CHANGES IN FUNCTIONAL ACTIVITY OF MONONUCLEAR PHAGOCYTES IN MICE OF DIFFERENT AGES

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The causes of the immunological immaturity of newborn animals have not yet been established. One of them may be immaturity of cells of the mononuclear phagocytic system (MPS). It has been shown, for instance, that the immunological immaturity of newborn mice can be attributed to the absence of antibody-synthesizing cells, and not to a deficiency of cells digesting the antigen, i.e., macrophages [2]. The digestive function of macrophages is performed by their lysosomal apparatus.

To study the mechanism of immunologic immaturity of newborn mice, in the investigation described below the ability of cells of the MPS to carry out phagocytosis was studied and activity of cathepsin D in their lysosomal apparatus was investigated in animals of different ages, starting with the first day of life.

EXPERIMENTAL METHOD

CBA mice of different ages — 1, 3-5, 14, 21, 28, 30, 45, and 60 days — were used in the experiments.

Peritoneal exudate cells (PEC) were obtained from unstimulated mice by flushing out the peritoneal cavity with medium No. 199 containing heparin. Sheep's red blood cells (SRBC), labeled with ^{51}Cr, were used as the antigen [3]. To study the uptake of SRBC-^{51}Cr by peritoneal or splenic macrophages, the macrophages were isolated from the cell suspensions by adhesion. The PEC were incubated in medium No. 199 in a concentration of $2 \cdot 10^6$ cells/ml in 15-ml samples for 1 h at 37°C. Spleen cells were incubated in medium No. 199 in a concentration of $1 \cdot 10^7$ cells/ml for 1.5 h at 37°C. After repeated washing, 0.2 ml of 20% SRBC-^{51}Cr in 15 ml of medium No. 199 was added to the resulting monolayers of macrophages. After incubation for 30 min at 37°C the monolayers were washed and the cells removed and resuspended in physiological saline. The uptake of SRBC-^{51}Cr was determined in the resulting cell suspension and expressed in cpm/mg protein. Protein was determined by Lowry's method.

To determine cathepsin activity in PEC and spleen cells homogenates of these cells were prepared and all work with them was done in the cold. The PEC were first sedimented by centrifugation at 1500 rpm for 10 min. The residue of PEC and spleen cells was homogenized in bidistilled water for 2 min. After homogenization the suspensions were incubated in bidistilled water at 4°C for 40 min to produce lysis of the cells and subcellular structures. After lysis, cell fragments were sedimented by centrifugation for 40 min at 4°C and at 15,000 rpm. The supernatant was drawn off very carefully, taking care not to disturb the residue, and the total content of cathepsins in it was studied (cytoplasmic fraction + lysosomal fraction). Activity of the cathepsins was determined by Anson's method [1].

EXPERIMENTAL RESULTS

In the experiments of series I activity of lysosomal enzymes in PEC and spleen cells was investigated in mice of the following age groups: 1 day (94 mice), 3 days (117 mice), 21 days (30 mice), 30 days (29 mice), 45 days (54 mice), and 60 days (20 mice). Enzymes of

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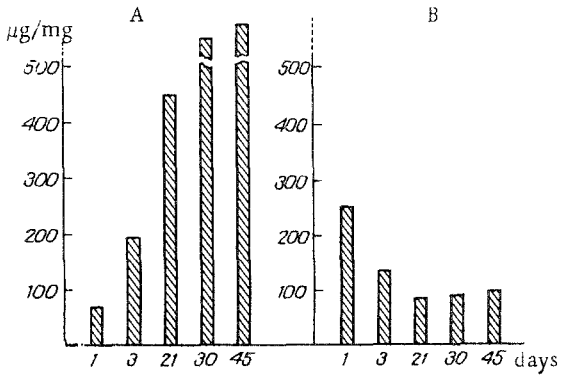

Fig. 1. Cathepsin activity in CBA mice at different ages. A) Cathepsin activity in PEC; B) cathepsin activity in spleen cells. Abscissa, age of mice (in days); ordinate, cathepsin activity (in μg tyrosine/mg protein).

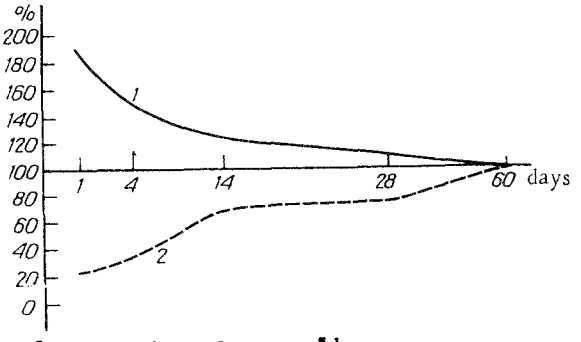

Fig. 2. Uptake of SRBC-^{51}Cr by macrophages of mice of different age groups. 1) Uptake of SRBC-^{51}Cr by peritoneal exudate macrophages; 2) uptake of SRBC-^{51}Cr by splenic macrophages. Abscissa, age of mice (in days); ordinate, uptake of SRBC-^{51}Cr (in % of control).

the cathepsin group were chosen as lysosomal enzymes. Total cathepsin activity in PEC was the same in different age groups. The lowest activity was found in mice aged one day. By three days cathepsin activity was increased by 2.5 times, and in mice aged 21 days activity was close to that in adult (60-day) mice (Fig. 1).

The opposite relationship was observed when cathepsin activity was studied in the spleens of the same mice. Highest activity was found in mice aged one day, at three days it was reduced by half, and in mice aged 21 days the level of activity observed in adult mice (60 days) was reached (Fig. 1).

The experiments thus showed that low activity of lysosomal enzymes in PEC in the early stages of the neonatal period is accompanied by high activity of these enzymes at the same times in their spleen cells.

Besides studying the state of the lysosomal apparatus, we also investigated the ability of macrophages to take up SRBC in mice at different stages of immunologic maturity. Five series of experiments were carried out on mice aged one, 4-5, 14, and 28 days. Since these series of experiments were carried out at different times and since SRBC-^{51}Cr labeling also differed, each series was accompanied by its own group of control animals (adult mice, aged 60 days).

Uptake of SRBC-^{51}Cr by macrophages was studied after incubation of the cells for 30 min *in vitro*. As will be clear from Fig. 2, uptake of antigen by macrophages of different origin differed in mice of each age group. The most active uptake of SRBC was by peritoneal exudate macrophages of mice aged one day (182% of the control). PEC from day-old mice, incidentally, were less capable of adhesion than PEC of adult mice, for the quantity of protein detectable in the final cell suspension in the first case was only one-tenth of that in the second case (mean values 0.01 and 0.1 mg protein/ml), although the initial number of cells placed in the tubes for adhesion was the same in both cases ($2 \cdot 10^6$ cells/ml). With this in mind, it will be clear that the quantity of label taken up by each macrophage separately was considerably greater in the case of mice aged one day. Macrophages of mice aged 4-5 days also took up the antigen sufficiently actively (153% of the control), in mice aged 14 days activity was reduced somewhat (132% of the control), and in mice aged 28 days the phagocytic activity of the peritoneal exudate macrophages was equal to that in the control animals.

A different picture was observed when uptake of SRBC-^{51}Cr by splenic macrophages was studied. It will be clear from Fig. 2 that splenic macrophages of mice aged 1-4 days possessed lower phagocytic activity (34%) than the control (adult mice), in animals aged 14 days activity increased to 63%, it remained at this level in mice aged 28 days, and reached 100% in mice aged 60 days, i.e., it was equal to the control.

Investigation of the ability of macrophages of mice to take up SRBC thus showed that high phagocytic activity of the peritoneal macrophages of newborn mice is accompanied by low phagocytic activity of the splenic macrophages of these mice.

The experiments showed differences in the functional state of the lysosomal apparatus and in the phagocytic activity of macrophages obtained from different organs. Activity of lysosomal cathepsins in mice on the first day of life was high in lysosomes of splenic macrophages. The ability of these cells to take up SRBC, however, was sharply reduced. High activity of lysosomal enzymes in spleen cells is probably essential for natural physiological processes such as the reutilization of the animal's own dying erythrocytes or the regulation of hematopoiesis to take place and to fulfill the role of a stabilizing factor, especially if it is recalled that the ability of splenic macrophages to take up foreign erythrocytes is sharply reduced in the neonatal period. Although the phagocytic activity of peritoneal macrophages of newborn mice is not disturbed but, on the contrary, is increased, they cannot digest the phagocytosed materials because of lack of lysosomal enzymes which participate in antigen digestion. Low activity of lysosomal enzymes in peritoneal macrophages may lie at the basis of the low reactivity observed in the neonatal period. This suggests that immaturity of the lysosomal apparatus of cells of the MPS is one of the mechanisms of immunologic incompetence.

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