SPACEFLIGHT EFFECTS ON HEMOPOIESIS OF LOWER VERTEBRATES FLOWN ON FOTON-M2

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ABSTRACT

Intact and operated newts *Pleurodeles waltl* flown on Foton-M2 for 16 days were used to study the effects of spaceflight as well as tail amputation and lensectomy on their hemopoiesis. The flight did not produce noticeable changes in the peripheral blood of non-operated newts. However, in operated animals, the number of lymphocytes increased whereas that of neutrophils decreased. There were no morphological differences in hemopoietic organs (liver and spleen) between flown non-operated and operated animals or their controls. However, in both non-operated and operated newts the liver weight and the number of hemopoietic cells in it increased. In contrast to non-operated newts, space-flown mammals typically showed significant changes in blood cell counts. Experiments with BrdU incorporation revealed labeled cells in the hemopoietic area of the liver as well as in blood and spleen. This observation gives evidence that the BrdU label can be used to study proliferation of hemopoietic cells.

Key words: *Pleurodeles waltl* newts, hemopoietic cells, BrdU

1. INTRODUCTION

The hemopoietic tissue can serve as a sensitive indicator of environmental effects on the human and animal body. Humans cannot sustain long-duration space missions if they develop hemopoietic problems. When exposed to the space environment, humans and animals showed noticeable blood changes such as decreased erythrocyte and lymphocyte counts, increased neutrophil count, reduced plasma volume, enhanced spontaneous hemolysis of red blood cells and their shortened life cycle [1, 2, 3, 6, 7, 11]. Rats flown on Cosmos biosatellites displayed lowered counts of stem hemopoietic cells (CFU-S), committed hemopoietic (CFU-GM, BFU-E, CFU-E) and stromal (CFU-F) progenitor cells [5, 12, 13].

Lower vertebrates, including amphibians, can be well used in experiments flown on unmanned spacecraft because their handling and support are less demanding than that of mammals. Post-flight examination of hemopoiesis of lower vertebrates and comparative analysis of hemopoietic responses of animals from different taxonomic groups to the space environment yielded important data. Newt experiments flown on Bion-10 and Bion-11 showed that hemopoietic responses of intact and surgically treated (the animals whose forelegs and tails were partially amputated before flight) animals were different [4, 8]. Hemopoietic cells of space-flown newts maintained their capability to synthesize DNA and form erythroid and granulocytic colonies on acetate-cellulose membranes (ACM) in the peritoneal cavity of irradiated recipients [4, 9].

This paper summarizes the results of investigating blood, liver and spleen of newts before and after the Foton-M2 flight. Our studies addressed blood cell counts, hemopoietic cells in the liver as well as liver and spleen histology. Some of the Foton-M2 newts underwent 1/3 tail and lens removal prior to launch. This allowed us to study their hemopoietic responses to the space environment as well as to the surgical intervention (Regeneration experiment).

2. MATERIALS AND METHODS

The experiments were performed on adult newts *Pleurodeles waltl* some of which were untreated and others underwent surgical intervention. The intact animals were subdivided into three groups each of which included 5 animals: flight (F-Int), synchronous control (SC-Int), and basal control (BC-Int). The Regeneration experiment was conducted using newts that 10 days before launch were subjected to surgical removal 1/3 of their tails and lenses: 15 flight animals (F-Reg), 5 basal controls (BC-Reg), and 15 synchronous controls (SC-Reg). The animals were anesthetized, operated and handled in strict adherence to the Russian Academy of Sciences rules of animal care and use. The Foton-M2 flight continued for 16 days. The synchronous control experiment was initiated 48 hours after launch to simulate downlinked temperature variations onboard the spacecraft. When the experimental animals were delivered to the Institute of Developmental Biology, blood smears were

prepared, livers weighed, and blood-forming cells in the liver counted. Liver and spleen morphology was examined using histological sections. Some smears and sections were used to measure BrdU incorporation. More detailed description of BrdU delivery and immunological identification can be found in the papers by E. Almeida et al. and by E. Grigorian et al. published in the 27th Annual IGP Meeting Proceedings.

3. RESULTS AND DISCUSSION

The major hemopoietic organs of newts as a representative of tailed amphibians are liver, spleen and blood, which support granulopoiesis, lymphopoiesis, and granulopoiesis and erythropoiesis, respectively. Bone marrow is not involved in the process - it is actually fibrous tissue containing adipocytes.

3.1. Peripheral Blood

The following formed cells were detected in the peripheral blood of intact newts: neutrophils, eosinophils, basophils, lymphocytes and monocytes. In contrast to blood cells of mammals, mature erythrocytes and thrombocytes of newts contained nuclei. Also, poorly differentiated (blast) cells, large and medium-size lymphocytes, as well as erythroblasts and mitotic cells were found in newts.

Blood cells of BC-Int animals included lymphocytes that amounted to almost 50% of all white blood cells. Neutrophils made about 38% and included cells of varying degree of maturity: myelocytes (M), metamyelocytes (mM), bands (B) and segmented (S) forms with mature forms being predominant (Table 1). This is confirmed by the ratio of (M+mM+B): S = 0.4+0.2. The ratio of lymphocytes to neutrophils was on the average 1.9+0.9. Small counts of blast cells were also found. Comparative analysis of blood cells of newts from the F-Int, SC-Int and BC-Int groups did not show any significant differences in the neutrophil and lymphocyte counts or the ratios of maturing neutrophils to mature segmented forms (M+mM+B): S and lymphocytes to neutrophils (L: N). It can therefore be concluded that the spaceflight did not impact the hemogram of intact newts. This observation is in agreement with the Bion-11 data: no noticeable changes were detected in blood cell counts of newts flown for 14 days [8].

In contrast, the space-flown newts that underwent surgery (F-Reg) showed a decrease in neutrophils and an increase in lymphocytes compared to BC-Reg and SC-Reg animals. Since the controls were also subjected to 1/3 tail and lens removal, the F-Reg changes can be attributed to the spaceflight effects. However, the ratios of maturing to mature neutrophils (M+mM+B): S in the F-Reg, BC-Reg and SC-Reg newts were similar (Table 1).

The regenerating newts flown on Bion-10 for 12 days also showed changes in the ratios of blood cell forms. Bion-10 changes were however opposite of the Foton-M2 shifts: neutrophils increased and lymphocytes decreased [4]. The difference may be explained by the severity of surgical intervention: the Bion newts had the proximal area of forelegs and part of the tails removed whereas the Foton-M2 animals had only part of the tail removed. Bion-10 and Foton-M2 biosamples were fixed on launch day 13 and 17, respectively. It was previously reported that the counts of different leukocytes (lymphocytes, neutrophils, monocytes, basophils and eosinophils) in the peripheral blood of newts varied at different regeneration stages [10].

In summary, Bion-10 and Foton-M2 newts experienced the effects of both spaceflight factors and tissue repair that was stimulated by blood loss. It can therefore be inferred that changes in peripheral blood parameters that can occur in the space environment are associated with the condition of the hemopoietic tissue.

3.2. Liver

The hemopoietic tissue consisting of 10-15 rows of cells is located along the edges of the liver and covered by a connective tissue capsule. In addition, hemopoietic areas can be found around blood vessels and bile ducts in the parenchyma. The hemopoietic areas are not separated from the parenchymal tissue due to which hemopoietic cells are directly adjacent to hepatocytes. In the liver, granulocytopoiesis is predominant; granulocytic cells at different differentiation stages as well as mitotically dividing cells can be seen. Histological examination of the liver of Foton-M2 newts did not show any significant differences between flight and control animals or between operated and non-operated newts.

The liver weight of BC-Int, SC-Int and F-Int newts was essentially the same (Fig. 1). However, the liver weight of SC-Reg animals was significantly lower than that of BC-Reg and the liver weight of the F-Reg animals was close to that of BC-Reg. The number of hemopoietic cells in the F-Int and F-Reg newts was almost identical and significantly higher than in the SC-Int and SC-Reg animals. This suggests that an increase in the number of hemopoietic cells in the flown animals (F-Int and F-Reg) compared to SC groups is more likely to be induced by the spaceflight effects rather than by hemopoietic responses to surgeries (Fig. 2).

3.3. Spleen

In the Foton-M2 newt spleens, the red and white pulp was distinctly visible. The system of connective-tissue trabecules was lacking. The white pulp was represented by lymphoid follicles consisted of lymphocytes at various stages of maturity and lymphoid cords. The red pulp contained primarily mature erythrocytes and mononuclear cells (probably, small lymphocytes). No foci of active erythropoiesis were seen. No morphological differences between space-flown and control newts or between operated and non-operated animals were detected.

Table. Percentages of leukocytes of different types in the peripheral blood of Foton-M2 newts

Group of newts	N					Ba	Co	Mo	L	(M+Mm+B):S	L:N
	M	mM	B	S	total						
BC-Int	l.8±0.3	3.4±1.3	5.2±2.8	27.8±l.4	38.4±17.2	4.3±1.3	6.4±2.4	0.2±0.1	50.4±14.9	0.4± 0.2	1.9±0.9
(n-3) sc-Inl	0.4±0.2	l.4±0.7	l.8±0.8	12.0±2.9	15.6±3.9	9.3±4.1	8.8±5.2	0.5±0.4	65.4±5.1	0.3±0.1	4.7±1.6
(n-3) F-Int	1.1±0.7	l.3±0.4	1.0±0.3	10.2±5.5	13.8±6.2*	4.6±2.8	5.6±1.4	0.4±0.1	74.4±4.9*	0.5±0.3*	7.9±4.6*
in i) BC-	2.6±1.6	4.5±1.6	2.7±1.2	10.4±0.8	20.5±4.2	5.6±1.7	10.0±0.7	0.5±0.7	63.7±3.4	0.90±0.4	3.3±1.0

Reg (n-3)	1.9±2.3	2.3±0.6	3.2±0.9	8.4±1.7	15.8±2.2	7.4±1.3	5.0±1.9	0.5±0.2	65.6±4.5	1.0±0.4	5.6±0.8
SO Reg (n-8)											
F-Reg (n-7)	1.1 ±0.3	2.2±0.8	1.9±0.7	2.5±0.8	7.7±2.0*	3.5±1.2	10.1±2.3	0.4±0.2	78.0±4.4 *	3.3±1.3*	11,1±3.0"

Designations: N - neutrophils, M - myelocytes, mM - metamyelocytes, B - band neutrophils, S - segmented neutrophils, Ba -basophils, Eo - eosinophils. Mo - monocytes, L - lymphocytes.

*Differences are insignificant.

*- significant differences from the control groups (0.01<P<0.02)

*-significant differences F-Reg vs SC-Rcg (*P*=0.05) and F-Reg vs BC-Reg <0.05<*P*><0.02)

** - significant differences F-Reg vs BC-Reg (*P*<0.05)

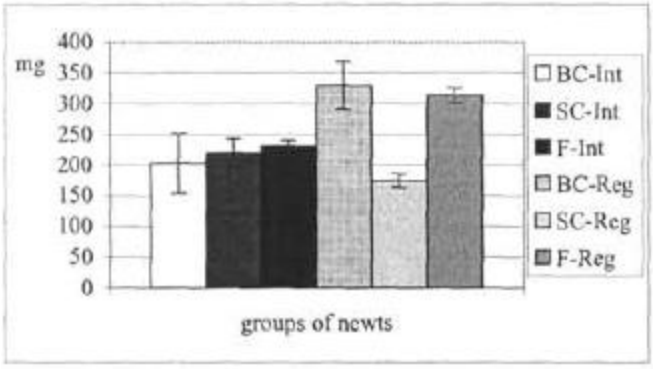

Fig. 1. Liver weight of space-flown and control newts.
F-Rcg vs SC-Rcg *P*<0.00l. SC-Rcg vs BC-Reg 00.1<*P*<0.01. Differences F-Int vs BC-Int and SC-Int and F-Rcg vs BC-Reg are insignificant.

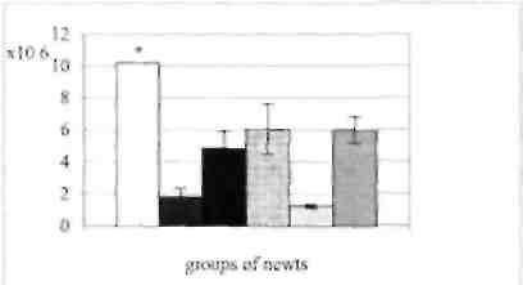

Fig. 2. Hemopoietic cell number in the liver of space-flown and control newts.
Designations as in Fig. 1.
* - Average value for the pooled content of five livers. F-Int vs SC-Int 0.01<*P*<0.05, F-Reg vs SC-Reg *0.001<P<0.01.* BC-Reg vs SC-Reg *P=0.02.* Differences F-Reg vs BC-Reg arc insignificant.

3.4. BrdU incorporation in hemopoietic cells

Pre-flight experiments demonstrated BrdU labeled cells in the hemopoietic areas of the liver as well as in the peripheral blood and spleen. This indicates that the BrdU delivery by the tested procedure can be used to measure proliferation of the hemopoietic tissue.

4. CONCLUSION

Comparison of the Bion-10, Bion-11 and Foton-M2 findings gives evidence that blood cell counts in the peripheral blood of space-flown newts may change only in response to the stimulation *of* organ/tissue regeneration caused by amputation and concomitant blood loss. Lack of blood changes in newts distinguishes them from mammals and indicates that hemopoietic responses of animals of different taxonomic groups to spaceflight effects are dissimilar. It should be however noted that the number of hemopoietic cells increase in the livers both non-operated and operated

newts flown onboard l'oton-M2.

5. ACKNOWLEDGMENTS

This work was supported in part by MCB.

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