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EFFECT OF INTENSIVE PHYSICAL EXERCISE ON MACROPHAGAL FUNCTIONS

N. P. Voronina and D. N. Mayanskii

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The functional state of phagocytic cells of the reticuloendothelial system (RES) and, in particular, its chief compartment — the mononuclear phagocytic system (MPS), essentially determines resitance to trauma, blood loss, burn toxemia, various forms of circulatory shock, and so on [4]. After treatment of animals with various substances stimulating RES function (microaggregated human albumin, glyceryl trioleate, quinones, a combination of estrogens and glucocorticoids), in many cases tolerance to stress increased, whereas after blockade or depression of the phagocytic activity of the resident macrophages (Mph) mortality increased [10]. It was shown previously that after sudden cooling (to −7°C) [9], acute physical exercise [1], and administration of hydrocortisone in a dose of 125 mg/kg [5, 6], the clearing function of the RES was abruptly depressed, with a corresponding lowering of resistance to stress; the ingestive function of the RES was depressed after acute stress, moreover, because of depression of the phagocytic function of the Lupffer cells (KC) of the liver, whereas clearance of the blood of pulmonary interstitial Mph showed a compensatory increase. Meanwhile, the functional activity of Mph from different compartments of the MPS during stress has received little study.

TABLE 1. Number of Monocytes in Blood, PMph, AMph, and Sinusoidal Cells of the Liver in Control and after IPE (M ± m)

Experimental conditions	PE				BAW		
	total number of leukocytes	absolute number of monocytes	total number of cells	PMph	total number of cells	AMph	number of sinusoidal liver cells per 1000 hepatocytes
	·10⁹/liter		·10⁶				
Control	6,8±0,8 (6)	0,7±0,09 (6)	14,5±1,1 (15)	14,2±1,0 (15)	9,2±0,8 (5)	8,2±0,4 (5)	335,2±8,2 (7)
IPE	4,8±0,6 (6)	0,4±0,05* (6)	12,0±1,8 (6)	11,8±1,0* (6)	12,1±0,9* (5)	11,0±1,0* (5)	326,9±5,2 (7)

Legend. *p < 0.05 compared with control. Here and in Tables 2 and 3, number of animals given in parentheses.

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TABLE 2. Functions of PMph and AMph in Control and after IPE (M ± m)

Experimental conditions	PMph					AMph			
	percent of Mph in monolayer phagocytosing		percent of Mph reducing	cathepsin D activity		percent of Mph phagocytosing MG	percent of Mph reducing nitro-BT	cathepsin D activity	
	SRBC	MG	nitro-BT	free	total			free	total
Control	19,4±2,1 (5)	62,8±4,1 (5)	6,4±0,9 (5)	7,7±0,7 (27)	13,8±0,9 (27)	12,1±0,8 (9)	14,5±0,7 (9)	5,2±0,6 (5)	13,6±3,5 (5)
IPE	24,4±4,3 (5)	70,4±3,7 (5)	10,0±0,5** (5)	6,1±0,6 (13)	10,9±0,7** (13)	18,6±1,7** (10)	22,9±1,4* (9)	6,8±0,9 (5)	14,7±2,9 (5)

Legend. *p < 0.001, **p < 0.01 compared with control. Here and in Table 3, cathepsin D activity expressed in micromoles tyrosine/min/g protein.

The aim of this investigation was to compare the properties of Mph in the liver, lungs, and peritoneal exudate (PE) in control animals and after intensive physical exercise (IPE).

EXPERIMENTAL METHOD

Experiments were carried out on 92 Wistar rats and 71 CBA mice. IPE consisted of enforced swimming in a tank at 32-34°C by rats for 3.5 h and by mice for 1 h, carrying a load equal to 4% of body weight. The total leukocyte count was determined and the absolute number of monocytes counted. Peritoneal Mph (PMph) were obtained after the mice had swum for 1 h by the method in [8], in irrigating the peritoneal cavity of the mice with heparinized Hanks' solution 3 days after parenteral injection of 4% starch suspension. Alveolar Mph (AMph) were obtained by the method in [13] in our modification. The number of cells in PE and in the bronchoalveolar washings (BAW) was counted in a Groyaev's chamber. For differential counting, cells of PE and BAW were deposited on coverslips, fixed with methanol, and stained by Pappenheim's method. Their functional activity was determined relative to ingestion of sheep's red blood cells (SRBC) and methacrylate granules (MG) 0.9 μ in diameter. For this purpose coverslips with a monolayer of the viable Mph were placed in 0.9 ml of Hanks' solution with 5% bovine serum albumin to which was added, in some cases, 0.1 ml of a 5% suspension of SRBC, and in others 0,1 ml of a suspension of MG at the rate of 100 particles per cell. After incubation for 1 h at 37°C the preparations were washed to remove uningested particles, fixed in methanol, and stained by Pappenheim's method. The number of Mph binding and ingesting SRBC or taking up MG (at least three granules per cell) was counted under the microscope with a magnification of 1000. Ability of Mph to generate the superoxide anion-radical and other active forms of oxygen was judged by the results of the nitro-BT test [3]. For this purpose coverslips with a viable cell monolayer were transferred for 30 min into bottles with incubation medium containing 0.9 ml of Hanks' solution and 0.1 ml of 0.2% nitro-BT ("Chemapol," Czechoslovakia). After incubation the preparations were washed and fixed in methanol, and the cell nuclei were counterstained with 1% carmine solution. The number of diformazan inclusions in the preparations was counted. The number of sinusoidal cells per 1000 hepatocytes and the number of KC phagocytosing Mark P-100 iron carbonyl particles (1-1.5 μ in diameter) were counted in sections through the rat liver 4-5 μ thick, stained with hematoxylin and eosin, under a magnification of 1000. In lung sections the number of phagocytic Mph in the interstitial tissue was counted in 10 fields of vision [9]. For this purpose, 1 ml of a 10% suspension of iron carbonyl particles in 5% isotonic starch solution was injected intravenously after IPE. The ingestive functions of KC and Mph in the interstices of the lung were determined 2 h after injection of the particles. KC were isolated from the rat liver by magnetic fractionation [2]. For this purpose, 2 h after intravenous injection of a suspension of iron particles the animals were killed and the liver was perfused in situ with cold 0.25 M sucrose solution with 0.01 M EDTA, pH 7.4. All subsequent procedures were carried out at 2-4°C. KC, loaded with iron particles, were separated from hepatocytes in the field of an electromagnet with an intensity of 1000 Oe. Free and total (in the presence of Triton X-100 in a final concentration of 0.1%) catepsin D activity was determined [7] in the KC thus obtained, and also in lung homogenates, AMph, and PMph. Total protein was determined by Lowry's method [12]. The results were subjected to statistical analysis by Student's t test.

TABLE 3. Functions of Pulmonary Interstitial Mph and Kupffer Cells in Control and after IPE (M ± m)

Experimental conditions	Percent of phagocytic Mph in interstices of lung	Cathepsin D activity in lung homogenate		Number of phagocytic KC per 1000 hepatocytes	Cathepsin D activity in KC		
		free	total		free	total	free / total
Control	9,0±1,4 (7)	0,25±0,05 (5)	0,49±0,05 (5)	210,1±9,8 (7)	1,6±0,3 (6)	5,7±0,6 (6)	0,29
IPE	40,6±3,5 (8)	0,38±0,02** (5)	0,84±0,11** (5)	100,2±9,8* (7)	8,6±2,2** (6)	9,6±2,0** (6)	0,83

Legend. *p < 0.01, **p < 0.05 compared with control.

EXPERIMENTAL RESULTS

Whereas the leukocyte count in the rats' blood after swimming for 1 h was increased by 1.7 times, after IPE for 3.5 h it showed a tendency to fall. The absolute number of monocytes under these circumstances was reduced by 1.8 times. The total number of PE cells also had a tendency to fall, mainly due to a decrease in the number of PMph in the exudate. Meanwhile, the number of cells in BAW was significantly increased by 1.3 times after IPE, due to an increase in the number of AMph and neutrophils. The total number of liver sinusoidal cells in the stressed animals differed only a little from the control (Table 1).

The ingestive functions of PMph and AMph were activated a little after IPE. For instance, binding and ingestion of SRBC by PMph increased by 1.3 times, and of MG by 1.2 times (Table 2). In the case of AMph, after IPE they began to ingest MG appreciably more actively than in control ($p < 0.01$). Incidentally, PMph exhibited much greater phagocytic activity relative to MG than to AMph. The number of diformazan-positive PMph in the nitro-BT test was increased after IPE by 1.5 times, and the number of AMph by 1.6 times. Free and total cathepsin D activity did not change significantly in PMph after IPE. In AMph there was a tendency for both free and total cathepsin D activity to increase (Table 2).

The study of fixed Mph in the liver and lung showed that the phagocytic power of the pulmonary interstitial Mph was increased by 4.5 times after IPE (Table 3). Simultaneously with an increase in the ingestive power of Mph in the lung, the phagocytic function of KC was inhibited. The number of KC in the liver phagocytosing colloidal iron particles fell by more than half (Table 3). Free and total cathepsin D activity in lung homogenates was increased after IPE by 1.5 and 1.7 times, respectively. A particularly great increase in free activity of the enzyme (more than fivefold) was observed in KC. Meanwhile, total activity was increased by 1.6 times (Table 3). An increase in the ratio of free to total cathepsin D activity was observed, evidence of the labilizing effect of IPE on the lysosomal membranes of KC.

The results are evidence that different classes of Mph react differently to IPE. In this connection it is worth noting that the authors previously showed that different classes of Mph react differently to cooling (−7°C) and to injection of a large dose of hydrocortisone [5, 6, 9], when the ingestive function of KC was inhibited whereas the phagocytic activity of Mph in other locations was activated at the same time: in some cases pulmonary interstitial Mph, in others splenic Mph. The total clearing capacity of the RES was reduced on account of depression of the phagocytic function of KC in the liver. This can be understood if it is recalled that KC accounts for 80-95% of the total blood clearance from foreign substances [6]. The increased sensitivity of KC to stress-induced changes may be connected with the higher density of receptors for glucocorticoids, mobilized during stress, on KC than on other classes of Mph. Meanwhile, KC are more sensitive to a deficiency of fibrinectin, synthesis of which is inhibited after various times of stress [15]. Activation of pulmonary Mph after IPE is difficult to explain at present. It can be tentatively suggested that it is connected with inadequate neutralization of endotoxins, which are constantly being absorbed from the intestinal lumen by KC in the liver [14]. Endotoxins are known to be powerful stimulators of pulmonary and other classes of Mph [11].

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PRODUCTION OF MONOCLONAL ANTIBODIES TO MAMMALIAN CELL NUCLEAR DNA BY IMMUNIZATION WITH STREPTOCOCCAL GROUP A POLYSACCHARIDE CONJUGATED WITH SYNTHETIC POLYELECTROLYTES

É. I. Drobyshevskaya, E. Yu. Pyt'eva,
E. V. Ryzhikova, N. A. Borodiyuk, I. M. Lyampert,
A. V. Nekrasov, and R. M. Khaitov

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Previous investigations showed the presence of antibodies reacting with DNA or nuclei of mammalian cells in the sera of animals immunized with various microorganisms, including group A streptococcus. It was suggested that this depends on the presence of cross-reaction between mammalian and microbial DNA [10]. It was shown at the same time that autoantibodies to DNA can be obtained by injection of a lipopolysaccharide (LPS), capable of inducing polyclonal activation of B-cells, into animals [8]. There is some evidence that antibodies to DNA are highly varied [12]. It has been shown by the use of monoclonal antibodies (McAb) that some of them can react specifically with individual antigenic determinants of DNA [15], and in other cases cross reactions have been found between DNA and cardiolipin, tubulin, and thyroglobulin [6, 7]. Hence the importance of obtaining McAb to antigens of cell nuclei by immunization with various microbial antigens.

It was shown previously that the polysaccharide (PS) from group A streptococcus (A-PS) contains a cross-reacting determinant, antibodies to which are autoantibodies and react with epithelium of thymus and skin [11]. It has been suggested that injury to the epithelium of the thymus may be the cause of immunoregulatory disturbances leading to the development of an autoimmune process [11].

A-PS is known to be a nonimmunogenic hapten. It was shown previously that conjugates of haptens with synthetic polyelectrolytes (PEL) induce marked production of hapten-specific antibodies [3, 4]. As a result of immunization with A-PS, conjugated with synthetic PEL, an immune response was obtained to A-PS, and McAb cross-reacting with the epithelium of the stratum basale of the skin and thymus were isolated [2]. By long-term immunization with the

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