DETECTION OF A SPECIFIC ANTIGEN OF AMYLOID FIBRILS IN MICE

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UDC 616-003.821-092-07:616-003.821-097.2-07

An antiserum was obtained against a preparation of mouse amyloid fibrils degraded with alkali. Bright fluorescence of amyloid was found in sections of mouse organs treated by the indirect Coons' method. The results of this investigation provide further proof of the existence of a specific antigen in amyloid fibrils.

Many plasma proteins such as immunoglobulins, complement, β-globulins, albumin, transferrin, haptoglobin, and fibrinogen have been detected by immunological and immunofluorescence methods in amyloid [2, 6]. Periodic rods, otherwise known as the P-component, selectively accumulating in amyloid masses [4, 16], have also been shown to be a normal constituent of the blood serum migrating in the zone of the α-globulins. The principal structural component of amyloid, namely its characteristic fibrils, by contrast with the periodic rods has been regarded as nonantigenic [7, 17]. It is only recently that the obtaining of an antiserum against degraded amyloid fibrils of man [5, 9, 11] and certain strains of mice [13] has been reported.* Meanwhile further evidence has been obtained in support of the origin of the fibrils from light chains of immunoglobulin [11, 14, 15]. Before the pathogenesis of amyloidosis can be explained, further information on the antigenic profile of the amyloid substrate is required.

The object of this investigation was limited to the detection of a specific antigen of amyloid fibrils.

EXPERIMENTAL METHOD AND RESULTS

The original material consisted of the spleen of BALB mice with experimental casein amyloidosis. Spleen tissue weighing 5 g altogether was carefully homogenized in a glass homogenizer, washed in 50 ml physiological saline, and then centrifuged at 16,000 rpm at 4°C. The procedure was repeated 8 to 10 times. The top layer of the residue, containing fibrils [8], was separated mechanically and washed a further 3-4 times in physiological saline. The resulting homogenate was stained with Congo red. It exhibited dichroism, and in the electron microscope it appeared to consist chiefly of typical amyloid fibrils (Fig. 1). About 100 mg of the crude mass of homogenate was incubated with 5 ml 0.1 N NaOH solution at room temperature for 3-6 h and dialyzed against distilled water for 2 days [18]. About 1 ml of a mixture of equal volumes of the preparation of partially degraded fibrils with Freund's complete adjuvant was injected into both popliteal lymph glands and subcutaneously in the dorsal region into rabbits. One month later the preparation was injected without the adjuvant subcutaneously or intravenously, and blood was taken at the end of a week. The antisera of several rabbits agglutinated erythrocytes sensitized with mouse serum proteins in low titers and formed a weak band with them in the agar diffusion test. After exhaustion of the antisera with the homogenate of the organs and the serum of intact mice the crossed reaction disappeared and only the precipitation band corresponding to the degraded amyloid fibrils remained (Fig. 2). Two of the four anti-

*The antisera provided for diagnostic purposes probably contained antibodies against serum protein and not against amyloid fibrils, since immunization was carried out with saline extract of a homogenate of amyloid organs [1, 3].

Laboratory of Age Pathology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 76, No. 12, pp. 99-102, December, 1973. Original article submitted February 7, 1973.

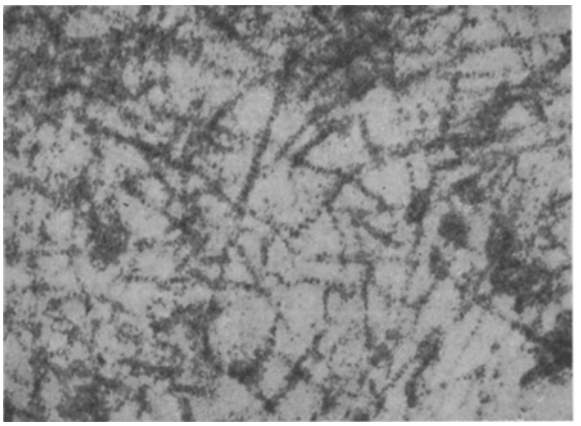

Fig. 1

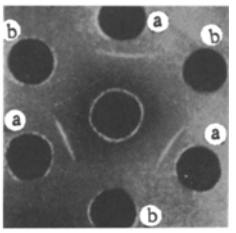

Fig. 2

Fig. 1. Homogenate of amyloid fibrils. Electron microscope, 101,000 ×.

Fig. 2. Agar diffusion test. Antiserum against preparation of degraded amyloid fibrils in center; a) preparation of degraded fibrils; b) mouse serum.

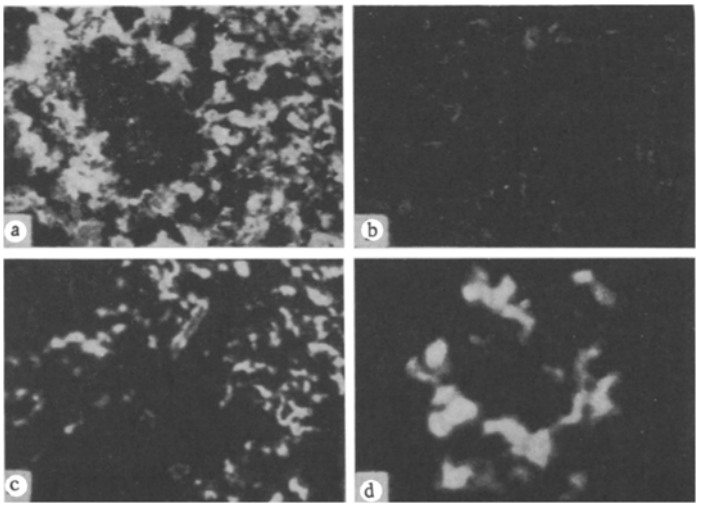

Fig. 3. Specific fluorescence of amyloid in sections of mouse organs treated with antiserum against degraded amyloid fibrils: in white and red pulp of spleen (a), along course of large vessels and capillaries in parenchyma of liver (c), in a renal glomerulus (d); in a control section of the spleen (b) treated with antiserum previously adsorbed with homogenate of amyloid organs, only background fluorescence remains.

sera were sufficiently active. The specificity of these antisera was also checked on sections of mouse organs. Frozen sections, fixed for 10 min in 96° ethanol, were treated by the indirect Coons' method using pure ass antibodies against rabbit γ-globulin.

Only amyloid in the organs investigated (spleen, liver, kidneys) gave off bright fluorescence which was completely inhibited by adsorption of the antisera with homogenate of amyloid organs or with purified fibrils (Fig. 3). Addition of undiluted mouse serum (containing the P-component), even in twice its volume, to the antiserum had virtually no effect on the intensity of fluorescence.

The results provide further confirmation of the antigenic individuality of amyloid fibrils, although the existence of their analog in normal tissues cannot be completely ruled out [6]. The need for preliminary alkaline hydrolysis of the fibrils can evidently be explained by the liberation of antigenic determinants

which are latent in native fibrils, increasing their immunogenic properties [10]. The ability of antisera to react with amyloid directly in the sections of organs, as other workers have described [5, 12, 13, 18], offers new opportunities for the identification of the cells responsible for synthesizing amyloid fibrils. The discovery of their hypothetical precursor, before it acquires ultrastructural organization, would be particularly important.

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