ELECTRON-MICROSCOPIC CHARACTERISTICS OF AMYLOID RESORPTION

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In experiments on CBA mice, in which an amyloid spleen was transplated beneath the capsule of the kidney in intact and amyloid mice, an electron-microscopic investigation was made of amyloid resorption. The results indicated that macrophages, performing the function of amyloidoclasts, participate in amyloid resorption. The dynamics of amyloidoclasia was studied.

KEY WORDS: amyloid resorption; macrophages; amyloidoclasts.

The mechanisms of amyloid resorption are of great interest. That resorption of amyloid can take place in principle has been demonstrated by several clinical-morphological and experimental investigations [3, 5-8, 10, 12, 19, 21]. It is considered that amyloid can be resorbed with the aid of leukocytes [13, 20] or giant cells [1, 2, 9, 11, 15, 16], which N. M. Lyubimov (1899) called amyloidophages [4]. Only a few papers have been published on the electron-microscopic investigation of resorption of amyloid fibrils by macrophages in tissue culture [17, 18], but naturally this is not identical with the processes of amyloidoclasia in vivo in animals or man.

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

EXPERIMENTAL METHOD

CBA male mice were used. Amyloidosis was produced in some (40) animals by subcutaneous injection of 1 ml of 5% casein solution in 0.25% sodium hydroxide solution six times a week for seven weeks. Pieces of "amyloid" spleen, measuring 2.5-3 mm, were implanted beneath the capsule of the upper pole of the kidney of 18 intact recipient mice (group 1) and 18 "amyloid" recipient mice (group 2). On the fifth, 15th, 30th, 60th, and 90th days after transplantation the animals were killed in batches of three or four. The graft was examined in the light microscope, in sections stained with hematoxylin–eosin, Congo red, and thioflavine T. Material fixed in a 1% buffered solution of osmic acid and embedded in Araldite was used for electron microscopy. Ultrathin sections were studied in the UÉMV-100K electron microscope.

EXPERIMENTAL RESULTS

Electron-microscopic investigation of the grafted "amyloid" spleen (histologically it looked like a "lardaceous" spleen) from the group 1 animals 5 days after transplantation showed macrophages, polymorphs, and platelets at the boundary with the zones of amyloid. The macrophages were large cells containing one or, less frequently, two or three elongated nuclei with slightly indented outlines and a uniform distribution of chromatin. In the cytoplasm, which was of low electron density, there were may free ribosomes and polysomes and the Golgi complex, as a rule, was hyperplastic.

The number of macrophages increased in the course of the experiment, their ultrastructure changed, and the changes in ultrastructure reflected the dynamics of amyloid resorption. The process began with

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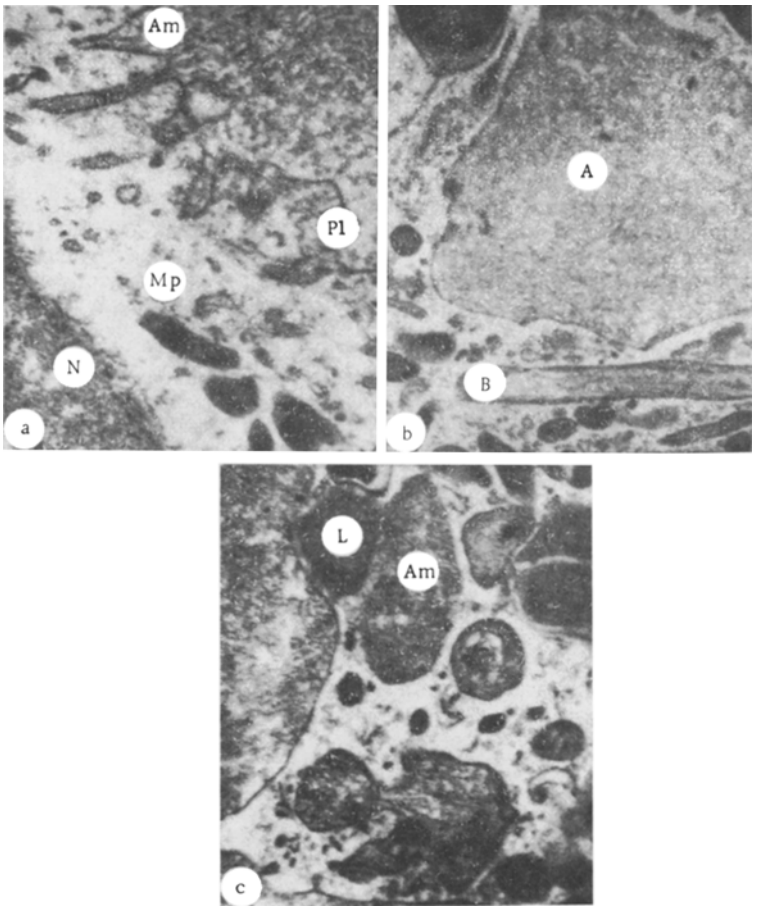

Fig. 1. Resorption of amyloid by macrophage: a) invaginations in plasmalemma (Pl) of a macrophage (Mp) containing amyloid fibrils (Am); N) nucleus of macrophage (30,000 ×); b) type 1 inclusions containing irregularly arranged amyloid fibrils (A) and type 2 inclusions, with regularly arranged amyloid fibrils (B), can be seen in the cytoplasm (15, 000 ×); c) phagolysosomes containing amyloid fibrils (Am) and lipid drops (L) – type 3 inclusions (30,000 ×).

the formation of invaginations, in which regular and irregular amyloid fibrils accumulated, in the plasmalemma. Later the plasmalemma above the invagination came together so that the collections of amyloid fibrils were surrounded by cytoplasm. They were bounded by a single membrane (Fig. 1a). "Loss" of the plasmalemma during phagocytosis evidently explains the lack of clarity of the cell membrane in certain parts of the macrophage. At different stages of intracellular "digestion" of amyloid in the macrophages, intracellular inclusions (phagosomes) of several types appeared. The type 1 inclusions were large. They contained irregularly arranged branching amyloid fibrils and were surrounded by a single membrane (Fig. 1b). The type 2 inclusions contained regularly arranged and tightly packed amyloid fibrils of different sizes and shapes. They had moderate or high electron density (Fig. 1b). The type 3 inclusions were phagolysosomes, formed by fusion of primary lysosomes with the type 1 or 2 inclusions; besides amyloid fibrils they also contained lipid drops (Fig. 1c). Myelin-like and lipid postphagocytic structures, sometimes large in size, formed the type 4 inclusions.

Investigation of the grafted "amyloid" spleen in the animals of group 2 showed signs of amyloid resorption later. Macrophages began to appear only on the 15th and 30th days of the experiment, and most of them contained only primary lysosomes. Collections of leukocytes and platelets were visible among the macrophages in the animals of both groups.

The results of these experiments show that resorption and complete intracellular digestion of amyloid by macrophages is possible and they also shed light on the dynamics of this process. Resorption is carried out with the participation of lysosomal hydrolases. The Golgi complex evidently plays an important

role in this process because its enzymes (galactosyl transferases and cerebroside sulfotransferases) are known to participate in the transformation of glycoproteins and lipids [14]. The accumulation of residual bodies in the macrophages is the morphological expression of the completion of amyloid resorption. The results of these experiments are in agreement with those of Shirahama et al. [18], who observed phagocytosis of amyloid fibrils by peritoneal macrophages and confirmed their observations cytochemically.

The present investigation provides morphological support for the view that macrophages with the function of amyloidoclasts exist. Most probably macrophages mainly of hematogenous origin are concerned with amyloid resorption, for in a transplanted spleen that was completely replaced by amyloid the cells were single and could hardly have played the role of fixed macrophages. The large numbers of polymorphs as well as macrophages in the areas of amyloid resorption may indicate the participation of these cells in the process also. The possibility cannot be ruled out that the platelets found in the same areas also facilitate resorption, by liberating biologically active substances as they disintegrate.

Analysis of the literature and of the results obtained in this experimental study of amyloid resorption provides support for the view that amyloidogenesis is the result of two opposite but interlinked processes of production and resorption of amyloid and that it depends on the ratio between the two populations of precursor cells later transformed into amyloidoblasts or amyloidoclasts. This ratio evidently determines both the direction of the process and the rate of its development.

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