AMYLOID RESORPTION IN THE SPLEEN GRAFTED INTO INTACT AND AMYLOID RECIPIENTS

V. V. Sura, V. V. Serov,
A. P. Chebyshev,
and A. Yu. Gritsman

UDC 616.411-003.821-089.843-07:616.441-003.821-036.84-092.9

Changes in amyloid spleens from CBA mice were studied when grafted into recipients of the following four groups: intact CBA mice, CBA mice with casein-induced amyloidosis, intact C57BL mice, and noninbred rats. Amyloid resorption in a syngeneic graft in recipients with amyloidosis was much less intensive than in intact animals. The intensity of amyloid resorption in intact recipients with syngeneic, allogeneic, and xenogeneic grafts increased with increasing heterogeneity of the transplanted material. The development of systemic amyloidosis (the so-called "transfer" of amyloidosis) was observed in some intact recipients after syngeneic transplantation of an amyloid spleen; the resorption of amyloid in the graft in these animals was less marked than in mice without "transfer." Administration of hydrocortisone into animals with a syngeneic graft of an amyloid spleen completely inhibited amyloid resorption in the graft.

KEY WORDS: transplantation of the spleen; amyloidosis; resorption of amyloid; hydrocortisone.

The study of amyloid resorption is interesting not only theoretically but also from the practical point of view in the search for ways of treatment of amyloidosis. The possibility of amyloid resorption has now been accepted, in principle, on the basis of clinical and morphological observations and experimental investigations [1, 3, 5, 13, 14, 16, 19]. Meanwhile, the resorption of amyloid in patients after treatment of the primary disease and in animals after discontinuation of the amyloidogenic treatment has been very rarely observed. It presumably takes place as a result of the spontaneous restoration of the mechanism for amyloid resorption which, in most cases, is in a profoundly depressed state.

The present investigation was carried out to study the conditions for amyloid resorption and to examine their possible role in the pathogenesis of amyloidosis. The state of a syngeneic graft of an amyloid mouse spleen was accordingly investigated in intact recipients and recipients with induced amyloidosis, as well as after allogeneic and xenogeneic transplantation into intact animals. The state of the spleen and kidneys of both donors and recipients also was studied.

EXPERIMENTAL

Adult male CBA and C57BL mice and noninbred rats were used. Amyloidosis was induced in the CBA mice (donors and recipients) by subcutaneous injection of 1 ml of 5% casein solution in 0.25% NaOH solution six times a week for 7 weeks. Pieces of amyloid spleen from CBA mice measuring about 2-3 mm^3 were grafted under the renal capsule of syngeneic intact recipients and recipients with amyloidosis 2-3 days after the last injection of casein, and similar pieces were grafted into C57BL mice and into rats. The state of the donors' spleen at the time of grafting, and the state of the graft, the kidney with the graft, and the spleen of the recipients 10, 30, 60, and 90 days after the operation were studied morphologically. The

Department of Internal and Occupational Medicine and Department of Pathological Anatomy, I. M. Sechenov First Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Tareev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 12, pp. 95-99, December, 1974. Original article submitted March 22, 1974.

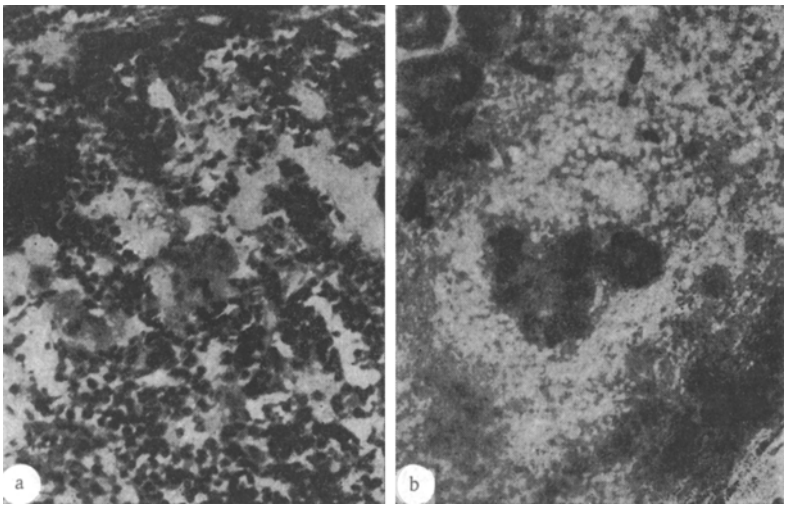

Fig. 1. Amyloid resorption in syngeneic amyloid spleen grafted under the renal capsule of an intact CBA mouse 30 days after transplantation: A) marked infiltration with macrophages, adsorbing masses of amyloid (hematoxylin–eosin, 180 ×); B) high acid phosphatase activity in cells adsorbing mass of amyloid in spleen grafted into intact mouse (azo-coupling test for acid phosphatase, 180 ×).

following methods were used: staining with hematoxylin–eosin, with Congo red, toluidine blue, and thioflavin T, the PAS reaction, and Brachet's reaction. The following enzymes also were studied histochemically: acid and alkaline phosphatase, α-glycerophosphate dehydrogenase, and succinate dehydrogenase.

RESULTS

Investigation of the state of the donors' spleen at the moment of transplantation revealed massive deposits of amyloid in the follicles and red pulp with atrophy of the cells (lardaceous spleen). In the syngeneic graft in the intact recipients, with no morphological manifestation of tissue incompatibility, fragmentation of the amyloid masses into separate lumps and changes in the staining properties of the amyloid were observed: diminished ability to stain with Congo red, decreased dichroism in polarized light, weakening of the PAS reaction, and weakening of metachromasia on staining with toluidine blue. Marked infiltration of the amyloid with macrophages, neutrophils, and giant cells, forming lacunae in the amyloid masses, was observed (Fig. 1A).

The enzyme–histochemical investigation showed that amyloid resorption was accompanied by increased activity of glycolytic (α-glycerophosphate dehydrogenase) and hydrolytic (acid phosphatase) enzymes in the macrophages. High acid phosphatase activity was observed in the masses of amyloid and the cells adsorbing it (Fig. 1B). With a decrease in the quantity of amyloid in the grafted spleen, hyperplasia of the lymphoid tissue took place, the number of fibroblasts increased, and eventually marked sclerosis of the capsule and tissues of the graft took place.

Only a very small decrease in the quantity of amyloid was observed in the syngeneic graft in the recipients with amyloidosis, by contrast with the intact recipients, within the period of observation; the amyloid was only moderately infiltrated with neutrophils and lymphocytes and there was some degree of hyperplasia of the lymphoid tissue of the graft (Fig. 2).

In allogeneic and xenogeneic transplantation the amyloid resorption had the character of a foreign body reaction, with the formation of numerous giant cells with congophilic granules in their cytoplasm. Amyloid resorption, amounting sometimes to its total disappearance, and the formation of fibrous tissue took place under these circumstances much more intensively and earlier (by the 30th-60th day after transplantation) than in syngeneic transplantation (by the 90th day).

Differences in the intensity of amyloid resorption also were found in a homogeneous group of animals, notably in the intact mice with a syngeneic graft. The intensity of amyloid resorption in the graft in the mice of this group depended on the development of systemic amyloidosis in the animals after transplantation.

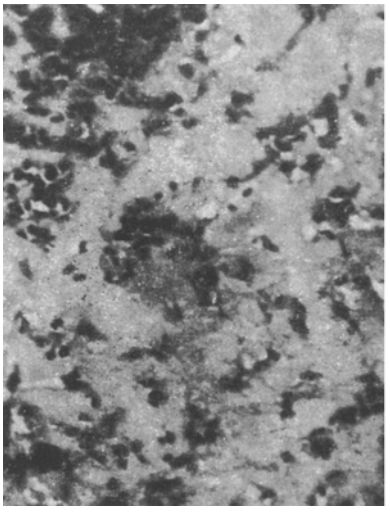

Fig. 2

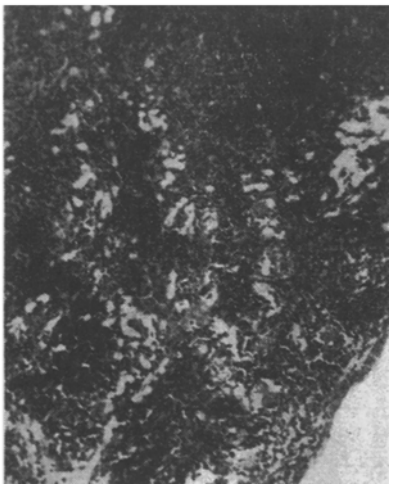

Fig. 3

Fig. 2. Massive deposits of amyloid without evidence of resorption in syngeneic amyloid spleen grafted beneath the renal capsule of a mouse with amyloidosis, 30 days after transplantation (hematoxylin–eosin, 180 ×).

Fig. 3. Syngeneic amyloid spleen grafted beneath the renal capsule of an intact C57BL mouse, 40 days after transplantation (hematoxylin–eosin, 80 ×). Above: marked resorption of amyloid (only separate granules remain); below: absence of resorption (lardaceous spleen) following administration of hydrocortisone.

In half of these recipients, for instance, after syngeneic transplantation of the amyloid spleen deposits of amyloid were found in the recipient's own spleen and kidneys, i.e., a "transfer" of amyloidosis as described by other workers [6, 10-12, 17, 18] had taken place. Amyloid resorption in the graft taken from such mice was less marked than in recipients in which no "transfer" phenomenon was found.

The results of this investigation thus confirm data in the literature to show the possibility, in principle, of amyloid resorption and they are evidence of the depression of this process in amyloidosis. The results also suggest that the irreversibility of the course of amyloidosis is to some extent due not only to the extreme inertness of the amyloid protein [4, 9, 20] but also to the general conditions created in the affected organism, which ultimately determine the absence of cellular reactions to the amyloid and the inactivity of the enzyme systems required for its resorption. Tolerance to amyloid in the affected organism of this nature is probably one of the causes of its accumulation.

A possible method of testing this hypothesis would be to study the effect of factors stimulating the development of amyloidosis on the process of amyloid resorption; glucocorticoids, injection of which, like that of other immunodepressants, stimulates amyloidogenesis [2, 7, 8, 12], are among this category of factors. Inhibition of amyloid resorption by treatment of this type could be interpreted as proof of the pathogenetic role of the inhibition of amyloid resorption in the development of amyloidosis. An experiment was accordingly carried out to study the effect of hydrocortisone on amyloid resorption in a syngeneic graft in intact recipients. The investigation was carried out on intact male C57BL mice with a syngeneic graft of an amyloid spleen. Subcutaneous injections of 100 μg hydrocortisone were given six times a week starting 24 h after the operation to half of these animals, while the remainder acted as the control. The state of the graft was studied 10 and 40 days after the operation. A well-marked picture of amyloid resorption was found on the 40th day in the control mice not receiving hydrocortisone (Fig. 3, above). By this time the cellular response characteristic of amyloid resorption could not be observed in the mice receiving hydrocortisone, in which the morphological picture corresponded to the lardaceous spleen (Fig. 3, below).

The experimental results show that one probable mechanism of the stimulation of amyloidogenesis during the administration of immunodepressants is inhibition of amyloid resorption by these drugs, although special investigations are required in order to confirm this hypothesis.

The results indicate that one cause of the accumulation of amyloid in amyloidosis could be a disturb-

ance of its resorption resulting not only from the high chemical stability of the amyloid protein but also from some of the features distinguishing the reticuloendothelial system in the affected organism.

LITERATURE CITED

1. A. I. Abrikosov, in: Collection to Commemmorate Twenty Five Years of Scientific Activity of N. N. Anichkov [in Russian], Moscow–Leningrad (1935), p. 14.
2. R. G. Arustamova, Effect of Antilymphocytic Globulin and Imuran on the Course of Experimental Amyloidosis. Author's Abstract of Candidate's Dissertation, Moscow (1973).
3. S. M. Evdokimov, Experimental Investigation of the Reversibility of Amyloidosis. Author's Abstract of Candidate's Dissertation, Moscow (1967).
4. T. S. Korneeva, Arkh. Pat., No. 6, 3 (1970).
5. B. I. Migunov, Arkh. Pat. Anat., No. 6, 100 (1936).
6. V. S. Rukosuev, Arkh. Pat., No. 10, 42 (1970).
7. V. V. Serov and N. A. Mukhin, Arkh. Pat., No. 4, 31 (1966).
8. I. A. Shamov, Probl. Tuberk., No. 7, 86 (1971).
9. P. Diezel and A. Pfleiderer, Arch. Path. Anat., 332, 552 (1959).
10. F. Hardt, Acta Path. Microbiol. Scand., 72, 446 (1968).
11. F. Hardt, Am. J. Path., 65, 411 (1971).
12. F. Hardt and P. Hellung-Larsen, Clin. Exp. Immunol., 10, 487 (1972).
13. A. Polliack et al., Brit. J. Exp. Path., 51, 236 (1970).
14. G. Richter, Am. J. Path., 30, 239 (1954).
15. G. Teilum, Ann. Rheum. Dis., 11, 119 (1952).
16. H. Waldenström, Acta Chir. Scand., 63, 479 (1928).
17. O. Werdelin and P. Ranlov, Acta Path. Microbiol. Scand., 72, 13 (1968).
18. J. T. Willerson et al., Arthr. Rheum., 12, 232 (1969).
19. G. Williams, J. Path. Bact., 94, 331 (1967).
20. G. Windrum and H. Kramer, Arch. Path., 63, 373 (1957).