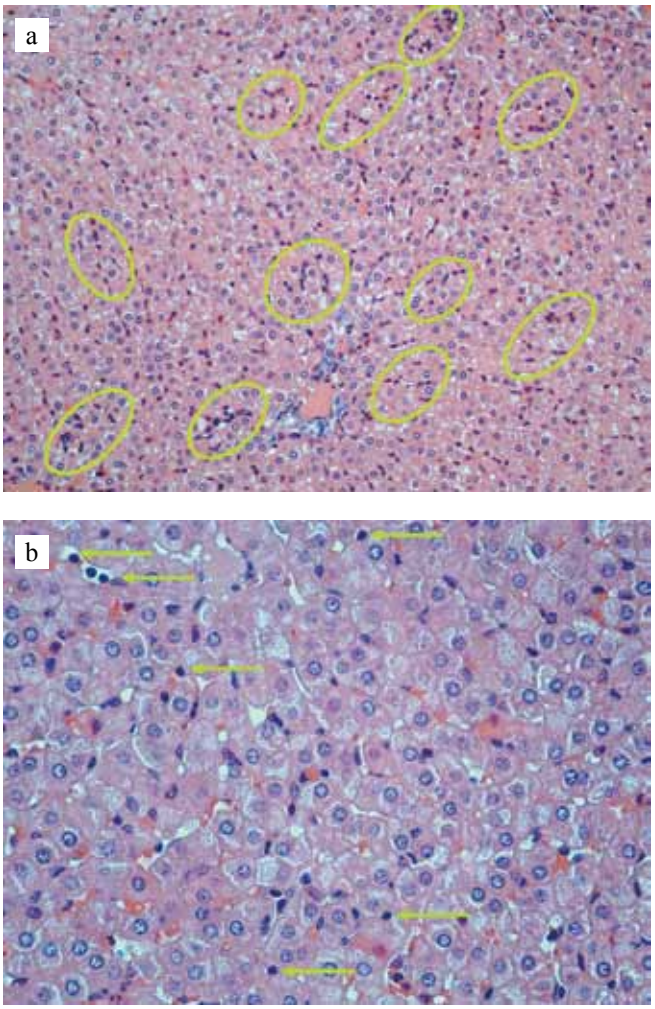

Fig. 3. Histological picture of the liver of a healthy guinea pig at day after 7 days of administration of tRNA obtained from a rat with extensive liver resection (xenogenic adoptive transfer): a) signs of pronounced sinusoidal cell activation (indicated by an oval). H&E stain. 200× magnification; b) lymphocytes in the sinusoidal lumen (indicated by arrows). H&E stain. 400× magnification

tRNA from BMCs of rats was injected intraperitoneally into intact xenogeneic recipients, showed that the histological picture of guinea pig liver significantly differed from the histological picture of the liver of allogeneic recipients (rats) in group 2.

It was established that for the mitotic activity of hepatocytes at the same observation periods (48 hours, 72 hours, and 7 days), MI values did not significantly differ from the initial level; they remained within 0.02 ± 0.01‰. Besides, histological preparations of the guinea pig liver at all studied periods showed diffuse activation of the liver sinusoidal cells, the presence of lymphocytes in sinusoids, as well as minor signs of infiltration of the liver portal tracts by inflammatory cells, which was especially pronounced at day 7 (Fig. 3, a, b).

Thus, it has been shown that xenogenic tRNA in a recipient's body during adoptive transfer does not induce mitotic and proliferative activity of hepatocytes but enhances hepatospecific immune response. Lymphoid cells, especially peripheral blood lymphocytes, are known to be carriers of regenerative signals in the body [4, 9], which are capable of targeted delivery of homologous and xenogenic RNA to cells [11]. The absence of a regulatory effect of activated tRNA on the mitotic activity of hepatocytes in group 3, apparently, can be associated with the fact that after contact with xenogeneic immune RNA, the recipient's lymphoid cells acquire new immunoregulatory properties and, upon contact with target organ cells, change the functional state of the RNA molecules of these cells [4]. Consequently, under the influence of xenogenic tRNA delivered to the cells, numerous regulatory protein-noncoding RNAs of the recipient liver cells become unable to exert regulatory effect on mRNA and activate translation and/or transcription of protein-coding genes at the level of the genome of these cells [6].

The mechanisms underlying the changes in the immunoregulatory properties of lymphoid cells in the recipient's body after their contact with immune RNA are not yet clear. However, incorporation of RNA into lymphoid cells should undoubtedly be one of the important factors for their subsequent activation.

In group 3 with introduction of xenogeneic tRNA in the recipient's liver, we detected activation of not only lymphocytes but also liver sinusoidal cells (due to the common mesenchymal origin with lymphocytes): Kupffer cells, endotheliocytes lining the liver sinusoids, perisinusoidal cells (Ito cells/stellate cells) and others.

Excessive activation of liver cells is what can explain the fact that when modeling liver damage by chronic CCl4 inoculation, induction of regenerative processes in the liver of mice using xenogeneic tRNA of rat liver is accompanied by a twofold increase in the amount of interlobular connective tissue and collagen at month 2 in comparison to the control [12]. Decreased number of necrosis foci in the liver was also noted. The authors believe that lower animal death may be associated not so much with increased mitotic activity of hepatocytes by this period, but with accelerated replacement of necrotizing liver cells by connective tissue and decreased intoxication.

When modeling adoptive transfer using xenogenic tRNA in the recipient's liver, the activity of liver cells of mesenchymal origin (sinusoidal cells) also significantly increases, as well as infiltration of the liver portal tracts by inflammatory cells, in the absence of activation of the mitotic activity of hepatocytes (see Fig. 2).

There is an opinion [13] that adequate exchange of regenerative information in the body is provided by production of two types of exosomes by immune cells: immune RNAs, which are involved in stimulating innate and acquired immunity mechanisms, and non-immune ones, through which RNA performs a remote synchronization of cell proliferation and differentiation processes. Based on the studies carried out, it can be concluded that

xenogeneic tRNA from BMCs stimulates predominantly immune regeneration mechanisms in the recipient's liver through activation of the inflammatory process. On the contrary, allogeneic (syngeneic) RNA predominantly enhances the mitotic and proliferative activity of parenchymal cells. These differences in the induction of regenerative processes in organs when using allogeneic (or syngeneic) and xenogeneic tRNA allow us to recognize that production and use of allogeneic tRNA preparations from BMCs is more effective, promising, and preferable than with xenogeneic tRNA preparations.

CONCLUSIONS

1. The adoptive transfer model allows to identify specific mechanisms for triggering the regenerative process when using allogeneic and xenogenic tRNA from BMCs.
2. The intrinsic ability of tRNA to hepatospecifically regulate regenerative and immune responses in the liver is expressed by predominant intensification of mitotic (proliferative) activity of hepatocytes when using allogeneic tRNA, and by intensification of immune-inflammatory reactions in the liver when using xenogeneic tRNA.
3. When choosing a source for tRNA isolation and application in the clinic, preference should be given to allogeneic sources of lymphoid cells, which effectively accelerate the processes of regenerative morphogenesis of cells of the damaged organ (liver).

The authors declare no conflict of interest.

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