Effect of Phagocyte Stimulation on Nonspecific Organism's Resistance to Shock

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Experiments on rats show a positive effect of prodigiosan phagocyte stimulation on humoral-cell interaction in the phagocytosis system in traumatic and burn shock accompanied by improvement of organism's resistance to extreme factors. Prodigiosan stimulation in the torpid phase against the background of altered phagocyte reactivity produced less pronounced and ambiguous effects.

Key Words: *shock; macrophages; neutrophils; fibronectin; phagocyte stimulation*

Our previous studies of the humoral and cellular components of phagocytosis at the early stages of traumatic and burn disease revealed complex structural and functional changes in stellate reticuloendotheliocytes (SRE), alveolar macrophages (AM), and neutrophils accompanied by shifts in the content of the nonspecific plasma opsonin fibronectin. It has been shown that macro- and microphage suppression against the background of hypofibronectinemia reflects the severity of postaggressive states and usually disappears in natural relieve of the shock [4-6]. These findings agree with the published data that organism's resistance to extreme factors strongly depends on the functional state of the phagocytosis system and plasma opsonizing activity [2,9,10,12,15]. In light of this, the search for new drugs that modulate phagocytic activity and improve nonspecific organism's resistance in shock is an important medical problem [4,8,11,13,14].

MATERIALS AND METHODS

Experiments were carried out on albino rats. To stimulate phagocytes, 0.005% prodigiosan (bacterial polysaccharide) was injected intraperitoneally in a dose of 25 μg/100g body weight 24 h before mechanical or thermal trauma or at the peak of the torpid phase of traumatic or burn shock. Phagocytic capacity of the hepatic reticuloendothelial system was assessed by the clearance of gelatinized casein ink [3]. To this end, elimination half-time and elimination rate constant were calculated and SRE containing ink particles were counted a histological preparations. Total cytosis and cytogram of the bronchoalveolar lavage were determined, and dead cells were counted using the methylene blue exclusion test [3]. Functional state of AM and peripheral blood neutrophils was assessed using 24-h *E. coli* 065 culture. The parameters of phagocytosis, phagocytic index, and phagocytic number were determined with allowance for changes in total AM and neutrophil counts and shift in differential leukocyte count [7]. Oxygen-dependent metabolism was assessed by tetrazolium nitroblue (TNB) reduction in spontaneous (sTNB) and induced (iTNB) tests, coefficient of stimulation was also calculated. Plasma fibronectin was measured by enzyme-linked immunosorbent assay [1]. Samples for electron microscopy were prepared using routine techniques. The data were processed statistically using parametric ANOVA tests.

RESULTS

Prodigiosan induced the accumulation of mononuclear cells in the microcirculatory bed of the liver and lungs; the number of mature SRE and AM, as

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TABLE 1. Number and Functional Activity of Macro- and Microphages in Shock-Related Damage and Phagocyte Stimulation (Mean Deviation from the Control, %, n=9-10)

Experimental conditions	Phagocytizing SRE								Neutrophil leukocytes					
	AM	total	dead	PA	PI	sTNB	iTNB	SC	total	PA	PI	PCC	sTNB	iTNB	SC
Stimulation in intact rats	42.6	77.4	8.6	25.9	19.4	85.3	223.5	76.6	26.8	14.5	49.5	30.5	136.1	70.0	-29.0
Torpid phase without stimulation	-30.0 -35.7	423.1 100.0	628.6 271.4	-45.7 -17.1	-31.6 -20.7	69.9 312.6	-25.2 93.1	-55.9 -47.5	188.1 302.4	-4.0 -20.5	-22.8 -17.4	36.2 -10.5	514.1 928.8	292.3 -1.8	24.5 -9.4
Shock+stimulation	-14.3 -8.6	25.8 12.9	-40.7 8.6	-25.0 -8.8	-15.8 -18.3	33.3 -15.7	79.6 42.6	38.3 106.6	169.7 288.1	-1.3 -2.7	-12.1 -10.5	66.4 23.7	-44.9 27.4	-6.2 -15.8	54.4 -4.1
Shock relieve without stimulation	-11.4 -14.3	492.3 22.6	742.8 14.3	-30.5 -37.9	-30.8 -48.2	-26.3 -5.2	-54.1 -15.8	-37.5 -29.1	169.1 310.3	4.6 -15.9	-36.3 -18.6	-18.7 36.4	271.0 252.2	453.7 276.5	37.9 -0.5
Shock relieve+stimulation during torpid phase	-2.9 -5.7	132.2 251.6	85.7 657.1	-26.9 -35.4	-28.9 -46.2	5.2 -15.8	8.8 -4.3	4.2 -2.5	247.4 606.2	28.7 -49.2	38.2 -46.6	-6.6 122.0	74.4 170.2	81.7 39.5	-5.8 -0.9

Note. Numerator and denominator correspond to traumatic and burn shock, respectively. PA: phagocytic activity, PI phagocytic index; PCC: phagocytosis completion coefficient.

well as peripheral blood neutrophil count and fibronectin content increased significantly. In the majority of experiments, increased phagocytic and metabolic activities of phagocytes with hypertrophy and hyperplasia of cell organelles were noted, which was accompanied by accelerated blood clearance (Table 1, Fig. 1).

After prodigiosan stimulation, individual reactions to mechanical and thermal injuries were similar, the shock was prolonged and mild, the total lethality decreased by 20%, and its maximum was delayed in comparison with nonstimulated controls. Under these conditions the number of sinusoidal cells considerably surpassed that in the control; the number of phagocytizing SRE and clearance rate increased. However, despite marked fibronectinemia, phagocytosis remained inhibited, the total number of AM increased slowly, while the number of dead AM decreased in comparison with nonstimulated animals. In the cytogram, the subpopulation of young and typical AM increased in parallel with a decrease in the number of defective macrophages. Functional activity of AM decreased in the sTNB-test and increased in the iTNB-test resulting in a significant increase in the stimulation coefficient in comparison with the control and shock only. Phagocytic activity of AM was suppressed: phagocytic activity and phagocytic index surpassed those in nonstimulated animals to a lesser extent but did not return to the control values.

Marked neutrophilia was observed both in stimulated and nonstimulated rats. The mean parameters of phagocytic activity and phagocytosis completion coefficient slightly increased against the background of relatively low phagocytic index, spontaneous activity of neutrophils was reduced, while their response to shock-associated damage was preserved. In parallel, blood concentration of immunoactive fibronectin increased.

Electron microscopy of liver samples showed predominance of young active SRE with numerous processes, compactly arranged cell organelles, and the signs of active endocytosis and biosynthesis in different zones of hepatic lobules. Intracellular structures of AM were characterized by hypertrophy and hyperplasia, which indicates mobilization of phagocytosis for biosynthesis in the majority of lavage macrophages. Blood neutrophil population remained heterogeneous; however, polymorphonuclear leukocytes with extended pseudopodium-like cytoplasmic processes became more abundant. These cells were characterized by granules adjacent to the cell membrane, clarified matrix and well-defined contours of cell organelles, which probably indicate moderate physiological activation of these cells. Macro- and

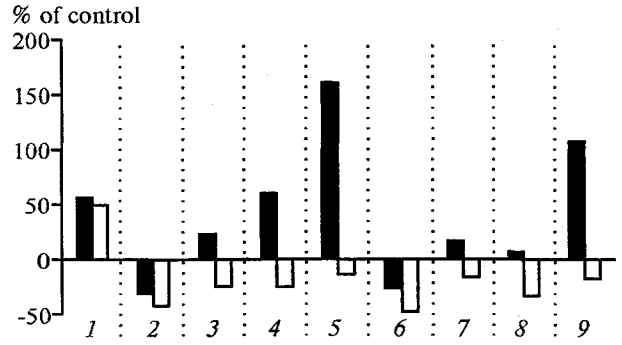

Fig. 1. Content of plasma fibronectin (dark bars) and elimination rate constant (light bars) in shock-associated damages and phagocyte stimulation. *1*) stimulation in intact rats. Traumatic shock: torpid phase (*2*), after stimulation (*3*), relieve without (*4*) and after stimulation (*5*). Burn shock: torpid phase (*6*), after stimulation (*7*), relieve without (*8*) and after stimulation (*9*).

microphages with destructive changes were uncommon. It should be noted that these structural, ultrastructural, and functional changes in phagocytes as well as variations in the blood fibronectin content were observed only in 75-80% prodigiosan-treated rats. This suggests that the stimulating effect of prodigiosan depends on the initial reactivity of phagocytes. Analysis of individual observations showed that the total number of neutrophils and their phagocytic activity increased only in individuals with high initial potential of these cells.

This relationship was most pronounced in experiments when prodigiosan stimulation was performed at the peak of the torpid phase of traumatic or burn shock. Under these conditions stimulation induced less pronounced and ambiguous effects. In approximately 50% rats stabilization of clinical and laboratory parameters was followed by complete recovery from the shock. Other animals with severe shock, in whom prodigiosan had practically no effect on the dynamics of shock, died.

These findings suggest that accumulation and short-term activation of macro- and microphages, an element of immediate adaptive reaction, associated with post-traumatic toxemia can exhaust functional reserves of phagocytes in the torpid shock phase so that additional stimulation of these cell became ineffective. Thus, the efficiency of modulation of phagocyte reactivity for improving nonspecific organism's resistance to shock-associated damage depends on individual reaction to extreme factors which is determined by the initial functional state of the phagocyte and blood opsonin systems.

REFERENCES

1. G. A. Ermolin, E. E. Efremov, E. V. Filimonova, *et al., Vopr. Med. Khimii*, No. 6, 123-126 (1986).
2. A. N. Mayanskii and D. N. Mayanskii, *Essays on Neutrophil and Macrophage* [in Russian], Novosibirsk (1989).
3. I. Ya. Uchitel', *Macrophages in Immunity* [in Russian], Moscow (1978).
4. G. M. Kharin, *Kazan' Med. Zh.*, No. 3, 199-202 (1994).
5. G. M. Kharin and A. M. Sabitova, *Byull. Eksp. Biol. Med.*, **123**, No. 5, 541-544 (1997).
6. G. M. Kharin and A. Z. Shakirova, *Ibid.*, **122**, No. 11, 577-581 (1996).
7. E. N. Shlyakhov and L. P. Andriesh, *Immunology* [in Russian], Kishinev (1985).
8. Yu. M. Shtykhno and I. P. Titova, *Pat. Fiziol.*, No. 3, 10-14 (1981).
9. J. Barroso-Aranda, R. H. Chaver, and J. C. Mathison, *Am. J. Physiol.*, **266**, No. 2, H413-H421 (1994).
10. A. J. Botha, F. A. Moor, E. E. Moor, *et al., Shock*, 3, No. 3, 157-166 (1995).
11. K. Decker, *Eur. J. Biochem.*, **192**, No. 2, 245-261 (1990).
12. H. Liehr, *J. Clin. Chem. Clin. Biochem.*, **25**, No. 4, 211-212 (1987).
13. D. J. Loegering and L. M. Commins, *Circ. Shock*, **25**, 325-332 (1988).
14. *Pathophysiology of the Reticuloendothelial System.* Ed. B. M. Altura and T. M. Saba, New York (1983).
15. S. M. Reichard and A. C. Reese, in: *Reticuloendothelial System: a Comprehensive Treatise*, New York-Lond. (1985), Vol. 7B, pp. 429-473.