COADAPTATION OF ENZYME SYSTEM OF LYMPHOCYTES AND NEUTROPHILS IN INTACT MICE AND MICE WITH INFECTIOUS DISEASES

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Unity of the organism as a whole presupposes a definite orderliness and interaction of systems. Organization of systems is achieved through complex combinations of regulatory mechanisms, functioning at different levels of organization of the living organism. Meanwhile interconnection and integration permit the essential freedom of some elements of the system relative to others. Integration of the organism may be characterized by functional interaction of its component elements, which in the special case is expressed as correlation between enzymes. If the concrete system of the blood is examined, complexes can be distinguished in which the features are combined and mutually determined not only by internal cellular processes, but also by the influence of the diverse regulatory mechanisms of the entire status of the organism [7, 10]. Terent'ev [12] has called these systems of correlating features correlation pleiads. The need to study integration, as well as the relative autonomy of cellular systems, is determined by previous investigations, For instance, it has been found that clear correlation exists in rats during hypoxia between enzyme activity of lymphocytes and the myocardium [2], but inadequate strength of the correlation pleiad of levels of activity of certain enzymes in lymphocytes heralded a lethal outcome of staphylococcal toxemia in mice [5]. It was accordingly decided to study correlation between levels of activity of certain enzymes of lymphocytes and neutrophils at three levels of organization: subcellular — correlation between activity of enzymes within lymphocytes and neutrophils, intercellular — coordination of metabolic processes of two types of blood cells (lymphocytes and neutrophils) and, finally, temporal coordination of levels of metabolism in blood cells before and after infection.

EXPERIMENTAL METHOD

Experiments were carried out on C57BL, CBA × C57BL, and noninbred mice Activity of the following dehydrogenases was determined in peripheral blood lymphocytes and neutrophils by Nartsissov's quantitative cytochemical method [9]: succinate dehydrogenase (SDH, 1.3.99.1), α-glycerophosphate dehydrogenase (α-GPDH, 1.1.2.1), NAD-dependent α-glycerophosphate dehydrogenase (α-GPDH-NAD, 1.1.1.8), malate dehydrogenase with NAD as coenzyme (MDH-NAD, 1.1.1.37), malate dehydrogenase with NADP as coenzyme (MDH-NADP, 1.1.1.40), glucose-6-phosphate dehydrogenase (G6P 1.1.1.49), $NADPH_2$-diaphorase ($NADPH_2$-d, 1.6.1.1), lactate dehydrogenase (LDH, 1.1.1.27), and also the hydrolytic enzyme acid phosphatase (AcP, 3.1.3.2), by the azo-coupling method [14], its activity expressed as an index [15].

The cytochemical investigation was conducted on C57BL and CBA × C57BL mice before and 3 h and 1, 2, and 8 days after intraperitoneal infection with a culture of *S. paratyphi* B. Noninbred mice also were tested before and 3 days after infection (intraperitoneally) with a living culture of *S. typhimurium*. Coefficients of correlation were calculated between the values of enzyme activity obtained. To characterize the degree of coordination of the enzyme systems as a whole, a mean coefficient of correlation was calculated with the use of the auxiliary parameter: $Z(r) = {}^1/_2 \ln [(1 + r)/(1 - r)]$. Differences between coefficients of correlation were discovered with the aid of the cumulative sigma [13].

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TABLE 1. Correlation between Parameters of Blood Cell Enzyme Activity at different Levels of Integration in Mice

Intracellular correlations in lymphocytes and neutrophils			In intact mice		Intercellular correlations			
blood cells	lymphocytes		neutrophils		lymphocyte – neutrophil			
enzymes	n	r	n	r	enzymes	n	r	p
SDH-LDH	80	0,197	69	0,406**	α-GPDH·NAD	59	0,788	0,001
SDH-AcP	81	0,083	80	0,172	α-GPDH	59	0,620	0,001
LDH-CP	80	0,206	69	0,156	$NADPH_2$-d	100	0,603	0,001
MDH·NAD					G-6-P	40	0,507	0,001
MDH·NADP	40	0,300	38	0,050	MDH·NAD	40	0,413	0,01
MDH·NAD-AcP	40	0,308	40	0,288	LDH	69	0,390	0,01
MDH·NAD					AcP	152	0,298	0,01
$NADPH_2$-d	40	—0,140	21	—0,509*	SDH	80	0,295	0,01
$NADPH_2$-d								
AcP	40	0,190	51	—0,288*	Av. value of r	599	0,455	0,001
$NADPH_2$-d								
G-6-P	40	—0,117	21	0,197				
AcP	40	0,222	40	0,119				
G-6-P								
MDH·NAD	40	0,207	40	0,079				
Av. value of r	521	0,188		0,223				

	n	Before infection	3rd day after infection		p
MDH·NAD	40	0,413	0,605		
AcP	40	0,125	0,474		
G-6-P	40	0,507	0,781		
Av. value of r	120	0,357	0,637	I=2,92	0,01

Legend. n) Number of animals, *p < 0,05, **p < 0.01. r) Coefficient of correlation.

EXPERIMENTAL RESULTS

Table 1 gives coefficients of correlation between levels of enzyme activity of lymphocytes and neutrophils. Coordination between enzyme activity in blood cells of each type is relatively low. This was due to the multicomponent nature of the metabolic system (rigid correlation is possible only in a two-component system), including the fact that none of the correlation pairs contained enzymes belonging to the same metabolic pathway. Nevertheless, the mean coefficient of correlation, reflecting the total strength of interconnection between individual parameters of enzyme activity, is statistically accurate and is evidence both of the general integration of the cellular system as a whole and also of the relative autonomy of its components. The degree of coordination of metabolism in lymphocytes and neutrophils is close, but on comparing individual correlation pairs, certain distinguishing features may be noted. In neutrophils, for instance, glycolysis and the Krebs' cycle (LDH — SDH) were more closely coordinated in neutrophils. The negative correlation between $NADPH_2$-diaphorase and malate dehydrogenase activity will be noted. This correlation can be interpreted as competition between the dehydrogenases for reduced NADP, for this nucleotide may either be oxidized by $NADPH_2$-diaphorase or it may be utilized in the oxidative carboxylation of pyruvate into malate (maintenance of the Krebs' cycle). Negative correlation between activity of $NADPH_2$-d and AcP in the neutrophils also is interesting. This correlation is difficult to explain on the basis of functional interaction between individual metabolic pathways in the cell. Evidently in this case we have a reflection of cytochemical differentiation of the neutrophils in the course of maturation: mature cells of the neutrophilic series are characterized by a higher AcP level [1]. During maturation of the cells the level of AcP activity falls, but ability to destroy microorganisms is increased. Neutrophils, unlike lymphocytes, are also characterized by a relatively high degree of regulation of enzymes with $NADPH_2$-d, which is of great importance for the metabolic ensemble of the cell, if the specific role of this enzyme in the bactericidal function of the granulocytes is recalled [11, 16, 17].

In the whole system of intracellular metabolism individual concrete correlations cannot be regarded as rigidly fixed In accordance with the principles of regulation of enzyme activity by feedback mechanisms [7], phasic changes must be expected in both the value of the coefficient of correlation and its sign. Transformation of the coefficient of correlation of this kind we discovered when determining the strength of correlation between $NADPH_2$-d and α-GDPH-NAD, when a change in correlation between levels of enzyme activity from small and negative ($r = -0.263$, $n = 35$) to small and positive ($r = 0.345$, $n = 22$) was observed in two groups of mice at different times of the investigation. Fluctuations in the strength of correlation also were noted

between other cell enzymes. Such fluctuations in the level of integration of the enzyme systems of lymphocytes may be determined by the nonhomogeneity of individuals forming the population, which is brought to light by the action of pathological factors [5].

Coordination of enzyme activities at the intercellular level in the lymphocyte—neutrophil system is characterized in some stages of metabolism by quite strong levels of correlation. It is a striking fact that the greatest rigidity in coordination is observed between enzymes of the α-glycerophosphate shunt and $NADPH_2$-d. The existence of correlations between levels of enzyme activity of lymphocytes and neutrophils can be explained if, on the one hand, factors facilitating its onset are taken into account and, on the other hand, factors leading to labilization of these correlations are considered. The fact that intracellular organelles have common functions (integration of the organism at the subcellular level) can be placed among factors of the first category, together with common aspects of neurohumoral and hormonal regulation; factors of the second category include the performance of their specific functions by the cells and, consequently, the specificity of cellular metabolism, and the unequal sensitivity of different types of blood cells to regulatory and damaging influences. The high degree of correlation between enzymes of the α-glycerophosphate shunt, which is particularly sensitive to the action of thyroxine [6], is therefore not an accident, It is possible that the increase in the degree of coordination of enzyme systems between lymphocytes and neutrophils after exposure to infection (the 3rd day of the incubation period, Table 1) may also be explained by a common influence. The similar response in the strengthening of coordination of enzyme systems was observed between the lymphoid organs of animals under the influence of an injected antigen [8]. The presence of correlation between levels of activity of $NADPH_2$-d in lymphocytes and neutrophils can partially explain the prognostic informativeness of this enzyme relative to the outcome of the infection in mice [4].

The temporal connection between levels of enzyme activity of blood cells belongs to a higher level of regulation. Correlation between enzymic activities of neutrophilc and lymphocytes can be regarded as coadaptation of cells and the enzymic basis of cellular cooperation. It will be clear from the data given above that although the enzymic status of the blood cells is by no means completely reduced to agreement, nevertheless we can distinguish unique channels of communication (relatively high correlation connections). It is striking that one such channel should include the α-glycerophosphate shunt. Besides carrying out shuttle transport from cytosol into mitochondria, this enzyme system also regulates the level of glycerophosphate, a metabolite which is a component of phospholipids that are essential ingredients of cell membranes. Destructive processes, accompanied by the appearance of free phospholipids and their degradation products, lead to an increase in the substrates of the glycerophosphate shunt, and consequently, they supply the cells with energy. The inflammatory reaction, with its neutrophilic and lymphocytic phases, activated immediately after such destruction, is realized by cells that have been tuned into biochemical resonance. Another channel of communication deserving attention is $NADPH_2$-diaphorase In the lymphocyte and neutrophil this enzyme performs different physiological functions: in the neutrophil it is included in the enzyme system for destroying microorganisms, whereas in the lymphocyte its more probable role is in the regulation of anabolism (we know that $NADPH_2$ is necessary for many synthetic processes) The existence of temporary connections between enzyme activities can be explained by preservation of individual components of the system in a certain state. We studied correlation of levels of activity of some enzymes present in leukocytes before and at certain time intervals after exposure to the infectious agent. Calculations showed that 3 h after exposure to infection $NADPH_2$-d activity in the lymphocytes was independent of its initial level ($r = 0.064$, $n = 30$); some degree of correlation between these times also was preserved in α-GPDH activity ($r = 0.379$, $n = 15$, $p < 0.1$), and the somewhat higher initial values of the enzymic parameters determined the level of SDH activity 3 h after exposure ($r = 0.451$, $n = 18$, $p < 0.1$). Correlation between levels of activity of α-GPDH and SDH of lymphocytes in the following time intervals were found to be not very strong: before and 1, 2, and 8 days after infection. Coefficients of correlation between these parameters varied from 0.037 to 0.661. The exception was a temporary connection between AcP activity of lymphocytes, whose initial level was inversely proportional to the level of its activity 1 and 8 days after infection ($r = -0.752$ and $r = -0.730$; $p < 0.05$). This correlation indicates that the highest AcP activity in the lymphocytes during infection will occur in cases with initially low values.

The low level of correlation between the enzyme systems of the blood cells with time may be due, besides to other to the physiological heterogeneity of the mouse population studied, which included individuals that undoubtedly differed in the duration of their phasic response to outside influences. Differences in the individual reactivity of the organism imposed limitations in the prognosis of the outcome of the pathological process, if that prognosis is formed in the early stage of onset of the infection. If the outcome of an infectious process can be predicted within certain limits from the premorbid state of metabolism of the blood cells, after exposure the prognosis based or level of enzyme activity will be possible later [3].

Consequently, the introduction of systems concepts into the cytochemical analysis of neutrophils and lymphocytes revealed the unique role of status of the blood cells, involved consecutively in the inflammatory process, and also enabled elements of coadaptation of the two types of leukocytes to be determined to a certain degree.

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