KINETICS AND PROPERTIES OF CORTISOL-RESISTANT LYMPHOCYTE POPULATION FROM GUINEA PIG LYMPH NODES DURING PROTEIN SENSITIZATION

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UDC 612.428-064:615.357.453

Lymphocytes from the cervical lymph nodes of guinea pigs were incubated in medium No. 199 for 24 h in the presence of cortisol in concentrations of 20 and 100 μg/100 ml. The survival rate of the lymphocytes and their cortisol metabolism were determined and their nucleic acid concentration was estimated cytophotometrically. In intact and sensitized guinea pigs progesterone, in a concentration of 10^{-5} M, inhibited both the lytic action of cortisol and its metabolism. A marked decrease in cortisol metabolism by the lymphocytes was found from the sixth day after sensitization, and it did not recover until the 90th day. On the 17th-30th day the cortisol-resistant lymphocyte population was increased. In a concentration of 100 μg/100 ml cortisol reduced the nucleic acid concentration in lymphocytes both of the intact guinea pigs and also of the sensitized animals on the 17th-30th day, when the cortisol-resistant cell population was increased.

KEY WORDS: cortisol; cortisol-resistant lymphocyte population; sensitization; progesterone; nucleic acids.

Lymphocytes in the lymphoid organs and blood stream are heterogeneic with respect to their origin, function, relationship to mitogens, and other features. In relation to the lytic action of corticosteroids, two lymphocyte populations also are distinguished. One is destroyed by this hormone and is called corticosteroid-sensitive; the other population is stable, or corticosteroid-resistant [8]. The two populations differ in several features [6], but their role in the development of allergic and immunologic reactions has not yet been adequately explained. It was accordingly decided to study the kinetics and properties of the cortisol-resistant lymphocyte population during various allergic processes.

In this investigation the kinetics of the cortisol-resistant lymphocyte population in guinea pig lymph nodes was studied during the development of protein sensitization and certain mechanisms of the resistance of the cortisol-resistant population to the lytic action of cortisol were examined.

EXPERIMENTAL METHOD

Experiments were carried out on 80 male guinea pigs weighing 250-300 g. The animals were sensitized with normal horse serum in a dose of 0.05 ml subcutaneously and were used in the experiments on the 6th, 17th-30th, 60th, and 90th days. The guinea pigs were killed by taking blood from the heart, followed by air embolism. The cervical lymph nodes were removed and placed in a Petri dish containing medium No. 199 in Hank's solution. The lymph nodes were thoroughly homogenized to obtain a cell suspension which was centrifuged at 600 rpm and washed twice or three times with medium. Films prepared from this suspension were stained with azure–eosin for determination of the cell composition. The viability of the cells also was determined by the trypan blue test. The original suspension was diluted to a concentration of $(1.51\text{-}2.25)\cdot 10^6$ lymphocytes/ml and transferred in volumes of 3 ml to flasks containing cortisol in a final concentration of

Department of General Pathology, N. I. Pirogov Second Moscow Medical Institute. Allergologic Scientific-Research Laboratory, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 68-71, January, 1977. Original article submitted July 9, 1976.

20 and 100 g/100 ml. Parallel flasks were prepared without the hormone for use as the control. The flasks with lymphocytes were placed in a specially designed shaker to ensure constant mixing of the lymphocytes with the medium, after which they were incubated at 37°C for 24 h. After incubation the number of cells was counted in a Goryaev's chamber, the percentage of surviving lymphocytes was determined, and the lytic action of the cortisol was accordingly estimated. The original and residual concentrations of cortisol were determined fluorimetrically and the intensity of its metabolism per 10^7 lymphocytes was calculated from the difference. Changes in the relative nucleic acid content in the lymphocytes were estimated cytophotometrically using an apparatus designed at the Laboratory of Immunology, Tuberculosis Research Institute, Ministry of Health of the Kazakh SSR, after straining the films with gallocyanin and chrome alum by a modified method of De Boer and Sarnaker [2].

EXPERIMENTAL RESULTS

In the experiments of series I optimal conditions for incubation of the lymphocytes were determined. One such condition was the choice of an adequate glucocorticoid. This was important because different workers have used different substances and have obtained contradictory results under identical conditions [3-5]. To choose the preparation, the lytic action of cortisol, cortisol acetate, cortisol-21-succinate, and cortisone in a concentration of $2.76 \cdot 10^{-6}$ M were tested. The survival rate of the lymphocytes on incubation for 24 h in the presence of each glucocorticoid was 58.9, 83.4, 95, and 98%, respectively. Cortisol thus had the strongest lympholytic activity. The action of cortisol acetate was much weaker, and that of cortisol-21-succinate and cortisone was virtually absent. All the remaining experiments were therefore carried out with cortisol. It was also found that after incubation for 24 h the survival rate of the lymphocytes with cortisol in a concentration of 100 μg/100 ml became stabilized, and prolonging the incubation to 48 h caused no further decrease in the percentage of surviving lymphocytes, although in a concentration of 20 μg/100 ml this percentage continued to fall a little. The optimal concentration of lymphocytes was $(1.26\text{-}2.50) \cdot 10^6$ cells/ml medium, for within these limits both the percentage of surviving lymphocytes and the intensity of cortisol metabolism remained constant.

In the experiments of series II the kinetics of the cortisol-resistant lymphocyte population and the metabolism of cortisol by the lymphocytes in intact and sensitized guinea pigs were studied (Table 1).

As Table 1 shows, on the sixth day of sensitization the survival rate of the cells was the same as in the control although the intensity of cortisol metabolism by the lymphocytes was already significantly reduced. On the 17th-30th day the cortisol-resistant lymphocyte population was increased, and this also was accompanied by a significant decrease in the intensity of cortisol metabolism. By the 60th day of sensitization the cortisol-resistant population was back to its normal size. The intensity of cortisol metabolism, however, still remained low and did not return to normal until the 90th day.

As Pytskii [1] has found, the blood plasma concentration of 11-hydroxycorticosteroids in sensitized guinea pigs is not increased and does not differ from that in the control. Consequently, the possible destruction of the cortisol-sensitive lymphocyte population, which could lead to relative preponderance of the cortisol-resistant lymphocyte population, has no role to play in this case. It can accordingly be concluded that the increase in cortisol-resistant population was an independent process connected with activation of immune mechanisms.

TABLE 1. Effect of Different Concentrations of Cortisol on Survival Rate of Lymphocytes and Metabolism of This Hormone by Lymphocytes of Intact and Sensitized Guinea Pigs (M ± m)

Group of animals	Number of guinea pigs	Survival rate of lymphocytes, %		Cortisol metabolism, μg/10^7 lymphocytes	
		20 μg/100 ml	100 mg/100 ml	20 μg/100 ml	100 mg/100 ml
Intact	32	73,2±1,60	57,3±1,74	0,401±0,017	0,931±0,040
Sensitized:					
6 days	6	67,9±4,24	49,5±3,43	0,178±0,024	0,422±0,078
P		>0,05	>0,05	<0,001	<0,001
17—30 days		82,7±2,74	71,0±2,93	0,121±0,020	0,375±0,045
P		<0,01	<0,01	<0,001	<0,001
60 days	6	74,0±3,44	60,3±4,64	0,206±0,029	0,588±0,049
P		>0,05	>0,05	<0,001	<0,001
90 days	6	77,05±2,15	54,9±3,97	0,347±0,045	1,077±0,140
P		>0,05	>0,05	>0,05	>0,05

TABLE 2. Changes in Nucleic Acid Concentration during Incubation of Lymphocytes from Lymph Nodes of Intact and Sensitized Guinea Pigs (M ± m)

Group of animals	Number of guinea pigs	Content of nucleic acids per lymphocyte, conventional units			
		Before incubation	after incubation for 24 h		
			without cortisol	with cortisol, 100 μg/100 ml	significance of difference, P
Intact	5	14,34±0,115	14,04±0,127	12,22±0,168	<0,001
Sensitized for 17-30 days	5	15,16±0,33 >0,05	15,51±0,290 <0,02	14,18±0,105 <0,001	<0,01 —

TABLE 3. Effect of Progesterone on Survival Rate of Lymphocytes and Cortisol Metabolism by Lymphocytes of Intact and Sensitized Guinea Pigs (M ± m)

Group of animals	Number of guinea pigs	% of surviving lymphocytes		P	Cortisol metabolism, $\mu g/10^7$ lymphocytes		P
		with cortisol, 100 μg/100 ml	cortisol + progesterone		with cortisol, 100 μg/100 ml	cortisol + progesterone	
Intact	11	52,9 ±2,72	78,4 ±2,30	<0,001	1,023 ±0,07	0,641 ±0,12	<0,01
Sensitized for 17-30 days	12	66,5 ±2,07	87,0 ±2,10	<0,001	0,416 ±0,05	0,189 ±0,057	<0,01

Investigation of the nucleic acid content (Table 2) showed that it was higher in the lymphocytes of sensitized guinea pigs after incubation than in intact animals. However, cortisol significantly reduced their concentration in the intact guinea pigs and also in the sensitized guinea pigs at a time when their cortisol-resistant lymphocyte population was increased.

It will be noted that in the sensitized animals the mean content of nucleic acids was significantly greater after incubation than in the lymphocytes of the intact guinea pigs.

Although progesterone does not possess the biological properties of cortisol, it competes with it for receptors in the cytoplasm, and so inhibits the effects of cortisol [7]. The ability of progesterone in a concentration of 10^{-5} M to inhibit destruction of lymphocytes by cortisol was therefore investigated. Progesterone itself, in that concentration, had no effect on the survival rate of the lymphocytes. The addition of progesterone to cortisol (Table 3) led to a significant increase in the survival rate of the lymphocytes and depressed the metabolism of this hormone by the lymphocytes in intact guinea pigs.

Consequently, not only the lympholytic effect of cortisol, but also its metabolism takes place after binding of the hormone with the appropriate receptor in the cytoplasm. In sensitized guinea pigs progesterone has virtually the same protective action and inhibits cortisol metabolism considerably. The development of sensitization in guinea pigs is thus accompanied by a decrease in the ability of the lymphocytes in the lymph nodes to metabolize cortisol, and by a small but significant increase in the cortisol-resistant lymphocyte population on the 17th-30th day. The mechanisms of these changes require further study.

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