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RESPONSE OF PULMONARY MACROPHAGES TO HYDROCORTISONE AND ADRENALECTOMY IN RATS

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The mononuclear phagocyte system (MPS) is under endocrine control [4]. In this connection macrophages have receptors for insulin, glucagon, somatomedin, parathormone, estrogens, and adrenalin [4, 7]. The writers showed previously that alveolar macrophages (AM) of rats possess a saturable (receptor) corticosterone binding system, and that the number of binding sites depends on the degree of activation of MPS [6]. Meanwhile reactivity of pulmonary macrophages during changes in the glucocorticoid level in animals has virtually not been studied, although the character of injury to lung tissue depends essentially on the functional state of the phagocytic cells of the bronchoalveolar tract [9, 10].

The aim of the present investigation was to study changes in the pulmonary compartment of MPS in response to injection of large doses of hydrocortisone and to bilateral adrenalectomy.

EXPERIMENTAL METHOD

Experiments were carried out on 95 Wistar rats of both sexes weighing 180-250 g. Subcutaneous injections of hydrocortisone (HC) acetate were given in a daily dose of 125 mg/kg subcutaneously for 7 days to the animals of group 1 in order to form a depot and to maintain a consistently raised glucocorticoid level in the animal [11]. Rats receiving daily injections of 1 ml of 0.85% NaCl solution in accordance with the same schedule served as the control for this group. The rats were killed 24 h after the last injection. Rats of group 2 underwent adrenalectomy, and the control group the corresponding mock operation, 7 days before investigation [15]. To stimulate MPS, animals of group 3 received an intravenous injection of zymosan in a dose of 0.1 g/kg, and the corresponding control group received 1 ml of 0.85% NaCl solution; the animals were killed 5 days after the injection. Rats of group 4 were stimulated with zymosan 24 h after the last injection of HC, and in the control the rats received 0.85% NaCl solution instead of HC. Blood clearance of colloidal carbon was estimated by the method in [12] and the number of phagocytic cells in the interstices of the

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TABLE 1. Parameters of Functional Activity of AM and Cell Composition of BAW (M ± m)

Parameter	Group of animals			
	1	2	3	4
Number of phagocytic cells in interstices of lungs per area of 140,000 μ²	6,3±0,56* / 24,0±2,30	53,2±5,46* / 15,4±1,93	49,0±5,00* / 15,0±1,40	15,4±3,58* / 52,4±3,13
Number of BAW cells ($\cdot 10^6$) per gram of lung tissue	34,9±4,90* / 6,6±0,74	3,6±0,41 / 2,9±0,37	11,4±1,67* / 4,3±0,64	21,7±3,60* / 7,2±0,30
Number of AM in BAW ($\cdot 10^6$) per gram	12,5±3,70* / 5,2±0,44	2,7±0,49 / 1,7±0,13	6,9±1,20* / 3,4±0,45	14,5±2,40* / 5,7±0,58
Number of neutrophils in BAW ($\cdot 10^6$) per gram	20,0±3,80* / 0,3±0,11	0,1±0,06 / 0,1±0,03	0,1±0,06* / 0,01±0,00	3,2±0,55* / 0,7±0,10
AM in monolayer phagocytosing methacrylate granules, percent	5,7±4,25* / 19,3±0,43	36,0±5,87 / 29,8±2,93	42,7±1,81* / 20,0±0,92	7,2±1,10* / 26,3±1,22
AM in monolayer reducing nitro-BT, percent	3,3±1,48* / 10,7±2,49	24,5±3,33* / 11,3±0,82	25,6±1,12* / 10,2±1,53	11,3±3,91 / 20,0±1,23

Legend. Numerator shows values of parameters in experiment, denominator – in control. *p = 0.05, compared with control.

lungs was counted [5] simultaneously with the total number of cells in bronchoalveolar washings (BAW) and the number of individual cell forms in a monolayer stained with azure and eosin [5]. Blood for determination of the leukocyte count and formula was obtained from the retro-orbital sinus. The BAW cells were diluted to a concentration of $0.5 \cdot 10^6$ cells/ml in medium 199 with 20% serum for nutrient media. The cell suspension was transferred in volumes of 0.5 ml into chambers made by gluing polyethylene tubes, 0.5 cm in diameter and 2.5 cm high, to slides. The cells in the chambers were allowed to settle for 1 h at 37°C. The incubation medium with nonadherent cells was then decanted and the monolayer rinsed twice with Hanks' medium. The potential bactericidal power of the cells was determined by the nitro-BT test [3]. Into chambers containing a viable monolayer, 0.2 ml of 0.02% nitro-BT in Hanks' medium was poured. After incubation at 37°C for 30 min the monolayer was rinsed with 0.85% NaCl solution, dried, and fixed for 3 min in methyl alcohol. The cell nuclei were stained with carmine. The percentage of AM containing inclusions of reduced diformazan was counted in the preparations. The phagocytic power of AM was judged from accumulation of methacrylate granules (d = 0.9 μ; Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences). For this purpose, 0.2 ml of Hanks' medium with methacrylate granules was added to the monolayer of BAW cells at the rate of 100 particles per cell, and the mixture was incubated for 1 h, rinsed, dried, and stained by Pappenheim's method.

EXPERIMENTAL RESULTS

The clearing function of MPS was impaired in the rats of group 1. The rate of clearance of the blood from colloidal carbon was reduced by 1.7 times compared with the control. The number of phagocytic cells in the interstices of the lungs was 3.8 times less than in the control (Table 1). The number of blood leukocytes ($16.4 \cdot 10^3 \pm 1.8 \cdot 10^3/mm^3$) was more than doubled, and 90 ± 1.7% of them were neutrophils (control 18 ± 2.5%; p = 0.001). The number of cells in BAW was increased more than fivefold (Table 1). In BAW preparations from animals receiving HC, neutrophils predominated (experiment 56.6 ± 7.5%, control 1.1 ± 0.5%). The relative number of AM was reduced by more than half (experiment 36.2 ± 8.4%; control 88.6 ± 1.6%; p = 0.001), although their absolute number was greater than in the control (Table 1). The percentage of AM reducing nitro-BT and phagocytosing methacrylate granules reflected a definite reduction of the bactericidal and ingestive power of AM in animals treated with HC (Table 1).

On the 7th day after adrenalectomy (group 2) there was evidence of activation of the clearing function of MPS. The rate of clearance of the blood was 1.4 times higher in the experimental animals than in the control (p = 0.005). The number of phagocytic cells in lung sections also was considerably greater than in the control. Marked monocytosis was observed in the blood (experiment $1.46 \cdot 10^3 \pm 0.6 \cdot 10^3/mm^3$; control $0.53 \cdot 10^3 \pm 0.03 \cdot 10^3/mm^3$; p = 0.002). In the experiments, 1.4 times more cells were washed out of the lungs than in the control, mainly represented by AM, for which the values of parameters of the nitro-BT test were twice as high as in the control (Table 1).

After intravenous injection of zymosan the adrenalectomized animals died almost immediately, so that it was impossible to study their cellular responses.

The rate of clearance of colloidal carbon from the blood of the rats of group 3 (stimulated by zymosan) was increased threefold ($p < 0.001$). This was combined with a more than threefold increase in the number of phagocytic cells in the interstices of the lungs.

The number of leukocytes in the blood was increased (experiment $11.2 \cdot 10^3 \pm 1.5 \cdot 10^3/mm^3$, control $6.9 \cdot 10^3 \pm 0.9 \cdot 10^3/mm^3$; $p = 0.005$). Significantly more cells were washed from the bronchoalveolar tract, mainly due to doubling of the absolute number of AM. In the monolayer, the AM ingested methacrylate granules and reduced nitro-BT much more intensively (Table 1). In animals treated with HC, the rate of clearance of the blood after intravenous injection of zymosan (group 4) was only half of that in the control ($p = 0.016$), but the number of phagocytic cells in the interstices of the lungs was only one-third of that in the control (Table 1). The total number of leukocytes in the blood ($8.07 \cdot 10^3 \pm 1.3 \cdot 10^3/mm^3$) was two times lower than in the control due to a decrease in the numbers of all types of cells ($p = 0.002$). However, BAW contained significantly more cells than in the control, chiefly on account of AM and neutrophils. Parameters of ingestion of methacrylate granules and of the nitro-BT test in the monolayer of AM were lower by two-thirds and one-half, respectively (Table 1).

Thus the effect of stimulation by zymosan was manifested much less strongly in animals receiving large doses of HC beforehand. The rate of clearance of the blood from foreign particles, the phagocytic activity of the interstitial cells of the lungs, and parameters of the nitro-BT test were restored to their normal values, but did not exceed them, as they did in the control animals after stimulation with zymosan. The recruiting of a large number of neutrophils into the alveoli of the lungs after injection of large doses of HC was most demonstrative, and was caused by an increase in numbers of the whole pool of eluted cells. The number of neutrophils in the blood was many times greater than normal. Glucocorticoids are known to accelerate maturation of granulocytes and the release of mature neutrophils into the blood stream from the bone marrow, similarly to what is observed in stress [1, 2]. The lungs are one of the principal sites for sequestration of neutrophils, and under certain conditions this may disturb the resistance of the lung tissue [8], for neutrophils generate mediators of inflammation and cytotoxic factors, including O_2^-, $\uparrow O_2$, H_2O_2, and $\cdot OH$, which directly damage the endothelium of the lung capillaries [14]. Another factor which must be taken into account is that HC depresses the ingestive and bactericidal power of AM and of neutrophils [13]. Thus chronic stress or artificially induced hypercorticism can create favorable conditions for complications of inflammatory processes in the lungs. Measured stimulation of MPS (in this case with zymosan) can bring to light the latent reserves of the system, which are considerably depressed in animals with a raised glucocorticoid level.

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