EFFECT OF ANTILYMPHOCYTIC SERUM ON THE COURSE OF EXPERIMENTAL AMYLOIDOSIS

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Antilymphocytic globulin was found to have an accelerating action on experimental (casein) amyloidosis in experiments on C57BL male mice. The omission of the preamyloid phase and the rapid development of amyloidosis under the influence of antilymphocytic globulin are explained from the standpoint of Teilum's theory of amyloid production, according to which amyloidosis is the result of derangement of the protein-synthesizing function of the reticulo-endothelial system.

The frequency of amyloidosis and the severity of its prognosis explain the persistent search for methods of treating this complaint. Information regarding the role of transformation of cells of the reticulo-endothelial system and the role of immune reactions in amyloidosis account for attempts to study the action of substances with an affinity for reticulo-endothelial cells and with the action of immunodepressants on the course of amyloidosis. Most investigations on this subject have been concerned with the action of glucocorticoids, but their results, like those obtained in the study of the effect of cytostatics on developing amyloidosis, are contradictory [1, 2, 13, 14, 17-19, 22, 23, 25]. The few published reports of the inhibitory action of compounds of the 4-aminoquinoline series on amyloid production have suggested that these substances can be recommended for the clinical treatment of amyloidosis [3, 4, 5, 17].

Regarding the effect of antilymphocytic serum, now considered to be one of the most powerful immunodepressive agents, on the course of amyloidosis no definite conclusions about its character can be drawn from the few reports which have been published [10, 21].

This paper describes an attempt to assess the details of the course of experimental casein amyloidosis as affected by the simultaneous injection of antimouse antilymphocytic globulin (ALG).

EXPERIMENTAL METHOD

The ALG preparation consisted of a solution of globulin isolated by salt fractionation from the serum of a horse immunized with lymphocytes from the spleen and thymus of noninbred mice. The serum was adsorbed repeatedly with mouse erythrocytes until all hemagglutinins had disappeared. The titer of the 5% globulin solution in the lymphagglutination test was 1:640 and in the cytotoxic test 1:1280 (the preparation was made up in the laboratory of immunology, Moscow Research Institute of Epidemiology and Microbiology Ministry of Health of the RSFSR).

Experiments were carried out on male C57BL mice. The antigen was a 5% solution of sodium caseinate. Mice of the experimental group (ten animals) received 0.5 ml sodium caseinate solution by daily subcutaneous injection (six times a week) and at the same time they received ALG by intraperitoneal injection in a dose equalivalent to 6 mg protein (0.3 ml per injection).

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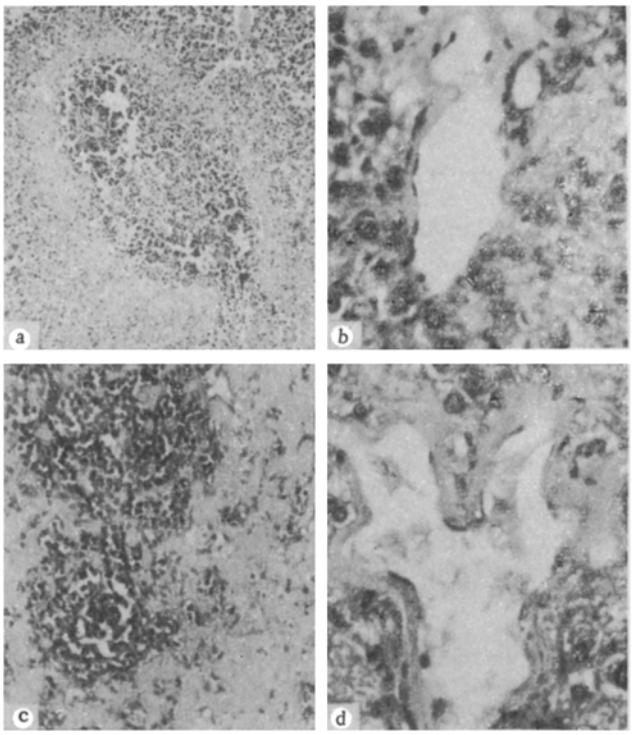

Fig. 1. Changes in spleen and liver of control (a, b) and experimental (c, d) animals on 45th day of experiment: a) "sago" spleen; b) amyloid in vessel wall in the portal tract of the liver; c) "lardaceous" spleen; d) accumulation of amyloid in vessel wall in the portal tract and in sinusoids of the liver. Hematoxylin-eosin. Magnification in a and c, 100×; in b and d 250×.

Mice which received sodium caseinate only (ten animals) formed the control group. These animals were sacrificed by decapitation after 25, 35, and 45 days. The spleen, lymph glands, liver, and kidneys were studied by histological, histochemical, and luminescent methods, enabling transformation of the reticulo-endothelial cells and amyloid to be detected. The degree of amyloidosis of the spleen was determined by a stereometric method using an ocular measuring grid. The results were calculated in relative percentages.

EXPERIMENTAL RESULTS

After 25 days some obliteration of the normal structural pattern was observed in the spleen and lymph glands as the result of marked proliferation of reticulo-endothelial cells, including many plasma cells. In the peripheral parts of single follicles of the spleen, deposits of amyloid material could be seen. Proliferation of the Kupffer cells was present in the liver. Amyloid was absent in the liver and kidneys.

At these same times, there was a marked decrease in the number of cells in the spleen and lymph glands of the experimental group of animals, and only small groups of monocytes were present in the central zones of the follicles. Proliferation of the Kupffer cells of the liver was absent. In the peripheral zones of the spleen follicles there were considerable deposits of amyloid, while in the kidneys amyloid was found in the intima of the arteries, mainly in the medulla.

After 35 days the reticulocyte-plasma cell transformation was clearly defined as before in the spleen and lymph glands of the control animals, while proliferation of the Kupffer cells was present in the liver. Accumulation of amyloid in the peripheral zones of the spleen follicles had the appearance of a narrow girdle, while amyloid was also found in the intima of the reneal medulla.

In the animals of the experimental group at these same times masses of amyloid occupied the peripheral zones of the spleen follicles and were also visible as individual aggregations in the red pulp also.

TABLE 1. Intensity of Amyloidosis of the Spleen in Experimental and Control Animals (relative percentages)

Time of experiment (in days)	Intensity of amyloidosis of the spleen	
	control animals	experimental animals
25	4	10
35	6,5	19,4
45	19,1	32,1

In the kidneys amyloid was deposited both in the intima of the cortical and medullary arteries and frequently also in the capillary loops of the glomeruli.

After 45 days masses of amyloid occupied only the peripheral zones of the follicles in the spleen of the control animals ("sago" spleen—Fig. 1a). Reticulocyte-plasma cell transformation was clearly present both in the residual central zones of the follicles and in the red pulp. In the kidneys amyloid was present in the intima of the cortical and medullary arteries and also in single capillaries of certain glomeruli, while in the liver it was found in the vessels of the portal tracts (Fig. 1b).

At these same times amyloid in the spleen of the experimental group of animals occupied both the follicles and the red pulp ("lardaceous" spleen—Fig. 1c), and small clusters of cells were present in the central zones of individual follicles. Much amyloid was present in the intima of the arteries, the arterials, and the capillary loops of most glomeruli. In the liver amyloid was found not only in the blood vessels of the portal tracks, but also in the sinusoids (Fig. 1d).

The results of the stereometric investigation demonstrate the high degree of amyloidosis of the spleen in the experimental animals receiving sodium caseinate and ALG by comparison with the control animals at all times of the experiment (Table 1).

The analysis of these experimental results showed that morphological changes in the organs of the control animals reflect the two successive phases of experimental amyloidosis described by Teilum [23, 24]: marked proliferation and plasmatization of the reticulo-endothelial system in the early stages of the experiment (the preamyloid stage), evidence of a high intensity of the protein-synthesizing and, in particular, the antibody-forming functions, followed by accumulation of amyloid successively in the spleen, kidneys, and liver (the amyloid stage), accompanied by a decrease in the number of reticulocytes and plasma cells in the lymphoid organs.

In the experimental animals that received ALG as well as sodium caseinate the morphology of the preamyloid stage was absent. The early and massive deposition of amyloid in the spleen, kidneys, and liver took place against the background of a marked decrease in the number of cells of the reticulo-endothelial system. ALG thus accelerated the onset of experimental amyloidosis considerably.

The mechanism of the accelerating action of ALG in amyloidosis is not absolutely clear, although there is no doubt about its general immunodepressive effect. On the one hand, in the explanation of this mechanism it is essential not to lose sight of the few reports which have been published on the role of cellular immunity in amyloidosis and, in particular, the results of experiments in which amyloidosis was transferred by immune cells [9, 21], on the reproduction of amyloidosis in cell cultures [6, 11], observations on patients with amyloidosis including the study of the blast-transformation reaction to injection of amyloid as an antigen [16], and the hypothesis [10] that immune tolerance to the specific immunogen (casein) may arise; on the other hand, it must not be forgotten that states accompanied by inhibition of small (immune) lymphocytes (such as thymectomy, whole-body irradiation, treatment with antimetabolites) lead to the acceleration of amyloidosis [7, 12, 13]. An interesting paper has been published by Kozlowski and Hrabowska [15] on the acceleration of experimental amyloidosis during administration of antibiotics (actinomycin D), which is evidently due to an increase in the quantity of breakdown products of nuclear protein. At the same time, the undoubted omission of the preamyloid phase and the rapid development of amyloidosis under the influence of ALG can be explained sufficiently well from the standpoint of Teilum's theory of amyloid production, according to which amyloidosis is the result of a derangement of the protein-synthesizing function of the reticulo-endothelial system.

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