Zoodiversity, **58**(5):429–434, 2024
DOI 10.15407/zoo2024.05.429

UDC 598.113.6

PATHOLOGICAL CHANGES IN THE LIVER PARENCHYMA OF REPTILES AS A FACTOR INFLUENCING THE PROCESSES OF HEMATOPOIESIS (ON THE EXAMPLE OF LACERTIDAE: *EREMIAS ARGUTA*)

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urn:lsid:zoobank.org:pub:71F58938-4CE9-4350-A0B5-22B6B8BBE81E

Pathological Changes in the Liver Parenchyma of Reptiles as a Factor Influencing the Processes of Hematopoiesis (on the Example of Lacertidae: *Eremias arguta*). **Akulenko, N. M.** — The influence of liver repair processes on haematopoiesis in scaly reptiles, in particular the steppe runner *Eremias arguta* (Pallas, 1773) is considered. Specimens with maximum and minimum liver parenchyma damage were selected from the same biotope and their liver myelogram indices were compared. It was shown that macrophage activation during liver repair stimulates lymphoid and myeloid haematopoietic progenitor cells in steppe runners. On the contrary, differentiation of erythroid cells at the normocyte stage is somewhat reduced, probably due to lack of resources.
Key words: Reptilia, liver, necrosis, hematopoiesis, macrophages.

Introduction

For successful division and differentiation of blood cells, vertebrates require action on progenitor cells by short-acting cellular mediators — colony stimulating factors, chalons, and similar macromolecules (Yaparla et al., 2016). In experiments with cell culture, it was shown that hematopoietic precursors respond to stimuli initially directed to the reproduction and differentiation of other cell types (Koibuchi et al., 2004, Mescher & Neff, 2005). It was also found that in the process of limb regeneration in poikilothermic vertebrates, phagocytic cells are activated, cellular mediators are released that stimulate the reproduction and activity of tissue macrophages, and defense mechanisms mediated by granulocytes and lymphocytes are activated (Mescher et al., 2017.) These data suggest that during the repair of extensive damage in the body, differentiation of individual hematopoietic lineages is activated automatically. At least, this can happen in a number of poikilothermic vertebrates (Alibardi, 2014, 2017; Mescher et al., 2017.)

Under the influence of anthropogenic pollution, pathological and compensatory changes occur in the liver of poikilothermic tetrapods. In particular, there are compensatory mechanism of accelerated proliferation and differentiation of hepatocytes. Unlike warm-blooded vertebrates, in frogs, extensive foci of necrosis are replaced by functional liver parenchyma, rather than fibrous formations (Akulenko, 2015). Complete regeneration of liver parenchyma in frogs is as interesting a phenomenon as limb regeneration in newts. This process involves melanomacrophage clusters, which are donors of pigments (Akulenko, 2021). Detailed studies of the tail regeneration process, including the participation of various cell lines in this process, were carried out on various species of the lizard family (Alibardi, 2014, 2017). At the same time, activation of some processes of specific and non-specific immunity was noted. It has been preliminary shown that the processes of tail regeneration in lizards do not have species specificity. Regeneration of internal organs in reptiles has been practically not studied; the relationship of this process with hematopoiesis and protective processes is unknown. In particular, amphibians and reptiles from polluted biotopes are characterized by liver regeneration with multiple foci of necrosis. We chose representatives of the species *Eremias arguta* (Pallas, 1773) as models. The pattern of liver response to pollution in scaly reptiles differs little, but the sensitivity to pollution in this species is quite high (Akulenko, 2015). Meanwhile, these processes are of interest as a theoretical model for studying the effect of nonspecific stimuli on hematopoiesis in vertebrates. Also, research in this area allows a better understanding of the mechanisms of regeneration in vertebrates.

Table 1. Indicators of myelograms of the liver in samples of steppe-runner, grouped depending on the degree of damage to the liver parenchyma, %

Indicators	Weak lesions of the parenchyma		Strong lesions of the parenchyma		Significance of differences	Correlation with the indicator "immature / mature myeloid cells"
	M	m	M	m		
Erythroid cells immature, sum	22	2.6	14	2.4	$p < 0.05$	
Erythroblasts	0.5	0.2	1.1	0.6		
Myeloid cells, sum	21	4.4	31	7.5		
Immature myeloid cells, sum	11	2.3	25	5.8	$p < 0.05$	
Lymphoid cells, sum	33	3.6	29	3.1		
Myeloid cells immature / mature ratio	1.8	0.7	8.6	3.0	$p < 0.05$	
Basophils	12	2.9	16	2.8		
Immature macrophages	0.4	0.3	2.9	0.8	$p < 0.01$	0.65 $p < 0.01$
Active macrophages	5.6	2.4	7.9	1.8		
Lymphoblasts	2.2	0.4	4.3	0.8	$p < 0.05$	0.64 $p < 0.01$
Plasma cells	1.7	0.3	3.7	0.8	$p < 0.01$	0.89 $p < 0.01$
Blasts, sum	4.5	0.8	9.2	2.1	$p < 0.05$	
Mitotic index	0.4	0.2	0.3	0.2		0.58 $p < 0.01$

Material and Methods

Studies of hematopoiesis were carried out on 20 mature specimens of steppe-runner, *Eremias arguta*, (8 males and 12 females, body length 4.5–5 cm) taken from moderately polluted biocenoses in the vicinity of the village Velyka Oleksandrivka, Kyiv Region. Animals were caught in the second half of August. After slaughtering the animals using ether anesthesia, histological preparations of the liver were made, as well as smears-imprints of the liver according to our modified method (Akulenko, 2021). Myelograms of the liver were counted on smears-imprints. Then, in Microsoft Excel, the integral indicators of myelograms were calculated for each animal, characterizing the intensity of hematopoiesis in the liver according to the previously described method (Akulenko, 2021). Basophilic granulocytes of amphibians and reptiles differ from eosinophils and neutrophils (heterophiles and azurophiles) in morphology, functions, and differentiate in other loci (de Carvalho et al., 2017), so they were not included in the "myeloid cells" column and are not were used to calculate myelopoiesis scores.

Correlation coefficients between certain indicators were determined according to the data of the general sample (20 steppe-runners) (see table 1). After studying the histological preparations, two samples were selected: animals with severe liver necrosis (7 specimens, 4 males and 3 females), animals with minimal manifestations of liver necrosis (7 specimens, 1 males and 6 females). For each sample the average values of the indicators, the error of the mean, and the significance of differences between the samples were calculated. All calculations were made in Microsoft Excel according to a previously developed method (Akulenko, 2021).

Results and Discussion

Interpretation of leukogram indicators in poikilothermic tetrapods is difficult because the concept of norm and pathology is very vague. Animals in natural biotopes are affected by many factors, such as dietary habits, environmental temperature, and the presence of infections and invasions (Stacy et al., 2011). As we noted, in poikilothermic tetrapods hematopoietic processes strongly depend on the season (Akulenko, 2012). In anthropogenically transformed landscapes, a new factor appears. It is a chemical pollution. The toxic effect of any specificity primarily leads to the liver parenchyma damage. We have described typical signs of such changes in hepatocytes of anurans (Akulenko, 2015). The combination of factors such as infections, nutrition, and pollution is individual for each population. In order to consider the impact of toxic liver damage on hematopoietic processes independently of other factors, it is necessary to compare animals that are in the same conditions. The best way out was to compare samples of animals taken from the same population (Akulenko, 2021).

To analyze the effect of changes in the liver on hematopoiesis, in this article we consider integral indicators: "immature erythroid cells, sum", "immature myeloid cells, sum", and "mature/immature myeloid cells, ratio". The first indicator most fully characterizes the intensity of erythropoiesis in the organ, and the next two reflect the intensity of myelopoiesis (Akulenko, 2021). According to a number of authors, cells with fine azurophilic granularity are present in the bloodstream of scaly reptiles (de Carvalho et al., 2017). We also found cells with fine amphophilic granularity typical of neutrophils in the bloodstream of the steppe-runner. A number of authors have established that such cells are similar to amphibian and mammalian neutrophils; these cells are actively involved in phagocytosis, which also makes them related to neutrophils (de Carvalho et al., 2017). Therefore, in our calculations, we considered this cell population as myeloid.

An analysis of the data given in the table 1 shows the difference between the samples with strong and weak changes in the liver of the steppe-runner. The influence of damage and repair processes in the liver affects all three hematopoietic lineages. In steppe-runner with pronounced manifestations of necrosis, erythropoiesis is significantly reduced, but indicators associated with cell-mediated immunity are significantly increased ("young macrophages", "lymphoblasts"). These myelograms do not fully reflect the functional activity of cells, but the enhanced differentiation of tissue macrophages and lymphocytes is evidence of the activation of these cell lines. The production of antibodies is stimulated too (column "plasmocytes"). The presence of significant correlations between the activation of granulocyte differentiation (indicator "immature myeloid cells, sum") and the reproduction of macrophages (indicator "young macrophages") and lymphocytes (indicator "lymphoblasts") is also an interesting fact. Based on our understanding of the immunity mechanisms, the primary should be the activation of macrophages, which directly remove the remains of necrotic cells from the liver (Grayfer & Robert, 2016; Paredes et al., 2019). It can be argued that the destruction of hepatocytes and the occurrence of foci of necrosis in the steppe-runner liver activate the mechanisms of cell-mediated immunity. It has been shown that activated tissue macrophages release factors that stimulate the proliferation of lymphocytes and granulocytes (Paredes et al., 2019). During the regeneration of the liver parenchyma in the foci of necrosis, the same mechanisms should be involved.

In steppe-runners with strong damage of the liver parenchyma, the primary factor is the activation of tissue macrophages. Macrophages clean the foci of necrosis from the hepatocytes remnants. The first manifestation of activation is enhanced differentiation of the progenitor cells and an increase in the number of young macrophages (table 1). Increase in the proportion of mature macrophages in liver myelograms is insignificant. Probably, the reason is that with an increased functional load, their life time decreases. Proliferation of lymphocytes (column "lymphoblasts"), production of antibodies (column "plasmocytes") and differentiation of granulocytes increases too.

Strengthening of protective reactions in the liver does not allow pathogenic microorganisms to use the remains of destroyed hepatocytes as a substrate. Therefore, stimulation of lymphocytes and granulocytes in areas of necrosis is partly an adaptive response.

The indicator "blasts, sum" reflects the number of the earliest progenitor cells of all types hematopoiesis. This indicator in steppe-runners with pronounced necrosis is significantly increased, which indicates the stimulation of early precursors. The number of erythroblasts was also insignificantly increased (table 1). Therefore, a reduced number of normoblasts in animals with strong manifestations of necrosis may be a consequence of a lack of plastic resources. A significant part of the free amino acids and iron compounds in the liver is spent on the regeneration of hepatocytes, enhanced differentiation of leukocytes (in both cases, iron is used as a coenzyme for a number of enzymes). Lack of plastic and energy resources may be the cause of reduced differentiation erythroblasts into erythrocytes.

Previously, we considered hematopoiesis in the liver of green frogs with minimal and maximal changes in the parenchyma (Akulenko, 2021). The results were completely different. In animals with severe lesions of hepatocytes, the only pronounced difference was a sharp decrease in the amount of pigments in the liver. At the same time, in samples of green frogs with severe and minor liver damage, no significant differences in the differentiation of blood cells were found. However, in a representative of reptiles, the effect of liver damage on hematopoiesis is pronounced.

Tail regeneration in lizards is a model for studying potential mechanisms of regeneration in vertebrates. During tail shedding and regeneration, numerous foci of necrosis, which are characteristic of liver damage, do not occur. However, this process also involves activation of the immune system and hematopoiesis processes (Alibardi, 2014). There is reason to believe that the activity of the immune system determines whether the regeneration process begins or whether the wound will simply heal with the formation of fibrous tissue (Bingqiang et al., 2021). An important indicator of this activity is the number of macrophages at the regeneration site. In the absence of numerous foci of necrosis, macrophages are not involved in the phagocytosis of cellular debris. However, preliminary data indicate that tissue macrophages at the regeneration sites have other functions that have not yet been explored (Vonk et al., 2023).

Conclusions

Lesions of the liver parenchyma in the steppe-runners have a significant impact on the processes of hematopoiesis and immune processes. Hepatocyte necrosis indirectly causes the activation of granulocyte differentiation in the liver. The primary link in the activation of myelopoiesis is the stimulation of tissue macrophages, which remove the remnants of necrotic hepatocytes. The inhibition of erythropoiesis may be caused by a lack of plastic resources.

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Received 25 June 2024
Accepted 16 October 2024