CHANGES IN ENZYME ACTIVITY OF LEUKOCYTES DURING AMYLOID FORMATION EXPERIMENTALLY AND IN PATIENTS WITH AMYLOIDOSIS

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In patients with secondary amyloidosis and in animals with experimental amyloidogenesis and amyloidosis (casein model) the activity of hydrolases, oxidoreductases, and myeloperoxidase was determined in neutrophils and lymphocytes of the peripheral blood. During the period of amyloid formation, hydrolase and myeloperoxidase activity in the neutrophis was reduced, especially in the stage of initial amyloid deposition. Changes in the activity of these enzymes in animals with developed amyloidosis coincided with those in the blood cells of patients with secondary amyloidosis. The results are discussed from the standpoint of the resorption theory.

KEY WORDS: amyloidosis; neutrophils; lymphocytes; enzymes.

It is natural to suggest that the development of amyloidosis must be preceded by a disturbance of the mechanisms of amyloid resorption. According to Spors [9], amyloid resorption takes place with the active participation of the neutrophils, in which high acid and alkaline phosphatase activity is found. This suggests that the leading role in the defragmentation of amyloid fibrils is played by the secondary and tertiary granules of the leukocyte, the functional state of which must change substantially in the process of amyloid formation.

The investigation described below was carried out to study these problems.

EXPERIMENTAL METHOD

The investigation was carried out on patients with secondary amyloidosis and on male C57BL mice (25-30 g) and male chinchilla rabbits (2.5-3 kg) with experimental casein amyloidogenesis.

Activity of alkaline (AlP) and acid (AcP) phosphatases [7] and of myeloperoxidase (MP) [4] was determined cytochemically in the blood leukocytes. The results were expressed by Kaplow's index [8]. Activity of mitochondrial α-glycerophosphate dehydrogenase (MGPD) and succinate dehydrogenase (SD) also was investigated [5]. The results were expressed as the number of formazan granules in 50 lymphocytes.

EXPERIMENTAL RESULTS AND DISCUSSION

In the early stages after the beginning of casein administration to the rabbits (1-5 injections) hydrolase and MP activity in the leukocytes was slightly increased, but starting from the 10th injection it fell gradually. Minimal enzyme activities usually were found by the 60th-70th day (25-30 injections) from the beginning of administration of casein. After 35-40 injections AlP and MP activity was increased, and after 45 injections AcP activity also was increased (Fig. 1).

Activity of AlP in the mouse neutrophils was completely absent, MP activity was reduced by half, and AcP activity was reduced by two thirds compared with the corresponding activity in the rabbits. Amyloid began

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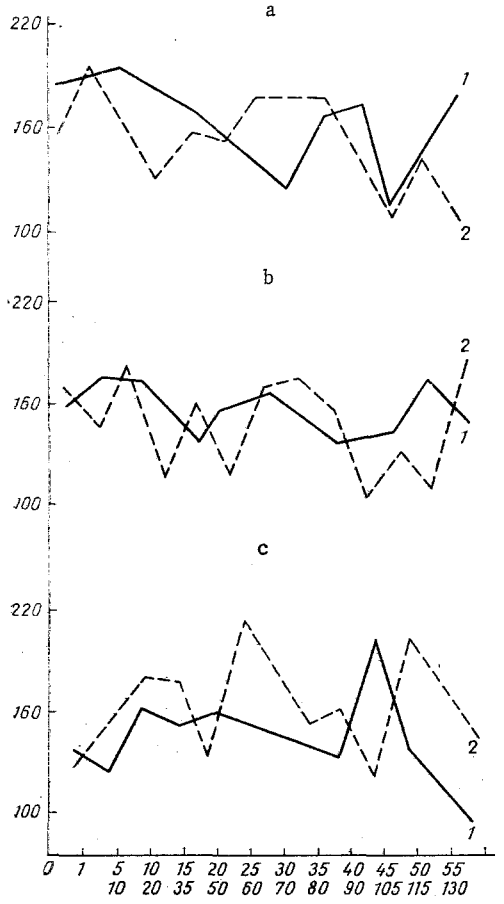

Fig. 1. Changes in activity of alkaline and acid phosphatases and myeloperoxidase in blood neutrophils of rabbits following repeated injections of casein. Abscissa: top row—injections, bottom row—days; ordinate, Kaplow's index. 1) Experiment; 2) control. a) Alkaline phosphatase; b) acid phosphatase; c) myeloperoxidase.

to appear in the mice by the 15th injection (on the 15th day of administration of casein). Injection of sodium caseinate into these animals led to a more rapid and severe fall in enzyme activity, preceding amyloid deposition (10-15 injections). During the period of development of amyloidosis in the mice (just as in the rabbits) there was an increase in AcP and MP activity, but later it fell (Fig. 2).

Casein and sodium caseinate also gave rise to definite changes in the peripheral blood lymphocytes of the animals. In mice the dynamics of dehydrogenase activity in the lymphocytes resembled that observed after a single injection of antigen [1-3].

The period of amyloidogenesis was thus characterized by disappearance of rhythmic cytochemical changes in the neutrophils of the rabbits, by a decrease in AcP, AlP, and MP activity (in both mice and rabbits) preceding the deposition of amyloid in the organs, and by the appearance of sudden fluctuations, in different directions, in the activity of these enzymes.

The dynamics of enzyme activity was less clearly visible in the lymphocytes, in agreement with the absence (at all stages of the experiment) of changes in the cell response to phytohemagglutinin (PHA).

Considering that the granules of neutrophils play an active part in defragmentation of fibrils [9], it will be understandable why in mice, in the absence of AlP activity (specific granulation) and with lower AcP activity (tertiary granules) and MP activity (primary granules) than in rabbits, amyloidosis develops much faster than in rabbits. In rabbits, however, the imbalance between reactions of different types of neutrophil granules evidently plays a role of special importance, and may contribute to deposition of amyloid in the organs. In the period of developed amyloidosis, on the other hand, activity of these enzymes rises and this evidently reflects the response of the organism to degenerative changes in the tissues.

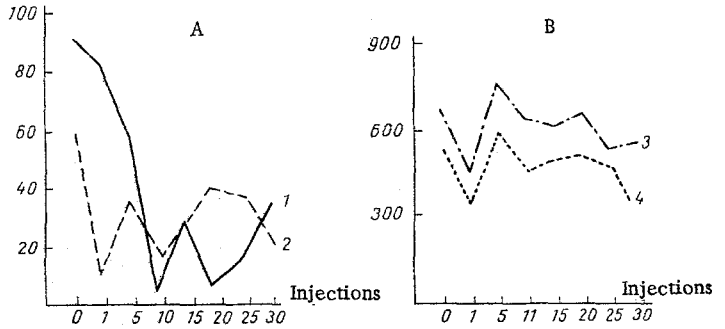

Fig. 2. Changes in myeloperoxidase, acid phosphatase, and dehydrogenase activity in mice during repeated injection of sodium caseinate. Abscissa, injections; ordinate, Kaplow's index (A), number of granules in 50 lymphocytes (B). 1) Myeloperoxidase in granulocytes; 2) acid phosphatase in granulocytes; 3) succinate dehydrogenase in lymphocytes; 4) α-glycerophosphate dehydrogenase in lymphocytes.

Such a considerable load on cells of the myeloid series as the disease progresses must lead to a sharp decrease in their functional capacity. High MP activity and low activity of hydrolases, especially AlP, are in fact observed in the blood neutrophils of patients with amyloidosis, and in some cases the latter is completely absent. Such cells can hardly participate in resorption processes, and this may perhaps explain the lack of response of the organism to amyloid already formed [6]. A reduction in the functional capacity of the neutrophils can arise at a certain stage of the original disease, for amyloidosis arises most frequently during prolonged destructive inflammatory conditions, in which neutrophils play an active role.

LITERATURE CITED

1. L. K. Katosova, "Cytochemical and histochemical study of lymphocytes and certain organs during experimental immunization and hypersensitivity of delayed type," Author's Abstract of Candidate's Dissertation, Moscow (1971).
2. I. A. Komissarova and N. M. Kudryashova, Pediatriya, No. 4, 31 (1970).
3. I. A. Komissarova and N. M. Kudryashova, Byull. Éksp. Biol. Med., No. 5, 81 (1972).
4. R. Lillie, Pathohistological Techniques and Practical Histochemistry [Russian Translation], Moscow (1969), p. 625.
5. R. P. Nartsissov, Arkh. Anat., No. 5, 85 (1969).
6. A. Policard and M. Bessis, Elements of Cell Pathology [Russian translation], Moscow (1970).
7. A. F. Goldberg and T. Barka, Nature, 195, 297 (1962).
8. L. S. Kaplow, Blood, 10, 1023 (1955).
9. S. Spors, Blut, 23, 14 (1971).