Acta Veterinaria(Beograd), Vol. 45. No. 5-6, 269-280, 1995.

UDK 619:612.619:612.41

THE ROLE OF THE SPLEEN IN HEMATOPOIETIC CELL PRODUCTION DURING THE ACUTE PHASE OF STERILE INFLAMMATION IN RATS

NEVENKA STOJANOVIĆ, GORDANA JOVČIĆ, MARIJANA PETAKOV, LIDIJA BILJANOVIĆ-PAUNOVIĆ, VESNA KOKO, VERA TODOROVIĆ, VERA TIŠMA and NADEŽDA MORARDET*

*Institute for Medical Research, Belgrade, Yugoslavia (Serbia) * INSERM, Institute Gustave Roussy, Villejuif, France*

(Received, 17. July 1995)

The participation of splenic hematopoietic cells in the acute phase of sterile inflammation was investigated using rats with polyvinylpyrrolidone (PVP) - induced sterile inflammation as a model in vivo. Changes within splenic granulocytic-monocytic (CFU-GM) and erytrroid (BFU-E and CFU-E) committed stem cells determined in vitro in cell cultures, as well as morphologically recognizable hematopoietic cells identified on May-Grunwald Giemsa smears and in histological sections, were estimated at different time intervals during the first 24 hours of the acute phase of inflammation. The data obtained demonstrated intensive production of cells belonging to different granulocytic cell compartments during the observed periods. Also, an increased production of monocytes was found although to much a smaller extent in comparison to granulocytic cells. Although no changes within peripheral blood erythrocytes were found, changes within different compartments of the erythroid cell lineage in the spleen indicated an increased production of erythroid cells during the acute inflammatory response. From the data presented it is obvious that different splenic hemopoietic cell lineages are activated during the acute phase of the inflammatory response implying significant participation of extramedullary hemopoiesis in the whole body host defense reaction.

Key words: spleen, sterile inflammation, hematopoietic cells.

INTRODUCTION

In the acute phase of the host response to an inflammatory agent different mechanisms are triggered in order to maintain an adequate reaction aimed at eliminating this "foreign guest". The existence of a network of cytokine-cells mutually co-ordinating corresponding changes directed to an effective defence reaction by the host, has been demonstrated (Jovčić et al, 1993) in rats with sterile inflammation induced by polyvinylpyrrolidone (PVP). The obtained data indicated

the involvement of some extramedullary produced regulators. Thus, to obtain insight into the whole body's host defense reaction to inflammatory agents, extramedullary hematopoiesis has to be taken into consideration to which the spleen contributes significantl ty in rodents.

MATERIALS AND METHODS

Experimental animals and induction of inflammation: Male Wistar rats weighing 200-220g were used in experiments where inflammation was induced using polyvinylpyrrolidone (Serva, Feinbiochemica, 20kD) as previously described (Hajduković et al., 1976). Briefly the rats were injected i. p. twice with 15ml of 3.5% PVP in sterile saline at an 18-h interval.Animals were killed 2, 4, 6, 18, 20, 24 hours after the first PVP injection, or 2 and 6 hours after the second one. Normal, nontreated Wistar rats were used as control animals.

Splenic cell suspension: The single cell suspension of the whole spleen of each rat made in Dulbecco's modification of Eagle's medium (DMEM) was used for the estimation of the total number of nucleated cells per spleen, progenitor cell assays and splenic cell differentials.

CFU-GM colony assay: The number of CFU-GM derived colonies was determined using a methylcellulose cell-culture system with 4×10^5 nucleated splenic cells per plate and a rat lung-conditioned medium CSF (Sheridan and Metcalf, 1973). After 7 days of incubation at 37^0C in a humidified atmosphere and 5% $C0_2$ in air, the number of colonies was counted and expressed as the number of CFU-GM per spleen (two to four plates were plated for each value for every rat).

Erythroid colony assay: The erythroid colony assay employed was a modification of the methylcellulose cell-culture technique described for mice by Iscove et al (1974), and Iscove and Sieber (1975). The final culture mixture consisted of 0,8% methylcellulose, 20% FSC, 1% bovine serum albumine, 2×10^{-5}M mercaptoethanol and 10% PKW-SCM (Basara et al.,1988) and for CFU-E it consisted of 0,8% methylcellulose, 30% FCS and 10^{-4} alphathioglycerol (Pavlović-Kentera et al., 1988). DMEM and Epo were used for both progenitor assays. The mixture, together with an appropriate number of splenic nucleated cells per ml, was plated in 35 mm Greiner plastic tissue culture dishes, and incubated at 37^0C in a humidified atmosphere of 5% CO_2 in air: 8 days for BFU-E and 2 days for CFU-E(two to four plates were plated for each value of every rat).

Splenic cells differential: Cells of the splenic cell suspension were stained by the May-Grünwald-Giemsa procedure and differentiated morphologically. Counted cells(1000 cells on each smear) were divided into the following compartements: metamyelocytes (META), granulocytes (GRAN), monocytes (MONO), erythroblasts (ERBL), orthochromatic blasts (ORTHO), lymphocytes (LYMPHO) and other cells including nonidentified cells.

Histological analysis: The middle part of the spleen was perpendicularly cut into several pieces and put into neutral buffered formalin. After processing in paraffin,4μm (micrometar) thick sections were stained with Giemsa stain. The

fields of granulocytic and erythroid differentiation in the red pulp of the spleen were estimated semiquantitatively on the scale 1+ to 4+.

Statistical analysis: Data are presented as a percentage of normal, control values. Student's t-test was performed on data representing the total number of each cell compartment per spleen mean values of 10-12 rats taken separately are presented in each experimental point.

RESULTS

As no significant changes within the total number of nucleated cells per spleen were found in any of the groups of rats, these data are not shown. All data were analyses using the total number of cells per spleen of each cell compartment but to clarify the presentation, data are presented as a percentage of normal values.

According to data presented in Figure 1, it is evident that splenic granulopoiesis is increased in the acute phase response due to the presence of inflammatory agent - PVP. CFU-GM numbers were increased significantly in comparison to normal values already 2 hours after PVP injection and tended to show increases during all observed periods, oscilheating, but without retuanng to normal values. Metamyelocytes decreased during the first 2 hors of the acute phase response and then started to increase continuously to exhibit above normal values at 6 hours after induction of inflammation. At 18 hours metamyelocytes were below normal values and stayed at the same level unit the 24th hour. Changes within metamyelocytes were accompanied by a consequent increase in granulocytes during the first six hours after induction of inflammation. After the second PVP injection given 18 hours after the first PVP injection, granulocytes decreased below normal values due to the increased demands for granulocytes caused by the new inflammatory stimulus. At 24 hours granulocytes had increased at the expense of metamyelocytes, the cells that precede granulocytes.

Data obtained for monocytes (Figure 2) demonstrated that 4 hours after the first PVP injection as well as 2 hours after the second PVP injection, these cells increased, i. e. when the inflammatory stimulus was repeated, a second significant increase of monocytes was found.

Cells belonging to the lymphocytic cell line did not change in number in any of the observed periods (Figure 2).

Changes within different compartments of the erythroid cell lineage (Figure 3) implied active stimulation of the production of cells belonging to this lineage. After the initial drop seen at 2 and 4 hours. After the first PVP injection, BFU-E showed a significant drop seen at the 6th hour. The same was observed after the secound PVP injection, i. e. at 20 and 24 hours. CFU-E as a transient population between BFU-E and the morphologically recognizable erythroid cell compartment behaved similarly to metamyelocytes. After an initial drop at 2 and 4 hours, CFU-E increased and oscillated around normal values until the 24th hour. CFU-E feed the erythroblast compartment and, as a consequence, a significant increase

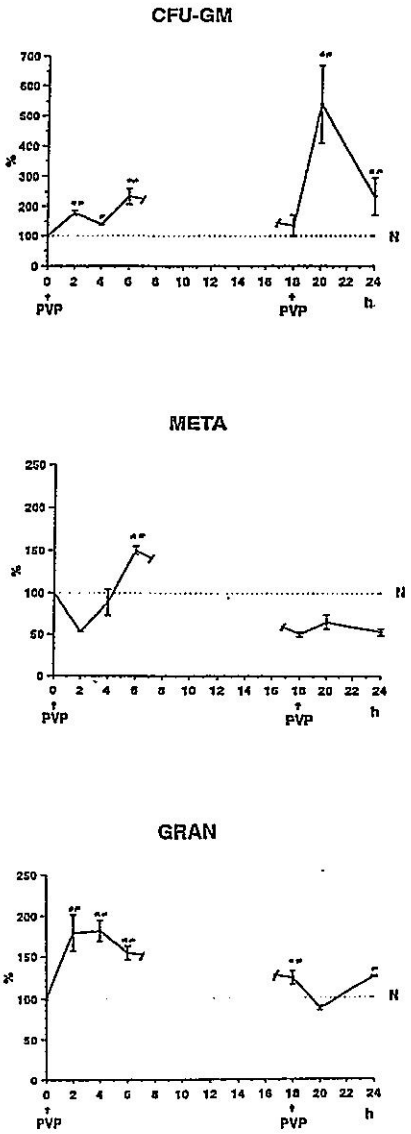

Figure 1. Granulocytis cells (CFU-GM, metamyelocytes-META, granulocytes- GRAN) in the total number of splenic cells 2, 4, 6, 18, 20, 24 h after induction of PVP inflammation presented as a percentage of those in normal, nontreated rats. N=100%. (mean values from three separate experiment. *p<0.01. Statistical calculations were performed on data representing the total number of each cell compartment.

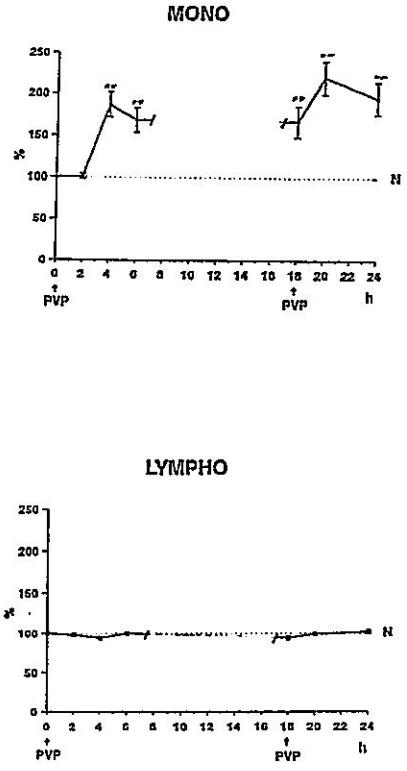

Figure 2 Monocytes (MONO) and lymphocytes (LYMPHO) in the total number of splenic cells 2, 4, 6, 18, 20, 24 h after induction of PVP inflammation presented as a percentage of those in normal, nontreated rats. N=100%. Shown data are mean values from three separate experiments. *$p<0.05$ **$p<0.01$. Statistical calculations were performed on data representing the total number of each cell compartment.

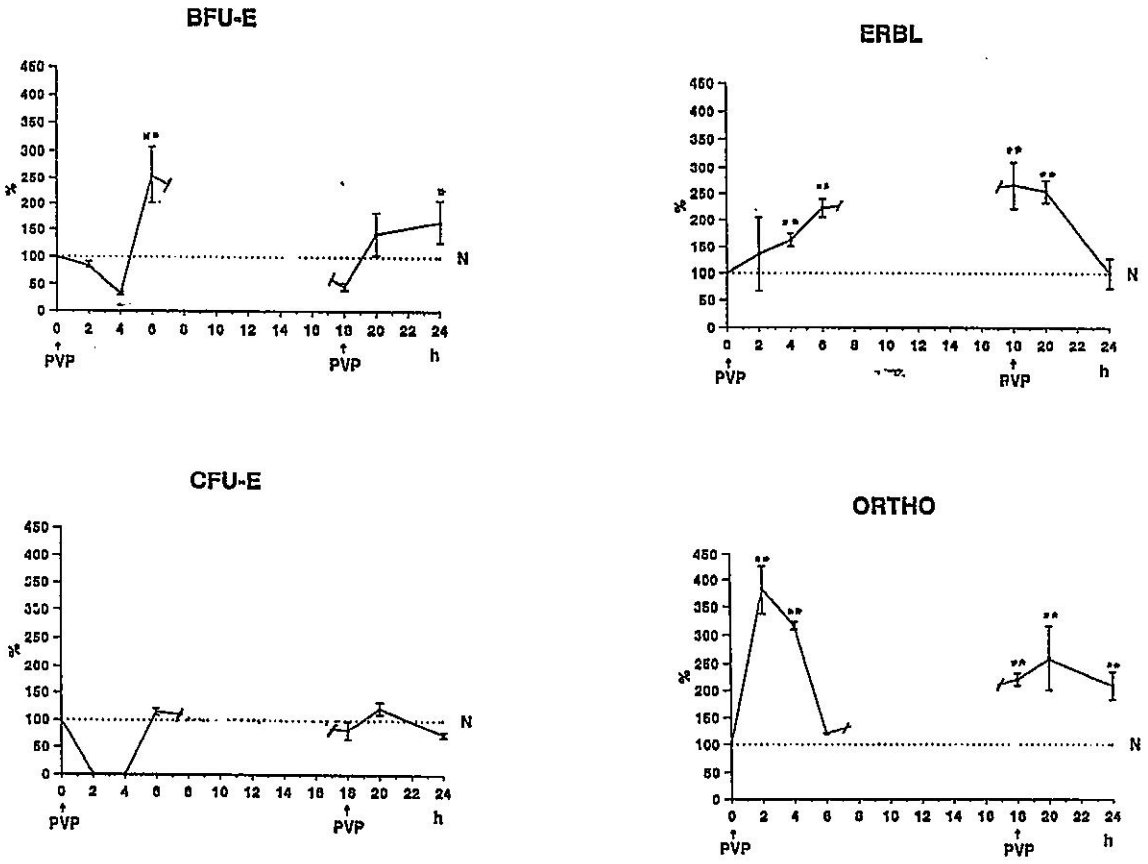

Figure 3. Erythroid cells (BFU-E, CFU-E, erythroblasts-ERBL, orthochromatic-ORTHO) in the total number of splenic cells 2, 4, 6, 18, 20, 24 h after induction of PVP inflammation presented as a percentage of those in normal, nontreated rats. N=100%. Shown data are mean values from three separate experiments. *p<0.05 **p<0.01. Statistical calculations were performed on data representing total number of each cell compartment.

of these cells was found up to 24 hours when the number of erythroblasts returned to normal values. Orthochromatic normoblasts which originate from erythroblasts were also increased during all observed periods.

Table 1. Semiquantitative histological estimation of the granulocytic and erythroid differentiation in red pulp from the spleen of rats killed at different time intervals after induction of PVP-sterile inflammation.

h / cell line	2	4	6	18	20	24
Granulocytic	+	+	+++	+	+	+
Erythroid	+	+	+	+	+	+

1. Semiquantitativescale used from 1+ -4+
2. No neutrophils were found

A semiquantitative evaluation of granulocytic and erythroid cells was performed on the histological sections. The data obtained (Table 1, Figure 4 and 5) demonstrated that two hours after induction of inflammation the fields with all stages of granulocytic cells became slightly increased. However, at the 4th, 6th and 18th hour the fields were larger and numerous mitoses were observed. At the 20th and 24th hours granulocytic fields decreased. Although all stages of granulocytic cells were not observed on the May-Granwald stained splenic cell smears, the fact that all stages of these cells were found on histological splenic sections during all the observed periods, directly confirm active granulopoiesis within the spleen. In the red pulp of the spleen the fields of erythroid differentiation became slightly increased 2 hours after the induction of inflammation. Each field consisted of all the stages of the erythroid differentiation. From the 2nd to the 6th hour slight oscillations in splenic erythroid cells were found, but from the 18th hour after induction of inflammation the fields of erythroid cells in all stages of differentiation were constantly increased.

DISCUSSION

Many authors (Athens, 1993; Loeffler and Wichmann, 1985) have demonstrated that the spleen is an active hemopoietic organ in variously disturbed physiological conditions in rodents (much more in mice than in rats). In such situations activation of the spleen is maintained towards compensation of suppressed hematopoiesis occuring within the bone marrow due to the treatment used. In the acute phase of the inflammatory response hematopoiesis within the bone marrow is stimulated. We have demonstrated (Jovčić et al., 1993; Stojanović et al., 1980) that in PVP induced sterile inflammation there is an increased demand primarily for granulocytes which results in a massive delivery of these cells at the site of inflammation (peritoneal cavity) accompanied by increased production of bone marrow granulocytic cells.

From the data presented in this paper it is obvious that in the same animals with PVP induced sterile inflammation at the same periods of time, splenic

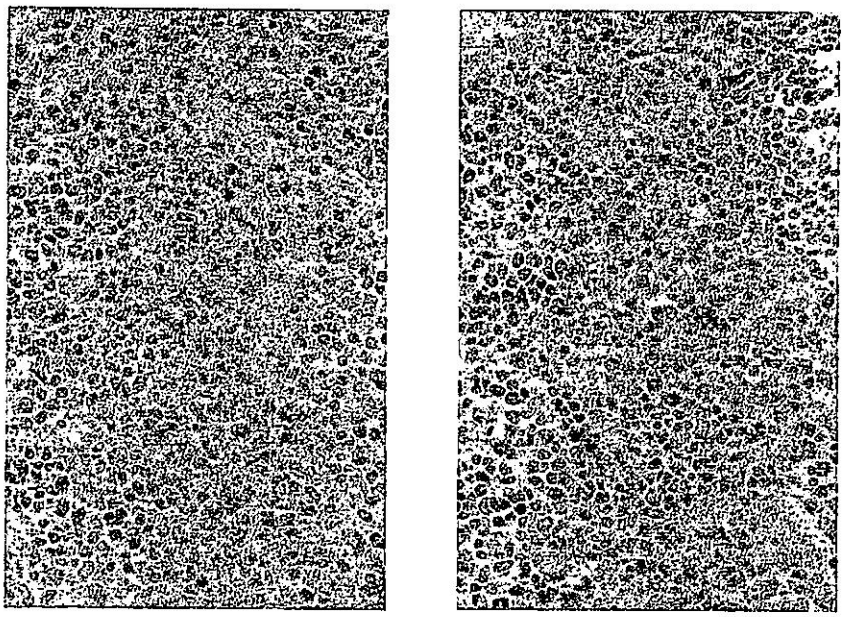

Figure 4a and b Spleen histology of a control rat (Figure 4a): normal proportion of erythroid and granulocytic cells with normal maturation sequence; haematoxylin and eosin, x 1000. Spleen histology of a rat after PVP injection (Figure 4b): granulocytic area with numerous rubricytes most of which are in the maturation phase; note paucity of scattered erythroid cells; Giemsa, x 1000.

granulopoiesis is also expanded. Splenic granulocytic cells were estiemated using morphological identification of cells on May-Grunwald-Giemsa stained smears, as well as on histological sections. The changes within different granulocytic cell compartments during the first 24 hours of the acute phase of inflammatory response directly confirm the effective production of splenic granulocytic cells. The early increase in the number of CFU-GM as well as the occurrence of numerous mitoses in the granulocytic cells seen in the spleen sections confirm the stimulation of de novo production of granulocytic cells. On the other hand, changes within the nonproliferative granulocytic compartment imply accelerated production of splenic mature granulocytes. The initial increase in the number of mature granulocytes until the 18th hour was followed by a drop in their number observed at the 20th hour. This indicates active involvement of the spleen in the delivery of granulocytes to meet the needs of the organism for these cells. If those results are taken together with our previous results concerning the kinetics of granulopoiesis within the bone marow in the same animal model

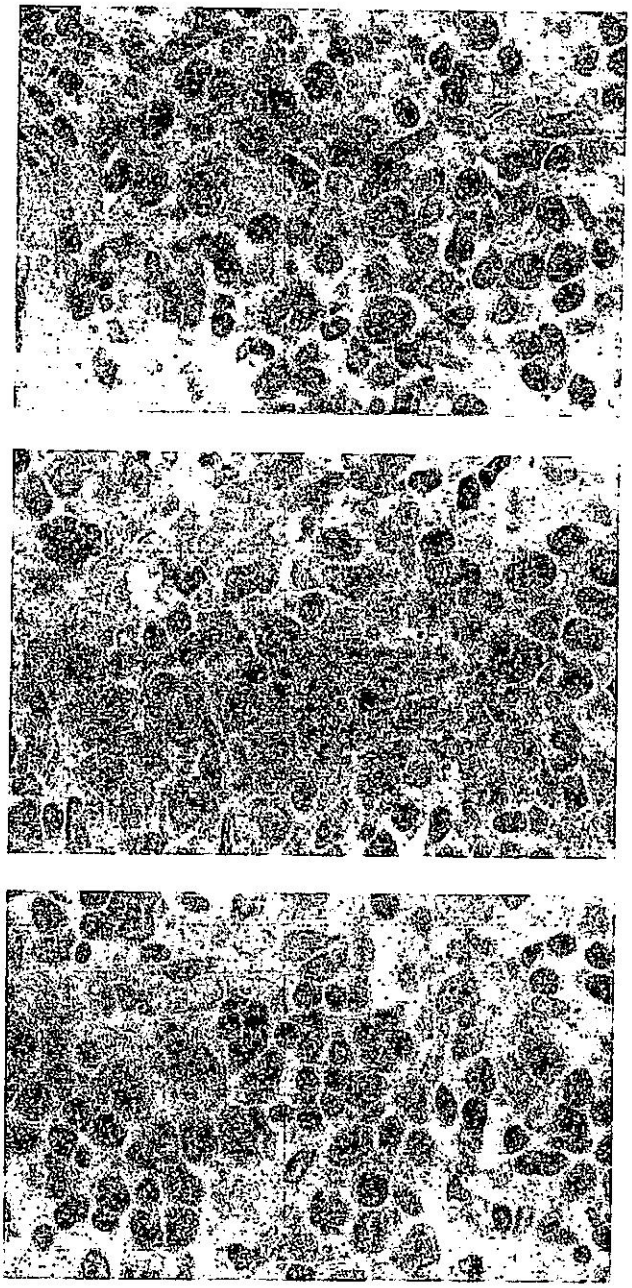

Figure 5a, b and c. Spleen histology of a control rat (a) and rats 18 h after PVP injection note that granulocytyc (b) and erythroid (c) cells in injected rats are much more numerous than in the control one (a); Giemsa, x 2500.

(Jovčić et al., 1993; Stojanović et al., 1980), it is obvious that both hemopoietic organs are involved together and simultaneously in the production of granulocytic cells during the acute phase of inflammation, since in the bone marrow parallel with the delivery of granulocytic cells from the storage pool the process of increased production of granulocytes occured, whereas in the spleen increased production of granulocytic cells preceds delivery of these cells.

Although monocytes are included in the inflammatory reaction in much smaller extents in comparison to granulocytes (Jovčić et al., 1993; Stojanović et al., 1980), their numbers were significantly increased in the spleen during the 24 hour observation period.

The increased production of splenic erythroid cells during the acute inflammatory response, which was confirmed by both techniques used for the morphologically recognizable erythroid cells, as well as by the determination of erythroid progenitors BFU-E and CFU-E could not be attributed to the increased demands for these cells, because no changes within peripheral blood erythrocytes were found in the same period of time. As our previous data concerning morphologically recognizable erythroid cells in the bone marrow of the same animal model demonstrated no significant changes, so the observed changes within different erythroid cell compartments in the spleen were not expected. On the other hand, it should be pointed out that in rodents the spleen is preferentially an erythropoietic organ especially in conditions of perturbed or damaged hematopoiesis (Loeffler and Wichmann, 1985; Lomax et al, 1990). One can suppose that even in conditions when granulopoiesis is primarily disturbed, as demonstrated in PVP treated rats, erythropoiesis also reacts owing to the large scale of mutual relations between these two cell lineages (sharing a common progenitor cell and sensitivity to some cytokines etc.) (Sachs, 1994; Suda et al., 1983; Suda et al., 1984). Additional experiments are needed to clarify whether increased splenic erythropoiesis is effective i. e. whether it results in an increased production of erythrocytes.

From the data presented here it is obvious that splenic cells belonging to different hemopoietic cell lineages are activated during the acute phase of the inflammatory response. They support evidence (Lomax et al, 1990; Stojanović et al., 1980), demonstating that the rodent spleen has the capacity to act as an expansible population and usually is the organ in which population changes are most readily evident.

Acknowledgement. This work was supported by a grant from the Serbian Research Foundation. The excellent technical assistance of Mrs. Kata Božanić, Mrs. Snežana Marković and Mrs. Milka Dokić is greatly appreciated.

REFERENCES

1. *Athens JW.* 1993. The reticuloendothelial (mononuclear phagocyte) system and the spleen. *In: Lee GR, Bithell TC, Foerster J, Athens JW, Lukens JN, eds. Wintrobe's Clinical Hematology, 9th ed. Philadelphia-London: Lea & Febiger, pp,* 311-325.

2. *Basara N, Biljanović-Paunović L, Pavlović-Kentera V.* 1988. The method for growing primitive erythroid progenitors (BFU-E) from rat bone marrow. *Exp Hematol 16,* 790-793.

3. *Hajduković S, Jovčić G, Stojanović N.* 1976. Granulocytic cell stimulation induced in sublethally irradiated mice by serum of leucophoretic rats. *Acta Vet. 26,* 137-240.
4. *Iscove NN, Sieber, F, Winterhalter KH.* 1974. Erythroid colony formation in cultures of mouse and human bone marrow: analysis of the requirement for erythropoietin by gel filtration and affinity chromatographon agarose concanavalin *A. J. Cell Physiol 83,* 309-316.
5. *Iscove NN, Sieber F.* 1975. Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. *Exp Hematol 3,* 32-35.
6. *Jovčić G. Stojanović N, Kataranovski M, Petakov M, Obradović P.* 1993. Acute sterile inflammation-correlation between cellular changes and extramedullary-produced regulators in vivo. *Ann Hematol 66,* 195-201.
7. *Loeffler M, Wichmann H-E,* eds 1985. Mathematical modeling of cell proliferation: stem cell regulation in hemopoiesis. *Vol I and II. Boca Raton FL: CRC Press* (for review).
8. *Lomax GL, Keyes DG, Kociba RJ.* 1990. Extramedullary hemopoiesis, spleen, rat. In: Jones TC, Ward JM, Mohr U, Hunt RD, eds. *Hemopoietic System. Berlin Heidelberg: Springer-Verlag, pp.* 232-235.
9. *Pavlović-Kentera V, Basara N, Jerkić M, Biljanović-Paunović L., Clemons GK, Milenković P, Sušić D. 1988. Erythrocytosis in spontaneously hypertensive rats. Exp Hematol 19,* 950-953.
10. *Sachs L.* 1994. The molecular control of haemopoiesis and leukaemia. *Europ J. Canc 30,* 852-860.
11. *Sheridan JW, Metcalf D.* 1973. A low-molecular-weight factor in lung conditioned medium stimulating granulocyte-monocyte colony formation in vivo. *J Cell Physiol 81,* 11-17.
12. *Stojanović N, Jovčić G, Petrović N, Hajduković S.* 1980. Experimental inflammation and granulopoiesis in rats in situ and in bone marrow cultivated in diffusion chambers. In: Baum SJ, Ledney GD, van Bekkum DW, eds. *Experimental Hematology Today, Basel: Karger,* pp. 245-252.
13. *Suda T, Suda J, Ogawa M.* 1983. Single cell origin of mouse hemopoietic colonies expressing multiple lineages in variable combinations. *Proc Natl Acad Sci USA 80,* 6689-6693.
14. *Suda J, Suda T, Ogawa M.* 1984. Analysis of differentiation of mouse hemopoietic stem cells in cultures by sequential upleting of paired progenitors. *Blood 64,* 393-397.

ULOGA SLEZINE U STVARANJU HEMATOPOEZNIH ĆELIJA U TOKU AKUTNE FAZE STERILNE INFLAMACIJE U PACOVA

NEVENKA STOJANOVIĆ, GORDANA JOVČIĆ, MARIJANA PETAKOV, LIDIJA BILJANOVIĆ-PAUNOVIĆ, VESNA KOKO, VERA TODOROVIĆ, VERA TIŠMA i NADEŽDA MORARDET

SADRŽAJ

Pacov sa sterilnom inflamacijom izazvanom polivinil-pirolidonom (PVP) je korišćen kao model za in vivo izučavanje uloge slezine u stvaranju hematopoeznih ćelija u toku akutne faze sterilne inflamacije (u periodu do 24-og sata). Praćene su promene u slezini u okviru matičnih ćelija opredeljenih za granulocitno-monocitnu lozu (CFU-GM) i eritrocitnu lozu (CFU-E i BFU-E), kao i promene u okviru morfološki prepoznatljivih ćelija ovih loza. Dobijeni rezultati su pokazali da se u akutnoj fazi sterilne inflamacije u slezini pacova odvijaju intenzivni procesi stvaranja ćelija granulocitne loze. Uprkos nepromenjenom broju eritrocita u perifernoj krvi analiza promena matičnih i morfološki prepoznatljivih ćelija eritrocitne loze u slezini ukazuju na stimulisanu eritrocitopoezu u ovom hematopoeznom organu. Na osnovu dobijenih rezultata može se zaključiti da u toku akutne faze inflamatornog odgovora dolazi do značajnog povećanja ekstramedularne hematopoeze u slezini pacova sa inflamacijom.