ABSENCE OF AMYLOID PROTEIN FROM THE ORGANS OF NORMAL MICE

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The indirect Coon's immunomorphological test was used to detect specific amyloid protein AA in the organs of mice with experimental amyloidosis and of normal mice (adult, newborn, and embryonic). By using pure antibodies against protein AA, minimal quantities of amyloid protein could be detected in the early stages of amyloid formation. No protein AA could be found in the organs of normal mice (adult, newborn, or embryonic).

KEY WORDS: *experimental amyloidosis; normal mice, pure antibodies against amyloid protein AA.*

Species-specific nonimmunoglobulin amyloid proteins (AA in the modern nomenclature) are found in fibrils deposited in different types of amyloidosis — experimentally in animals [5] and in patients with secondary and primary forms and with amyloidosis associated with Waldenstrom's macroglobulinemia or myelomatosis [4]. Serum amyloid proteins SAA, similar to fibrillary proteins in their antigenic properties and in their N-terminal amino acid sequence [2], but with a greater molecular weight, have also been found. It has been suggested that SAA is the precursor of AA, but the mechanism of conversion of the soluble into the fibrillary protein has not yet been explained. Protein SAA normally circulates in the blood in an extremely low concentration (about 90 ng/ml). In different pathological states (amyloidosis, infections, inflammation, lymphoproliferative processes), and also with age, the level of this protein rises to 2500 ng/ml [8]. After immunization of man [6] and animals [5] the SAA concentration rises sharply during the first 24-48 h, then gradually returns to normal unless the antigenic stimulus is repeated. On this basis, protein SAA belongs to the group of reactant proteins, and Linder et al. [6] have suggested that it is liberated from normal tissues as a result of their injury in inflammatory states. Using antibodies against human SAA these workers found large quantities of a protein similar to SAA, in the form of thin connective-tissue fibrils, in various organs of human embryos (the skin, liver, kidney, lung, and aorta) Embryonic fibroblasts produced this protein in tissue culture also. Strong autofluorescence of collagen and elastic fibers in the connective tissue of adult human organs made it difficult to detect the SAA-like protein. These workers thus concluded by stating that SAA-like protein is a normal component of developing extracellular connective-tissue fibers in human organs.

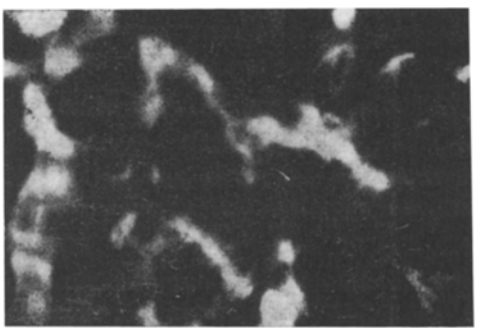

Fig. 1. Section through spleen of mouse with casein amyloidosis. Treatment with antibodies against mouse amyloid protein. Fluorescence of amyloid masses in red pulp. 270×.

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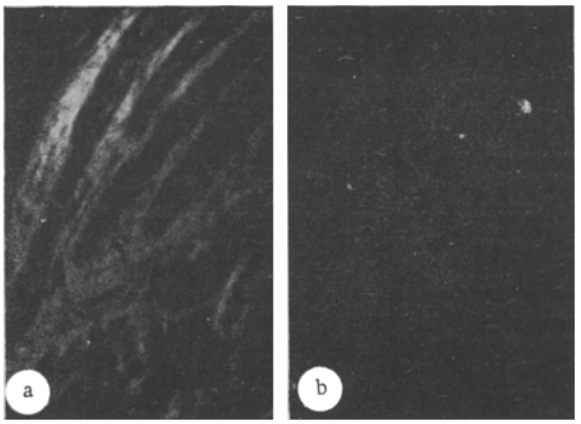

Fig. 2. Serial cryostat sections of lung of 19-day mouse embryo treated by indirect Coon's method. A) Section incubated with antiserum against connective tissue. Fluorescence of connective-tissue cells in wall of bronchus (top) and blood vessel (bottom left) and in lung parenchyma. B) Section incubated with antibodies against mouse amyloid protein. Complete absence of specific fluorescence; 100×.

However, it must be remembered that protein SAA consists of a low-molecular-weight peptide [7] identical in its antigenic properties to protein AA, and a so-called transport part, with no analog in protein AA. As yet this carrier protein has not been identified with any other known body protein. It could be this protein which Linder et al., [6] found in connective tissue. The present writer cannot accept the suggestion that immunofluorescence in sections of embryonic organs is due to the specific amyloid moiety in protein SAA.

In the investigation described below the appearance of specific amyloid protein in the organs of mice with casein amyloidosis (the analog of secondary human amyloidosis) was studied by means of the indirect Coon's test. In the first stage slices of organs (spleen, liver, kidney, heart) were incubated with pure antibodies against protein AA. As a first step rabbit antiserum was absorbed with normal plasma and with homogenate of normal mouse organs, hydrolyzed with 0.1 N NaOH (to remove contaminating antibodies against connective-tissue components), after which pure antibodies were isolated from it by means of a solid adsorbent [1]. In the second stage of the investigation slices were incubated with pure fluorescent antibodies against rabbit IgG. Mice were killed very early during the experiment — on the 2nd-3rd day (after one of two injections of casein). Normal mice (without amyloidosis) and mice with well marked experimental amyloidosis served as the controls. Serial sections of the organs in parallel tests were treated with antiserum against connective tissue. The results show that there is no amyloid protein in the connective-tissue structures of normal organs. In the experimental animals amyloid protein, detectable by the immunomorphological method, appears for the first time on the 2nd day of the experiment (in the intercellular spaces of the marginal zone of the spleen and in the blood filling the lumen of the sinuses of the spleen and liver). These intercellular "microfoci" of amyloid protein were not connected topographically with connective-tissue fibers, and their fluorescence was only a little weaker (like the fluorescence of the blood) than that of mature amyloid formed later (Fig. 1). Fibrous structures of amyloid protein, such as we observed, were not formed. Investigations of the organs of embryos (on the 10th and 15th days of gestation) and of newborn mice, conducted in the same way, gave negative results (Fig. 2A, B). The results thus demonstrate the absence of the specific antigen of amyloid in normal tissues, at least in amounts recordable by the immunofluorescence method.

It must be emphasized that antibodies against amyloid protein AA also specifically detect protein SAA [7], whereas antibodies against SAA do not always react with protein AA [3]. There are two possible explanations of this fact: either antibodies against the stronger contaminating antigens (for example, soluble connective-tissue proteins) predominate in the antiserum obtained against SAA, or antibodies reacting with the nonamyloid moiety of the mol-

ecule are formed in SAA. In the work of Linder et al. [6] it is impossible to rule out the presence of antibodies against connective-tissue components, for these workers used whole patient's serum as the source of antigen SAA (the SAA content was judged by the precipitation test in agar with antiserum against this protein). It was most likely fluorescence of connective-tissue proteins that these workers observed in the sections through the embryonic organs.

Despite the fact that the present results were obtained in experiments on mice, they ca be perfectly and properly compared with data on Linder et al. [6], for experimental amyloidosis is an analog of secondary amyloidosis, and human and mouse SAA proteins are similar in their properties. It seems unlikely that they are synthesized by different cells.

LITERATURE CITED

1. R. Axen, J. Porath, and S. Ernback, Nature (London), 214, 1302 (1967).
2. R. F. Anders, T. E. Michaelson, J. B. Natvig, et al., Scand. J. Immunol., 4, 397 (1975)
3. E. C. Franklin, J. Exp. Med., 144, 1679 (1976).
4. G. Husby, K. Sletten, T. E. Michaelson, et al., Scand. J. Immunol., 2, 395 (1973).
5. G. Husby, J. B. Natvig, K. Nordstoga, et al., Scand. J. Immunol., 4, 811 (1975).
6. E. Linder, R. F. Anders, and J. B. Natvig, J. Exp. Med., 144, 1336 (1976).
7. R. P. Linke, J. D. Sipe, P. S. Pollok, et al., Proc. Nat. Acad. Sci. USA, 72, 1473 (1975).
8. C. Rosenthal and E. C. Franklin, J. Clin. Invest., 55, 746 (1975).

IMMUNOCYTOCHEMICAL DETECTION OF THE NONFIBRILLARY STAGE OF AMYLOID FORMATION IN THE MOUSE MYOCARDIUM

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An indirect electron-microscopic immunoperoxidase method, with pure rabbit antibodies against fibrillary protein of mouse amyloid, was used to study casein amyloidosis in mice. In the early stages of development of amyloidosis deposits of finely granular material appeared in the mouse myocardium. These deposits contained an antigen similar to the fibrillary antigen of amyloid, but were without its fibrillary ultrastructure. The results of this investigation point to the existence of an early nonfibrillary stage of amyloid formation.

KEY WORDS: *amyloid; myocardium; immunocytochemistry.*

The obtaining of an antiserum against the specific antigen of amyloid fibrils — protein AA, the main component of the amyloid substance in secondary amyloidosis in man and also in experimental amyloidosis in several animals, including mice — has provided fresh opportunitie for the study of amyloid formation [1, 8, 10]. Immunologic investigations have shown that the serum of patients with secondary amyloidosis [9, 12], and also of monkeys, mink, rabbits, and mice with experimental amyloidosis [4, 11] contains a high concentration of a protein SAA corresponding antigenically to protein AA of amyloid fibrils. Protein SAA is probably a circulating precursor of fibrillary protein AA [9]. However, the possibility cannot be ruled out that both proteins are formed from a common precursor [4]. By the use of specific antiserum for the immunohistochemical study of the initial stages of experimental amyloidosis in mice it was discovered that the formation of amyloid with the typical staining properties is preceded by deposition of an antigen similar to fibrillary antigen in several organs [2, 10].

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