MODEL OF SEPSIS (CAECAL LIGATION AND PUNCTURE) IN RATS CAUSED BY MIXED AND PURE BACTERIAL CULTURES AND CHANGES IN WHITE BLOOD CELL COUNTS

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The number of leucocytes and immunocompetent cells, was investigated during a clinical form of sepsis in rats. The experiments were carried out on 104 male rats, Wistar strain, of body weight 190 to 240 g. The rats were divided into four groups: three with 28 animals and one control group with 20 animals. The animals were killed 12, 24, 72 or 120 hours after surgical intervention.

This consisted of caecal ligation and puncture (CLP), with inoculation of mixed bacteria or pure cultures of Escherichia coli or Staphylococcus aureus. They induced similar changes in the total leukocyte counts and percentages of different white blood cells. The significant leucopenia in the first half (early sepsis) of the examined period preceded significant leukosis in the rats with sepsis in the second half of the experiment (late sepsis). Also, there were significant alterations in the numbers of granulocytes and agranulocytes. Neutrophilia and lymphopenia dominated during the whole period.

Key words: leukocyte, sepsis, rats, E. coli, Staph. aureus

INTRODUCTION

Sepsis is a condition of heavy metabolic stress accompanied by great changes in the concentrations of cytokines, hormones and other biochemical and haematologic parameters in blood plasma (Yelich, 1990). Characteristic features are alterations in body temperature (>38°C and <36°C), heart frequency (>90 beats/minute), tachypnoea (<20 respirations/minute or hyperventilation with PCO_2 <32 mm Hg) and changes in the number of leukocytes (leukocytosis >12 x 10^9/ml or leukopenia <from 4 x 10^9/ml or more than 10% of non-mature neutrophils (Bone, 1991).

As mediators of cell interactions, cytokines, may influence proliferation and differentiation of cells. The proinflammatory, protective immunogenic response to infection and defence of the host in sepsis is regulated by tumor necrosis factor (TNF) and interleukin-1 (IL-1), by activation of neutrophils, macrophages and lymphocytes, reinforcing gene expression and the release of acute phase proteins and stimulating factor for granulocyte colonies (SFGK), with induction of the antiinflamatory cytokines IL-4, IL-6 and IL-10, IL-1 and transforming growth

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factor (TGF) β as well as reducing receptors specific for TNF and IL-1. Experiments on animals demonstrated partial involvement of these mechanisms. It is considered that prolonged activation of complement and local tissue inflammation may cause activation of macrophages, granulocyte aggregation in the microcirculation and tissue lipid peroxidase activity in different organs (Beutler, 1993; Cohn *et al*., 1991).

Peripheral blood cells have a decisive role in the defence of an organism from an infection. They provide functional immunological defence, carry oxygen and are important for maintaining homeostasis. Their number and half life changes according to the functional demands of the organism. The objective of our investigation was to induce a systemic inflammatory response in rats as a model of sepsis with mixed and pure bacterial culture and to determine the intensity of the inflamatory reaction by measuring the number of leukocytes immunocompetent cells.

MATERIAL AND METHODS

The experiment was carried out in 104 male rats, Wistar strain, of body weight 190 to 240 g. The rats were divided into four groups: three consisting of 28 animals each and one control group consisting of 20 animals. In order to monitor the development of sepsis, the animals were killed 12, 24, 72 or 120 hours after surgery.

Clinically visible sepsis in two groups of rats was provoked by inoculation of pure culture of *Escherichia coli* (EC) or *Staphylococcus aureus* (SA) into the previously emptied, ligatured and rinsed caecum (Stojanović*et al*., 2002). In the third group of experimental animals sepsis was provoked using the content of the ligatured and punctured caecum (mixed culture of microorganisms-MC) (Wichterman *et al*., 1980). The control group of animals was operated on by opening the abdomen.

Blood was punctured from the abdominal aorta and used to obtain the number of leukocytes and the differential leukocyte formula employing an automatic haematologic analisor (H-1-Technicon). The results were analysed on a PC IBM compatible computer with the program package "Statgraph" 3.0 Excel 97 and statistics 6.0. The descriptive statistics method, two-way analysis of variance and dependent and independent Student's t-test for small samples were used.

RESULTS AND DISCUSSION

Some important changes, in the haematological parameters, were detected, which pointed to an intense systemic inflammatory reaction in the rats with sepsis. Currently much intention is paid to pro- and anti-inflammatory cytokines which regulate the proliferation and differentiation of haematopoietic cells, in coordination with the reaction of the immune system, i.e. the control the inflammatory reaction, which can possibly explain the changes of our

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haematologic parameters (Florquin *et al*., 1994; Gerard *et al*., 1993; Lamy *et al*., 1993).

Significant leukopenia in the first half of our experiment proceeded to significant leukocytosis of the rats with sepsis in the second half of the experiment (Figure 1). Also, significant changes in the relative numbers of granulocytes and agranulocytes were noted during the time monitored, dominated by important neutrophilia and lymphopenia (Figure 2). Lymphocyte numbers fell significantly during the first phase (Table 1) and then returned to the control range, when neutrophil counts were significantly increased. (Table 2).
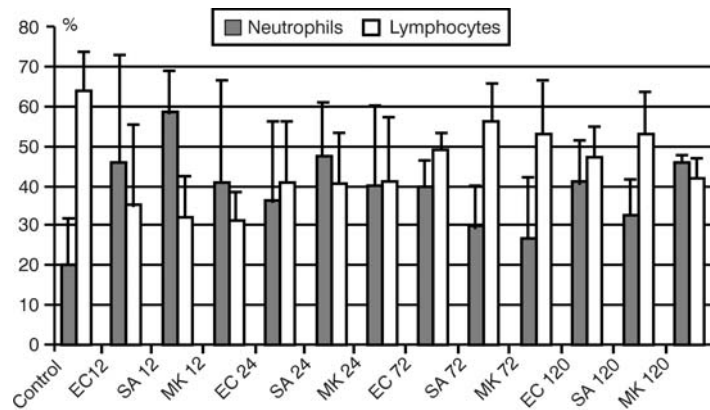

Figure 1. Development of leukocytosis in the experimental rats
MK – Mixed sepsis; EC - Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)
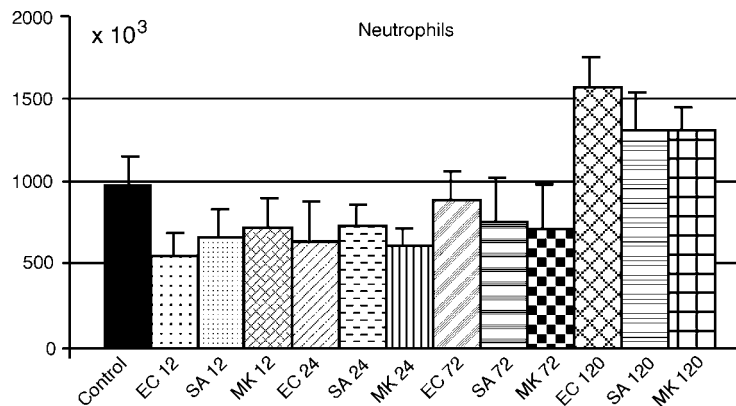

Figure 2. Changes in the relative numbers of lymphocytes and neutrophils in the experimental rats
MK – Mixed sepsis; EC - Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; 12, 24, 72, 120 – Time after surgery (h)

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Table 1. Differential counts of white blood cells in the early phase of sepsis (12th and 24th hour)

	control	MK-12	EC-12	SA-12	MK-24	EC-24	SA-24
leukocytes	4.95±0.74	2.26*±0.63	1.77*±1.13	3.11*±1.33	2.96*±2.16	3.49*±1.73	3.47*±1.19
neutrophils	1.02±0.75	1.00 ±0.71	0.66 ±0.49	2.13*±1.11	0.98 ±0.81	1.27 ±0.89	1.45 ±0.71
lymphocytes	3.13±0.42	0.74*±0.32	0.41*±0.18	1.07*±0.50	1.13*±0.90	1.41*±0.71	1.19*±0.51
monocytes	0.51±0.47	0.41 ±0.42	0.12 ±0.11	0.15 ±0.09	0.35 ±0.41	0.65 ±0.82	0.14 ±0.03
eosinophils	0.03±0.02	0.02 ±0.01	0.01 ±0.00	0.03 ±0.02	0.03 ±0.03	0.02 ±0.02	0.02 ±0.04
basophils	0.01±0.00	0.01 ±0.00	0.00*±0.00	0.01*±0.01	0.01 ±0.00	0.02 ±0.04	0.01 ±0.01
LUC	0.25±0.11	0.11*±0.05	0.09*±0.05	0.15 ±0.08	0.15 ±0.08	0.14*±0.04	0.17 ±0.05

MK – Mixed sepsis; EC - Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; LUC - Large unstained cells
The values represent the mean ± SD (n=5/group)
* p<0.05 compared with control

Table 2. Differential counts of white blood cells during the late phase of sepsis (72th and120th hour)

	control	MK-72	EC-72	SA-72	MK-120	EC-120	SA-120
leukocytes	4.95±0.74	5.80 ±2.35	7.61*±1.13	6.02 ±1.77	8.15*±1.64	6.59 ±2.97	6.17*±1.01
neutrophils	1.02±0.75	1.53 ±1.30	3.01*±0.25	1.93 ±1.02	3.79*±0.61	2.88*±1.84	2.02*±0.52
lymphocytes	3.13±0.42	3.09 ±1.46	3.78 ±0.81	3.33 ±0.85	3.54 ±1.06	3.04 ±1.02	3.35 ±0.94
monocytes	0.51±0.47	0.45 ±0.59	0.24 ±0.10	0.26 ±0.11	0.41 ±0.01	0.30 ±0.12	0.32 ±0.10
eosinophils	0.03±0.02	0.09 ±0.09	0.07*±0.04	0.08*±0.07	0.08 ±0.10	0.05 ±0.05	0.05 ±0.03
basophils	0.01±0.00	0.02*±0.01	0.01 ±0.01	0.01 ±0.00	0.05*±0.02	0.06*±0.01	0.05*±0.02
LUC	0.25±0.11	0.61*±0.28	0.51*±0.25	0.44*±0.13	0.33 ±0.04	0.32 ±0.13	0.41*±0.15

MK – Mixed sepsis; EC - Sepsis induced by *Escherichia coli*; SA – Sepsis induced by *Staphylococcus aureus*; LUC - Large unstained cells

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The statistically significant increases in number of large unstained cells (LUC) at 72h in all the groups of rats with sepsis, indicated the release undifferentiated cells into peripheral blood (Table 2). Eosinophilia was observed in the second half of the experimental protocol as well as basophilia 120h after surgery. In general these increases were statistically significant in comparison with control values (Table 2).Mild monocytopenia was apparent at 72 and 120h, as well as earlier in the group of SA rats (Table 1, 2). Thus this sepsis model in animals shows that haemotological changes include apoptosis of immune cells, as well as disorders in the function of regulatory cells during the inflammation response. Namely, besides apoptosis of immature thymocytes in the thymus during sepsis (Wang*et al*., 1994), Hotchkiss*et al*.1997, noted apoptosis of T- and B-cells, together with lymphocytes and plasmocytes in the spleen, ileum, colon, bone marrow and lungs. It is thought that besides glucocorticoids and TNF-alpha,the proinflammatory cytokines (IL-6 and IL-1) are also responsible for induction of apoptosis in the model of CLP rats**.** The immunosuppressive factors (TGF – beta, IL-4, IL-10, PGE2) are also secreted during sepsis (Hiramatsu*et al*., 1997a, 1997b). It seems that these molecules, which are released as part of the systemic inflammatory response during   sepsis, may be responsible for prolonged suppression of functional immune cells and this occurs, first of all, through induced apoptosis of these cells which significantly reduces their number.

The role of apoptosis in the disorder of regulatory cells during the inflammatory response is interesting (neutrophil granulocytes and cells of the mononuclear phagocyte system) (Deconick and Herve, 1990). Neutrophil granulocytes have the shortest life of all the leukocytes (72 hours) and are easily subject to apoptosis, as demonstrated in vivo and also in vitro. Neutrophil granulocytes are normally subject to apoptosis but now it is clear that the length of life and functional activity of mature neutrophils may be increased considerably by the action of proinflammatory cytokines (including G-CSF, GM-CSF, IFN-gamma, TNF-alpha, IL-2) (Cohn*et al*, 1991; Gillan*et al*.,1993). Besides the already mentioned cytokines, it is thought that glucocorticoids have a protective effect on neutrophils by postponing apoptosis. In this way agents which induce apoptosis in other cells, especially lymphocytes, prolong the life of neutrophils through postponing apoptosis. This also prolongs their functional activity, including secretion of toxic products. It is not entirely clear in which way inflammatory cytokines prevent apoptosis of neutophils, but it is supposed that it restores the number of receptors (CD64), improves oxidative metabolism and primary degranulation of neutrophil granulocytes (Rixen*et al*., 1996).

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MODEL SEPSE (CEKALNA LIGACIJA I PUNKCIJA) KOD PACOVA IZAZVANE MEŠANOM I ČISTIM KULTURAMA BAKTERIJA I PROMENE BELE KRVNE LOZE

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SADRŽAJ

U cilju praćenja promena hematoloških parametara (broja leukocita i procenta zastupljenosti imunokompetentnih ćelija) izazvali smo kliničku formu sepse kod pacova. Ogledi su izvedeni kod 104 mužjaka pacova, Wistar soja, telesne mase od 190 do 240 grama. Pacovi su bili podeljeni u četiri grupe od kojih su tri imale po 28 životinja dok je u kontrolnoj grupi bilo 20 životinja. Termini posmatranja i žrtvovanja životinja su bili: 12, 24, 72 i 120 časova posle hirurške intervencije.

U posmatranim terminima kod modela sepse cekalna ligacija i punktura (CLP), sa mešanom i čistim kulturama bakterija *Escherichia coli* i *Staphylococcus aureus*, uočene su slične promene hematoloških parametara: broja leukocita i procenta zastupljenosti ćelija bele krvne loze. Signifikantna leukopenija u prvoj polovini (rana sepsa) naših eksperimenata prethodila je signifikantnoj leukocitozi pacova sa sepsom u drugoj polovini eksperimenata (kasna sepsa). Takođe su uočene signifikantne promene apsolutnih vrednosti granulocita i agranulocita, pri čemu je tokom svih praćenih termina dominirala značajna neutrofilija sa limfopenijom.