EFFECT OF ANTIGENIC STIMULATION AND HUNGER ON RAT LIVER LYSOSOMES

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One of the most important links in the chain of reactions protecting the body against the effects of bacteria, viruses, and foreign substances is phagocytosis followed by intracellular degradation through the participation of lysosomes [1, 3]. Lysosomes also are responsible for formation of the immune response, in particular in the initial stage of immunogenesis [9, 10]. The writers showed previously that sensitivity of cells to viruses is to a certain degree dependent on genetically determined properties of lysosomal hydrolases [5]. There is good reason to suppose that functional changes in lysosomes can have a significant influence on immunogenesis.

Convincing proof has now been obtained of significant potentiation of functional activity and changes in the properties of lysosomal membranes of different types of cells with the transition to endogenous nutrition [2, 3].

This paper describes an attempt to study correlation between the character of the immune response, on the one hand, and activity of acid hydrolases and the properties of lysosomal membranes on the other hand. Antigenic stimulation (AS) with sheep's red blood cells (SRBC), in conjunction with total deprivation of food, was used as the experimental model.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 140-150 g. Animals of the experimental groups were kept for 9 days in individual metal cages with a wire mesh floor, completely preventing coprophagy, and they were totally deprived of food. Animals of the control groups were kept on the normal balanced animal house diet (ND). The rats were allowed water ad lib. AS of the experimental animals of group 1 was applied on the 4th day of starvation, to those of group 2 on the 1st and 4th days, by intraperitoneal injection of washed SRBC in a dose of 2 ml of a 20% suspension per rat. AS was applied at the same time to animals of the control groups. After 9 days the animals were killed. Immunologic reactivity of the animals was assessed by titration of antibodies in the blood serum (the dose of antigens in the hemagglutination test was 0.02 ml of a 3% suspension of SRBC) and by noting the degree of hyperplasia of the spleen. The total number of leukocytes was counted in the peripheral blood.

Liver homogenates, prepared by the standard method [3] using 0.25 M sucrose, pH 7.4, containing 0.001 M EDTA as the suspending medium, and the cytosol (105,000g, 30 min) were analyzed for total protein [6] and activity of four lysosomal hydrolases: β-galactosidase, β-glucuronidase, β-N-acetylglucosaminidase, and aryl sulfatases A and B, by spectrophotometric micromethods [3], using o-nitrophenyl-β-D-galactopyranoside, p-nitrophenyl-N-acetyl-β-D-glucosaminide, p-nitrophenyl-β-D-glucuronide, and p-nitrocatechol sulfate (from Sigma, USA) respectively as the substrate.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that starving the animals for 9 days led to some changes in the immune state. For instance, the total leukocyte count was 59%, whereas the weight index of the spleen (the ratio of its weight to body weight) and the antibody titer were 91 and 84% respectively of the control.

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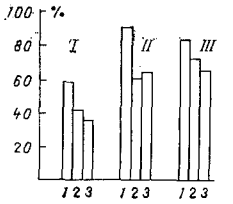

Fig. 1. Some parameters of immunity after AS and starvation. I) Leukocyte count; II) weight index of spleen; III) antibody titer (in % of corresponding control groups; 1) starvation; 2) starvation + single AS; 3) starvation + double AS.

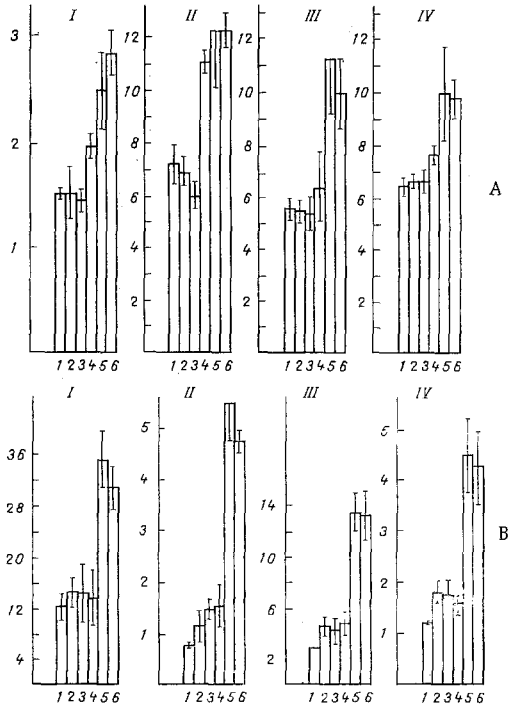

Fig. 2. Total (A) and nonsedimented (B) activity of rat liver lysosomal enzymes in response to AS and different dietary conditions. I) β-galactosidase; II) β-N-acetylglucosaminidase; III) β-glucuronidase; IV) aryl sulfatases A and B. 1) ND, 2) ND + single AS, 3) ND + double AS, 4) starvation, 5) starvation + single AS, 6) starvation + double AS. Ordinate: A) activity (in μmoles substrate/g protein/min), B) activity (in % of total).

In turn, very substantial differences between parameters of the immune response were found in animals exposed to AS after starvation and animals receiving AS and a normal diet. In that case, even with double AS, all parameters of the immune response in the group of hungry rats were reduced: the leukocyte count to 36-42%, the weight index of the spleen to 61-65%, and the antibody titer to 66-73% of the control.

The study of total and nonsedimented activity of lysosomal hydrolases showed (Fig. 2) that starvation caused activation of all enzymes up to 114-153% compared with the corresponding levels in intact animals. AS of the hungry animals induced an even greater increase in total activity, namely to 150-202%. Meanwhile total activity of lysosomal hydrolases after AS of animals receiving the normal diet was virtually unchanged at 83-103% of the control.

The character of the change in the level of nonsedimented activity on the basis of which the structural state of the lysosomes can be judged deserves attention. Both in animals receiving AS and a normal diet and in intact hungry rats an increase in nonsedimented activity of β-N-acetylglucosaminidase, β-glucuronidase, and aryl sulfatases A and B up to 144-199% and 130-209% respectively of the control was found. The combined effect of starvation and AS caused an extremely dramatic increase (by 3-7 times) in the level of nonsedimented activity of all lysosomal hydrolases studied.

During AS of rats receiving a normal diet, a decrease in immune reactivity was thus observed, accompanied by marked activation of the lysosomal system of the liver. The decrease in immune reactivity under conditions of an acute nutritional deficiency may be linked to a certain degree with a decrease in the intensity of antibody synthesis. The increase in total activity of lysosomal hydrolases, aimed during endogenous nutrition at redistributing the intracellular reserves of biopolymers of different kinds for replenishing the cellular reserves of the corresponding components, essential for meeting both structural and functional requirements [2, 3], is logical in this connection. It must be recalled that injection of an antigen against the background of an activated liposomal enzyme system may be the cause of changes in the character of transformation of the antigen and depression of its immunogenic properties. The sharp increase in the level of nonsedimented activity may be both the result of irreversible structural disturbances of the lysosomal membranes, caused by starvation [4, 7, 8], and the result of their oriented transformation and labilization, due to the need to release lysosomal hydrolases into the systemic circulation [9]. The combined action of factors of widely different physiological character probably causes mutual induction of the lysosomal apparatus, activation of which is compensatory in character when the immune response is depressed, and is aimed at ensuring the most complete realization of their protective function by the lysosomes.

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HYDROXYLATION PRODUCTS OF HYDROPHOBIC XENOBIOTICS AND CYTOCHROME P-450 STABILIZERS IN HEPATOCYTES

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When hepatocytes are cultured the terminal component of the mixed-function mono-oxygenase system, cytochrome P-450, undergoes spontaneous destruction [3, 10, 11]. The essential mechanism of its degradation is lipid peroxidation (LPO) [5, 6]; inhibitors of free-radical oxidation (4-methyl-2,6-di-tert-butylphenol and 2-ethyl-6-methyl-3-hydroxypyridine) prevent degradation of cytochrome P-450 in a primary hepatocyte culture [3]. Experiments on the microsomal fraction of the liver showed that during hydroxylation of certain xenobiotics, phenolic products are formed which behave as antioxidants, with ability to reduce the intensity of LPO [1, 2] and to protect cytochrome P-450 against destruction. In the investigation described below the validity of this hypothesis

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