EFFECT OF IMURAN ON THE COURSE OF EXPERIMENTAL AMYLOIDOSIS

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In experiments on C57BL mice the control animals received sodium caseinate and the experimental group received sodium caseinate together with imuran (azathioprine). Amyloid formation was sharply accelerated in the experimental animals, especially in the late period of the experiment (45 days). The severity of the amyloidosis in the spleen, estimated stereometrically, was significantly higher in the experimental than in the control mice. The accelerating effect of imuran is probably due to disturbance and distortion of globulin synthesis as a result of inhibition of proliferation of cells of the reticuloendothelial system responsible for this synthesis.

Evidence of the role of immune reactions and transformation of cells of the reticuloendothelial system in amyloidosis have led to attempts to study the course of experimental amyloidosis under the influence of various immunodepressants and drugs with affinity for reticuloendothelial cells.

Several papers have been published on the effect of corticosteroids [1, 5, 8, 14], antilymphocytic serum [6, 11, 13], and 4-aminoquinolines [5, 10] on amyloidosis. Information on the effect of cytostatic drugs on amyloidosis is scarce and contradictory: some workers describe their inhibitory action [9, 10, 12], others their accelerating effect on the development of amyloidosis [8, 15, 16].

In this investigation the course of experimental amyloidosis was studied in animals receiving imuran (azathioprine).

EXPERIMENTAL METHOD

Experiments were carried out on 40 male C57BL mice with a mean weight of 18-20 g. Mice of the control group (25) received a 5% solution of sodium caseinate, injected subcutaneously six times a week, as the antigen. Mice of the experimental group (15) received imuran in a dose of 2 mg by mouth at the same time as the sodium caseinate.

The animals were decapitated 25 and 45 days later. The blood serum proteins were investigated by biochemical methods (electrophoresis on paper). The spleen, lymph glands, kidneys, and liver were investigated by histochemical methods. The severity of amyloidosis of the spleen was determined stereometrically, using an ocular measuring grid. The results, calculated as relative percentages, were subjected to statistical analysis, using Student's coefficient [7].

EXPERIMENTAL RESULTS

Biochemical investigation of the blood serum proteins of the control animals 25 days after the beginning of the experiment revealed dysproteinemia: a decrease in the albumin level with a small increase in the β and γ globulins. A similar type of dysproteinemia was found in the experimental animals also.

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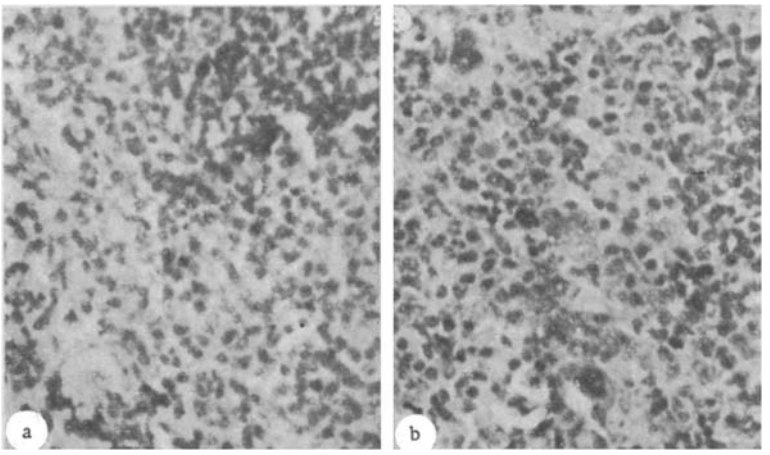

Fig. 1. Changes in spleen of control (a) and experimental (b) animals 25 days after injection of sodium caseinate: a) proliferation of reticuloendothelial cells in the red pulp and in the marginal zone of the follicles; b) giant cells among proliferating reticuloendothelial elements of the splenic pulp. Hematoxylin-eosin, 250×.

Histological investigation revealed proliferation of the endothelium of the sinuses and of the reticulum cells of the marginal zone of the follicles and of their germinal centers in the spleen and lymph glands of the control animals (Fig. 1a). Foci of deposition of amyloid material were seen in the peripheral zones of isolated splenic follicles of two animals. Amyloid also was found in the intima of arteries in the renal medulla. Only proliferation of the Kupffer cells could be distinguished in the liver.

At the same times the morphological changes in the organs of the experimental animals were similar to those in the control group. In the spleen and lymph glands, however, there were many macrophages among the proliferating reticuloendothelial cells (Fig. 1b). Amyloid was found in the spleen of two animals: in one as small deposits, but in the other as massive deposits in the peripheral zone of the follicles.

The character of the dysproteinemia was modified somewhat 45 days after the beginning of the experiment: the α_2 globulin level was higher in the mice of the control group, the β globulin concentration was increased as before, but in the animals of the experimental group the γ globulin concentration was lowered.

Histological investigation revealed deposits of amyloid in the spleen of the control animals which displaced the peripheral zones of the follicles, while in the intact portions of the follicles and in the red pulp reticulocyte-plasma cell transformation was clearly visible as before (Fig. 2a). Amyloid was found in the kidneys in the intima of the medullary arteries, while in the liver it was seen in the blood vessels of the portal tracts.

At these same times amyloid completely replaced many of the follicles in the spleen of the experimental animals and deposits of it could also be seen in the red pulp (Fig. 2b). Masses of amyloid in the kidneys were visible in the intima of the arteries and stroma of the medulla, and also in the capillary loops of the glomeruli; in the liver they were visible in the vessels of the portal tracts and along the course of some sinusoids. The intensity of amyloidosis of the spleen in the experimental animals, calculated stereometrically, was significantly higher than in the control group. For the 25th day of the experiment (five control and five experimental mice) $t = 3.54$ and for the 45th day (20 control and 10 experimental mice) $t = 3.3$; the difference between the experimental and control values in both cases was thus statistically significant.

It can be concluded from the results of the biochemical tests in the control and experimental groups that there is no significant difference in the character of the dysproteinemia in the animals of these groups that can be regarded as typical of experimental amyloidosis (a decrease in the albumin concentration and an increase in the globulin fractions).

The results indicating acceleration of the development of amyloidosis in animals receiving imuran can be represented as follows: imuran retards and inhibits proliferation of the plasma cells and disturbs RNA synthesis in their nuclei [2, 17]. After administration of antimetabolites, corticosteroids, actinomycin, and folic acid antagonists necrosis and disintegration of lymphoid tissue and acceleration of amyloidosis

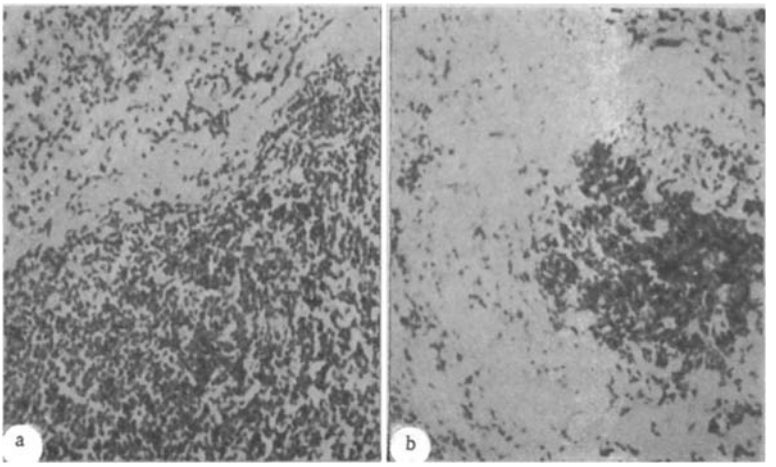

Fig. 2. Changes in the spleen of the control (a) and experimental (b) animals 40 days after injection of sodium caseinate: a) amyloid deposits in peripheral zones of splenic follicles coupled with reticulocyte-plasma cell transformation; b) massive deposits of amyloid in follicles and red pulp of the spleen. Hematoxylin-eosin, 100 ×.

have been found [16]. Marked karyorrhexis and features of acidophilic degeneration of cells of the reticuloendothelial system, accompanied by a more intensive deposition of amyloid in the spleen, liver, and kidneys have also been observed [18] in mice receiving casein and actinomycin D, whose effect on cells of the reticuloendothelial system is similar to that of antimetabolites. Destruction, disintegration, and changes in the metabolism of the cells of the reticuloendothelial system are evidently the cause of disturbance of intracellular protein synthesis and of the accelerating effect of imuran on amyloidosis. The effect of the cytostatic can evidently be reduced to prevention of the normal immune response to antigenic (casein) stimulation, reflected morphologically in suppression of reticulocyte-plasma cell transformation of the reticuloendothelium. The suppression of proliferation of the cells responsible for globulin synthesis leads to the exhaustion and, perhaps, the distortion of this synthesis, with the result that the development of amyloidosis is accelerated.

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