EFFECT OF ANTIGENIC STIMULATION AND STARVATION OF LYSOSOMAL PROTEINASES AND PROTEIN RENEWAL IN THE LIVER, SPLEEN, AND THYMUS IN RATS

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There is no question about the important role of the lysosomal apparatus of immunocompetent cells in immunogenesis, in immune responses, and in disturbances of immunologic homeostasis. Lysosomes may be involved in this situation in the formation of the immune response to an equal degree on account both of antigenic modification and of their participation in antibody formation and degradation [3, 5, 9]. In previous investigations the writers obtained proof of a significant increase in activity of several lysosomal hydrolases (β-galactosidase, β-N-acetyl-glucosaminidase, β-glucuronidase, aryl sulfatases A and B) and changes in the properties of lysosomal membranes in the rat liver during antigenic stimulation (AS) associated with the switching to endogenous feeding [7].

The aim of this investigation was to study the connection between the character of the immune response and the intensity of protein turnover in the liver and immunocompetent organs, on the one hand, and activity of lysosomal proteinases, which are the most likely participants in degradation of antigenic protein material and mechanisms of antibody production, on the other hand, using a model of combined exposure to starvation, which induces mobilization of the lysosomal apparatus, and of AS.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 200-220 g. Animals of the experimental groups were placed in individual metal cages with a wire netting floor, preventing any chance of coprophagy, on the 4th, 6th, and 8th days, and were completely deprived of food. Animals of the control groups were kept on the ordinary balanced animal house diet (BD). The rats were allowed water ad lib., and together with water, animals of the experimental groups received a combination of water-soluble vitamins corresponding to BD. Animals of the experimental groups were subjected to AS on days 0, 2, and 4 of starvation by intraperitoneal injection of washed sheep's red blood cells (SRBC) in a volume of 2 ml of a 20% suspension per rat. Animals of the control groups received parallel AS. The number of antibody-forming cells (AFC) in the spleen was determined 4 days after AS by Jerne's direct method [10]. Total complementary activity of the blood serum (CH_{50}) was determined nephelometrically [4]. Homogenates of liver, spleen, and thymus were prepared by the standard method [5], using 0.25 M sucrose, pH 7.4, containing 0.001 M EDTA, as the suspending medium, and total protein [8] and activity of the following six liposomal hydrolases were determined: cathepsins A, B, C and D by a spectrofluorometric method [2] and aryl sulfatases A and B by the method in [5], using N-carbobenzoxy-L-glutamyl-L-tyrosine, N-benzoyl-D,L-arginine-β-naphthylamide, glycine-L-phenylalanine-β-naphthylamide, hemoglobins, and p-nitrocatechol sulfate (Sigma, USA) respectively as the substrates. The half-life of total proteins ($T_{1/2}$) in the liver, spleen, and thymus was determined by injection of 1-[^{14}C]-D,L-leucine into animals of the corresponding experimental and control groups in a dose of 20 μCi/100 g body weight 1, 4, and 6 days before sacrifice. A sample of the test homogenate was precipitated with 5% TCA, transferred to a Synpor No. 2 membrane filter (Czechoslovakia), washed twice

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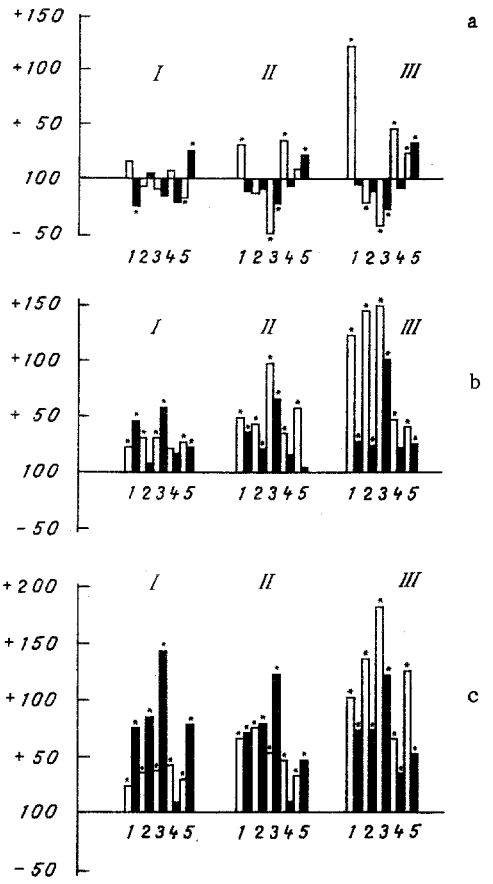

Fig. 1. Activity (in % of control) of lysosomal hydrolases in liver (a), spleen (b), and thymus (c) of rats during starvation and AS. 1) Cathepsin A; 2) cathepsin B; 3) cathepsin C; 4) cathepsin D; 5) aryl sulfatases A and B. I, II, III) 4, 6, and 8 days, respectively, of starvation. Unshaded columns — starvation; shaded columns — starvation + AS. *$p < 0.05$.

with 5 ml of 5% TCA, dried, and immersed in toluene scintillator (8 g PPO and 100 mg POPOP in 1 liter of toluene). Radioactivity was counted on a RackBeta 1215 scintillation counter. The value of $T_{1/2}$ was calculated on the basis of the degradation constants, which was obtained from dependence of LN of specific radioactivity of the sample on time elapsing after injection of the 1-[^{14}C]-D,L-leucine [14]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that starving the animals for all the time intervals chosen (4, 6, and 8 days) led to marked inhibition of the immune response, which was judged by the decrease in intensity of AFC production by 54, 85, and 34%, respectively, in agreement with existing data on the development of immunodepression after starvation [11, 12]. Besides changes in AFC production, there was also a sharp increase in total serum complement activity, which amounted to 277% on the 1st day, 256% on the 6th day, and 175% of the control on the 8th day. In view of information that activity of macrophages is increased during starvation and that they participate in the synthesis of several components of complement, the change in complement activity which was found would seem to be in order.

Investigation of lysosomal proteinase activity in the liver of the starving rats revealed a typical reciprocal change in activity of cathepsin A, D and B, and C and characteristic activation of aryl sulfatases A and B [1, 6]. Meanwhile generalized activation of all lysosomal hydrolases was observed in the spleen and thymus, and it increased progressively with an increase in the period of starvation, to amount to 140-280% of the control values by the 8th day (Fig. 1).

TABLE 1. Effect of AS and Starvation on AFC Production and Total Serum Complementary Activity (M ± m)

Parameter	Control + AS	Starvation + AS, duration of starvation, days		
		4	6	8
Number of AFC per 10^6 splenocytes	32,3±2,9	15,0±1,45*	4,8±0,41*	21,2±1,9*
Complementary activity (CH_{50})	38,3±3,7	106,1±10,5*	98,4±10,2*	66,9±3,5*

Legend. *$p < 0.001$. Here and in Table 2, mean results of 6-8 experiments shown.

TABLE 2. Half-Life of Protein ($T_{1/2}$, days) in Liver, Spleen, and Thymus of Rats during AS and Starvation (M ± m)

Organ	Control	Control + AS	Duration of starvation, days			Starvation + AS, days		
			4	6	8	4	6	8
Liver	5,01±0,28	9,46±0,24	6,72±0,35	5,98±0,11	7,46±1,20	4,45±0,28	5,0±0,14	10,99±0,46
Spleen	3,55±0,14	3,14±0,35	8,97±1,31	7,63±0,17	13,35±1,19	4,70±0,12	5,63±0,13	5,55±0,40
Thymus	3,91±0,30	3,94±0,22	5,44±0,11	4,16±0,16	8,60±0,53	6,71±0,22	4,76±0,12	5,58±0,32

As caused no significant changes in enzyme activity of the intact rats, except for a very small increase in cathepsin C activity in the spleen (by 28%) and cathepsin A activity in the thymus (by 23%; $p < 0.05$). Meanwhile AS virtually abolished the activation of cathepsins A and D discovered in the liver of the rats during starvation: compared with unimmunized animals the activity of these proteinases was reduced by 26-56% ($p < 0.05$) which led to an increase in cathepsin C activity (by 23-52%, $p < 0.05$) and in activity of aryl sulfatases A and B. Compared with the liver, AS induced weaker activation of cathepsins A and C (by 19%) and some decrease in cathepsin B activity in the spleen on the 4th day of starvation. In animals starved for 6 days, a moderate (by 10-35%) fall in the activity of all lysosomal hydrolases was found during AS, and an even greater decrease was found if the period of starvation was increased to 8 days. In that case a sharp (almost by half) reduction of activity of cathepsins A and B should be noted. Changes in activity of lysosomal and thymic enzymes during AS combined with short-term starvation (4 days) were very demonstrative. Thus in immunized animals marked activation of all lysosomal proteinases was revealed (by 35-80%; $p < 0.01$), except cathepsin D, as well as activation of aryl sulfatases A and B (up to 40%; $p < 0.05$). Lengthening of the period of starvation to 6 days abolished the activation of liposomal enzymes by AS, but by the 8th day of starvation the character of the change in enzyme activity in the thymus was similar to that in the spleen.

Determination of $T_{1/2}$ of total proteins in the liver, spleen, and thymus (Table 2) showed that in intact rats starvation caused a moderate increase of $T_{\frac{1}{2}}$ in the liver (by 19-49%; $p < 0.05$) and a very significant increase in the spleen (by 2.1-3.8 times). The greatest increase in $T_{1/2}$ in the thymus (up 220% of the control) was found on the 8th day of starvation. Immunization of the animals receiving BD caused no changes in the intensity of protein turnover in the spleen and thymus, but in the liver the value of $T_{\frac{1}{2}}$ increased by 88% ($p < 0.01$). It is noteworthy that in both the liver and spleen, AS of food-deprived animals was accompanied by an increase in the rate of protein turnover, as shown by a significant fall of $T_{\frac{1}{2}}$ in these organs by 16-34 and 26-48%, respectively, whereas in the thymus, on the other hand, $T_{\frac{1}{2}}$ of proteins was somewhat increased by the 4th and 6th day of starvation, but by the 8th day it amounted to only 65% of the value of $T_{\frac{1}{2}}$ in the unimmunized animals.

Shortening of $T_{\frac{1}{2}}$ of proteins in the liver and spleen during starvation and AS probably reflects an increase in activity of the macrophages [13], and in view of their role in synthesis of the components of complement and the decrease in lysosomal proteinase activity which we observed, the sharp rise in total complementary activity thus revealed can be interpreted as the result of combined stimulation of biosynthesis and a decrease in the rate of degradation of its individual components. Meanwhile the increase in the intensity of protein

renewal in the splenocytes in response to AS in the late stages (8 days) of starvation evidently lies at the basis of the relative increase in AFC production. In turn, marked activation of the lysosomal proteolytic system of the thymocytes may be due to their contact with antigenic material and its subsequent modification. At the same time, the total abolition, during AS, of induction of the lysosomal proteolytic system in response to AS, by contrast with induction of other lysosomal hydrolase [7] in liver, spleen, and thymus, can be interpreted as a compensatory reaction of the immunocompetent cells, aimed at the maintenance of immunologic homeostasis and, in particular, at regulation of intercellular interactions between macrophages and T and B lymphocytes, and of antibody synthesis.

Thus the character of the participation of the lysosomal proteolytic system in the realization of immunologic mechanisms is largely determined by the physiological state of the body.

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