ROLE OF RETICULO-ENDOTHELIAL CELLS OF THE SPLEEN IN THE SYNTHESIS OF AMYLOID FIBRILS IN TISSUE CULTURE

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Cultures of mouse spleen cells in the preamyloid stage and during amyloidosis were investigated. Under high power of the electron microscope the amyloid fibrils were globular in structure, with a subunit diameter of 40 Å. Globular particles of this diameter were found in cytoplasmic vesicles of the reticulo-endothelial cells. The role of globular material of the cytoplasmic vesicles in the formation of amyloid fibrils is postulated.

Reticulo-endothelial cells probably play an important role in the final stage of the pathogenetic process of amyloid fibril formation [8, 17, 18]. A basis for this conclusion is the constantly observed close structural relationship between cells of the reticulo-endothelial system and amyloid fibrils. The role of the reticulo-endothelial cells is either to participate in the conversion of "preamyloid," circulating in the blood stream into a fibrillary substance [17], or the synthesis of amyloid fibrils in situ. In the latter case the reticulo-endothelial cells either produced the soluble precursor of amyloid, which is converted into fibrils after leaving the cells [18], or they form amyloid fibrils in their own cytoplasm [15].

By studying the formation of amyloid fibrils in tissue culture, the possibility of a supply of "preamyloid" from the blood is ruled out. Only three references could be found in the literature to investigations of amyloid in tissue culture [1, 5, 12], and two of them [5, 12] used organ cultivation as the model. Cohen et al. [5] and Bari et al. [1], who used the electron-microscopic autoradiographic method, conclude that reticulo-endothelial cells may participate in amyloid synthesis de novo. Laufer and Tal [12] came to a similar conclusion by identifying intracellular amyloid in tissue culture by a series of reactions with dyes. These workers [12] observed the appearance of amyloid even if the primary tissue explant contained no deposits of amyloid.

The object of the present investigation was to continue the study of the role of reticulo-endothelial cells in amyloid formation in tissue culture.

EXPERIMENTAL METHOD

Amyloidosis was induced in male BALB mice weighing 18-20 g by subcutaneous injections of 0.5 ml 5% casein solution in 0.25% NaOH solution six times a week. The optimal number of casein injections before the appearance of the first signs of amyloid in the spleen was 15-16.

There were two series of experiments. In series I the animals were killed and the spleen removed after 12-13 injections, and in series II after 24-25 injections of casein. The spleens of normal BALB mice were used as the control.

The spleens, after removal, were rubbed through a steel sieve into medium No. 199. The cell concentration was adjusted to 4 million cells per ml suspension, and 2 ml of the cell suspension was placed in

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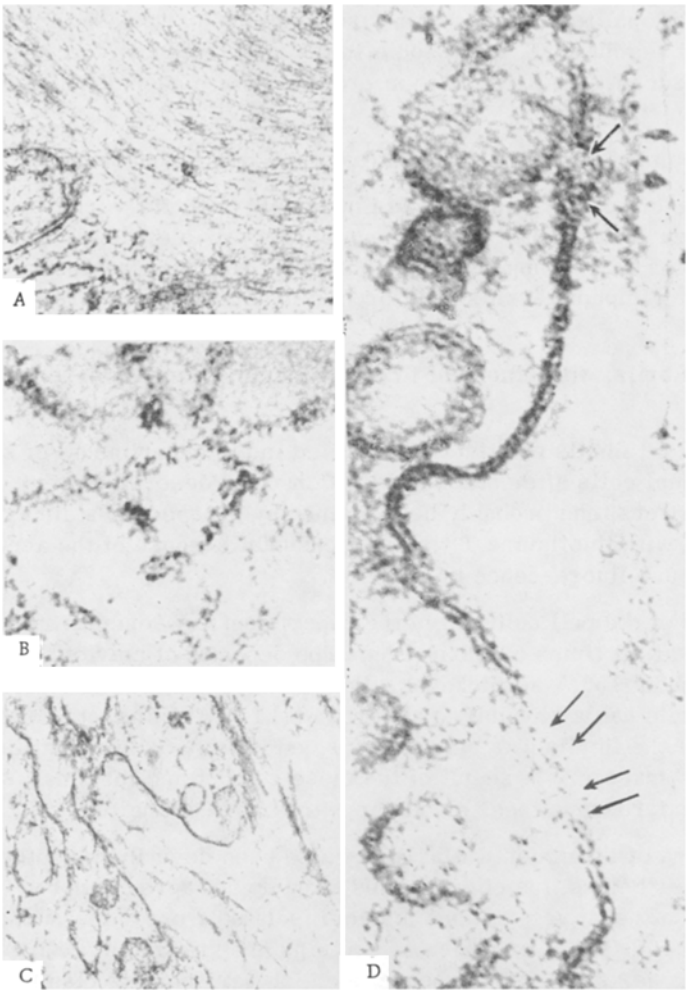

Fig. 1. A) intracellular microfibrils, 40,000 ×; B) globular structure of amyloid fibrils, 200,000 ×; C) invagination of cytoplasm of reticulo-endothelial cell, 50,000 ×; D) cytoplasmic vesicles of reticulo-endothelial cell. Zones where globular particles pass through the plasmalella are indicated by arrows, 220,000 ×.

flasks with cover slips and 10% bovine serum was added. The cell cultures were incubated at 37° C. The medium was changed every four days.

The cell cultures were investigated on the 7th, 14th, and 21st days of cultivation. For light microscopy the cell monolayer on the cover slips was fixed in 96° ethanol and stained by the Romanovsky–Giemsa method, with methyl violet, Congo red, and thioflavine T. For electron-microscopic investigations, cultures of cells on coverslips were fixed with 1% osmium tetroxide solution in veronal-acetate buffer, pH 7.4, for 15 min. Dehydration was carried out in acetone of increasing concentration, containing 0.5% uranyl acetate. The material was embedded in Vestopal W. Ultrathin sections, 400-500 Å in thickness, negatively stained with lead citrate, were examined in the JEM 6c electron microscope.

EXPERIMENTAL RESULTS

Similar results were obtained by electron-microscopic study of cultures of spleen cells under normal conditions and in the experiments of series I. On the seventh day of cultivation a mixed cell population was found, with predominance of round cells resembling lymphocytes and with numerous polymorphic macrophages. On the 14th and 21st days, the cell cultures contained mainly macrophages. They were extremely varied in shape, from polygonal to fusiform. The cytoplasm of the macrophages usually contained a varied

number of phagosomes, osmiophilic lipid droplets, lysosome-like particles, and microfibrils. The thickness of the microfibrils varied from 50 to 70 Å. In some cases large bundles of microfibrils occupied a considerable part of the cytoplasm (Fig. 1A). Similar microfibrils have been described in many types of cultivated cells [4].

The degree of development of the cell organelles of the macrophages varied considerably.

Because of the extreme polymorphism it was impossible to fit the varieties of macrophages discovered into concrete types of spleen cells. Ability to perform phagocytosis evidently cannot be used as a basis for the definition of macrophages as a homogeneous population of reticulo-endothelial cells [1, 5], for it has been shown that cells of the lymphoid series and fibroblasts become phagocytes under tissue culture conditions [7, 14].

Bundles of collagen fibrils, with traces of finely granular material, were frequently present in the intercellular spaces.

In no case were amyloid fibrils positively indentified in the experiments of series I. The dichroism of the cytoplasm of individual cells after staining with Congo red described previously [12] and sometimes observed by the present writers, can probably be explained by nonspecific staining of the intracellular microfibrils [11]. Staining with thioflavine T was unsuccessful because of the accumulation of large quantities of pigment possessing autofluorescence in the cells.

Electron microscopy of the cell cultures in the experiments of series II revealed deposits of amyloid in the intercellular spaces at all times of cultivation. The deposits of amyloid consisted of straight, chaotically arranged fibrils, about 100 Å in thickness. Under high power of the electron microscope (200,000-400,000 ×) the amyloid fibrils had a globular structure (Fig. 1B), the diameter of the globular subunits being 35-40 Å. The axis of the fibrils was evidently formed largely by two parallel chains of globular subunits, separated by a clear space. As a result of the close contact and the small size of the subunits, under low power of the electron microscope these chains appeared as two longitudinally arranged filaments.

The globular structure of the amyloid fibrils has also been described by other workers in ultrathin tissue sections [2, 8]. Nevertheless, the observations cited do not agree with the results of electron-microscopic investigations of negatively stained amyloid isolated from the tissue, with their evidence of 2 types of amyloid fibrils [3, 16]. However, globular proteins are known to be capable of aggregation in various ways [6, 10]. It can be assumed that the protein globular subunits visible in ultrathin sections form either fibrils [16] or pentagonal structures [3], depending on the method used to isolate the amyloid. Another possibility is that the amyloid fibrils consist of pentagonal structures [13]. In such cases the globular subunits of the amyloid fibrils could be the structures mentioned.

Some macrophages in the cell cultures in the experiments of series II had characteristic features, in the form of numerous cytoplasmic vesicles filled with material of average electron density, and numerous invaginations of the cytoplasm, containing amyloid fibrils. Since this last feature is found only in the reticulo-endothelial cells during amyloidosis [8, 17], the macrophages of this variety were regarded as reticulo-endothelial cells.

Small invaginations of cytoplasm of the reticulo-endothelial cells usually contain bundles of parallel amyloid fibrils (Fig. 1C) In these bundles, an intimate structural connection with the plasmalella was found for a large extent of its course, and their arrangement was palisade-like. The ectoplasm of the reticulo-endothelial cells usually contained garlands of vesicles from 700-1400 Å in diameter. Under high power of the electron microscope the contents of the vesicles had the appearance of densely packed globular particles about 35-40Å in diameter, with higher electron density than the adjacent matrix of the cytoplasm (Fig. 1D). Where the membranes of the vesicles merge with the plasmalella and, more commonly actually at the boundary with the plasmalella the release of globular contents from the vesicles was observed. In these areas the regular three-layered structure of the plasmalella was disturbed, evidently through the passage of large numbers of globular particles. These particles form aggregations on the outer surface of the plasmalella and in immediate contact with the amyloid fibrils.

It is difficult to judge with certainty at the macromolecular level the specificity of these globular particles, i.e., whether they are precursors of amyloid fibrils. However, the similar size of the globular particles of the cytoplasmic vesicles in the reticulo-endothelial cells and the globular subunits of amyloid fibrils thus suggest that this may be so. The mechanism of formation of the amyloid fibrils is

perhaps similar to that of synthesis of collagen fibrils by fibroblasts. The precursor of collagen is known to be supplied to the plasmalella of the fibroblasts in secretory vesicles. Collagen fibrils are finally formed outside the cell immediately by the plasmalella and, in particular, in invaginations of the cytoplasm [9].

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