Virchows Archiv A Pathol Anat (1989) 414:511–514

Virchows Archiv A
Pathological Anatomy and Histopathology

Amyloid enhancing factor (AEF) in the aging mouse

Tadaaki Yokota, Tokuhiro Ishihara, Hiroo Kawano, Mutsuo Takahashi, Yoshihiro Fujinaga, and Fumiya Uchino

First Department of Pathology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755, Japan

Summary. Amyloid enhancing factor (AEF) activity was tested in spleen homogenates from 52, 28, and 9-week-old untreated mice. Strong AEF activity was present in the supernatant of spleen homogenate from the 52-week-old mice. Only weak activity was observed in the supernatant from the 28-week-old mice, and none was seen in that from the 9-week-old mice. Crude spleen homogenates from the 52-week-old mice were subjected to Sephacryl S-300 gel filtration. AEF activity existed in the first gel filtration peak. These findings suggest that AEF increases in the spleens of aged mice and that AEF may be a substance of high molecular weight.

Key words: Amyloid – Amyloidosis – Amyloid enhancing factor – Aging

Introduction

Janigan and Druet (1968) reported that intraperitoneal injection of spleen cells and homogenates from azocasein-sensitized mice were able to induce significant amyloidosis in X-ray-irradiated recipients after a much shorter sequence of injections with azocasein. Since then, many attempts have been made to identify a factor, amyloid enhancing factor (AEF), that is capable of accelerating the deposition of amyloid fibrils. In experimental amyloidosis, AEF appears in amyloidotic organs from mice approximately 48 h before and during amyloid induction (Axelrad and Kisilevsky 1980). AEF has the ability to reduce the induction time of amyloid deposition in recipients and although AEF is present in organs from normal animals, its activity is very weak (Axelrad et al. 1982; Baltz et al. 1986). This work reports on AEF activity in murine spleen at three different ages.

Offprint requests to: Tadaaki Yokota

Materials and methods

AKR/J mice were obtained from Shizuoka Experimental Laboratory Animal Center (Japan) and were maintained in our laboratory. Fifteen mice of each group (9-week-old, 28-week-old, and 52-week-old) were sacrificed by ether anesthesia. Small pieces of the spleens, livers, kidneys and hearts were removed, fixed in 10% buffered formalin, and embedded in paraffin. The paraffin sections were stained with alkaline Congo red and tested by polarized light. Amyloid deposits were not detected histologically in any sections of the spleen, liver or heart from the 9-week-old, 28-week-old, and 52-week-old mice but were found in some of the sections of the kidneys from 52-week-old mice. The animals with amyloid deposits in the kidney were excluded from the extract process for AEF. The remaining portions of the spleens were stored at −80° C until use.

AEF was extracted from the individual organs according to the method of Axelrad et al. (1982). Briefly, the organs were homogenized with 8 ml 4 M glycerol, 10 mM Tris-HCl buffer, pH 7.6, per gram of tissue. The homogenates were shaken for 1 h at 4° C, then centrifuged at 25000 *g* for 1 h at 4° C. AEF activity in the supernatants was tested as follows; 0.5 ml of the individual supernatants were administered intraperitoneally to five 9-week-old recipient mice immediately before the subcutaneous injection of 0.5 ml of casein solution. Each mouse subsequently received two daily subcutaneous injections of casein and was killed 24 h after the last casein injection. Amyloid deposits in the spleen were examined in tissue sections stained with Congo red. Control animals were injected intraperitoneally with the supernatants alone, or were given three daily injections of casein only.

The supernatants from the spleens of 52-week-old mice were subjected to Sephacryl S-300 (Pharmacia Fine Chemicals, Uppsala, Sweden) gel-filtration in 4 M glycerol, 10 mM Tris-HCl buffer, pH 7.6. The individual fractions were extensively dialized (cut-off:3,500) against 10 ml phosphate-buffered saline, pH 7.2, in a dialysis tube (Spectra/Por3, Spectrum Medical Industries, Inc., USA), and were kept at −80° C. The protein concentrations of the fractions were measured by the technique of Lowry et al. (1951). After concentration by an ultrafiltration apparatus (Morukatto II-GC, SJGC 013 24; Nihon Millipore Kogyo, K.K., Japan), five mice were injected intraperitoneally with the concentrates with 200 μg protein, followed by casein injections as described above.

Table 1. Incidence of amyloidosis in recipients, receiving organ extracts and casein

Mouse Age	Extracted organs			
	Spleen	Liver	Kidney	Heart
9 weeks	0/5	0/5	0/5	0/5
28 weeks	1/5	0/5	0/5	0/5
52 weeks	5/5	0/5	0/5	0/5

Spleen, liver, kidney and heart extracts from each age group were administered to five 9-week-old mice and followed by injections of casein for 3 consecutive days

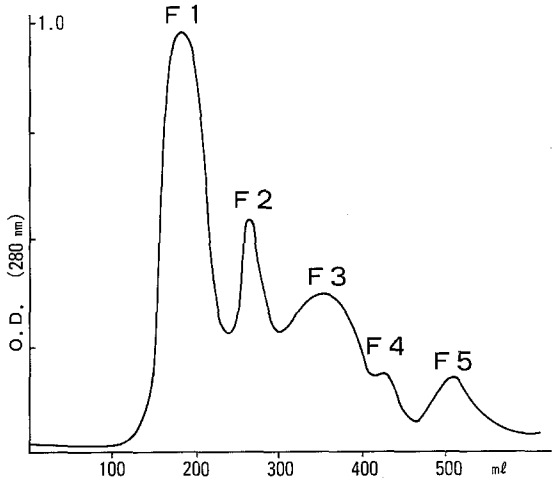

Fig. 1. Gel chromatography of the spleen homogenate from the 52-week-old mice

Table 2. Incidence of amyloidosis in recipients treated with fractions from gel filtration

	A	B	C
F1	5/5	0/5	0/5
F2	1/5	0/5	0/5
F3	0/5	0/5	0/5
F4	0/5	0/5	0/5
F5	0/5	0/5	0/5

(*A*) The fractions were administrated and followed by injections of casein for 3 consecutive days
(*B*) The fractions only were administrated
(*C*) Casein only was injected

Using an avidin-biotin-peroxidase complex method (Hsu et al. 1981), paraffin sections from amyloid-positive recipients were stained with the monoclonal antibody KM-268, which reacted with amyloid A (AA) protein as previously described (Yokota et al. 1987).

Results

Injection of the supernatant from the spleens of 52-week-old mice induced ring-like amyloid deposits in the splenic marginal zone of all recipients, and that from the 28-week-old mice in only one out of five recipients. Injection of the supernatant from the spleens of 9-week-old mice had no AEF activity (Table 1). Furthermore, AEF activity was not present in the supernatants of the livers, hearts and kidneys of any of the animals (Table 1). No amyloid deposit was detected in the spleens from control animals treated with the supernatants of the organ homogenates alone or only the casein injections.

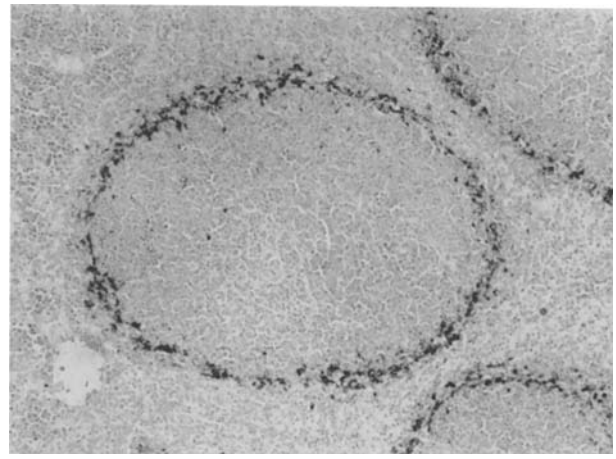

Fig. 2. The spleen of mice given AEF followed by injections of casein. Amyloid deposits in the marginal zone react positively with the monoclonal antibody against a synthesized short peptide corresponding to AA protein

An elution profile of the gel chromatography is shown in Fig. 1. Five fractions were obtained by gel filtration. When the individual fractions were given intraperitoneally, strong AEF activity was demonstrated in the first peak (F1) and weak activity in the second (F2), as shown in Table 2. No AEF activity was observed in the remaining three fractions. In the fourth and fifth fractions, a small amount of protein was detected.

Immunohistochemically, amyloid deposits reacted positively with the monoclonal antibody on the paraffin sections from all of the amyloid-laden recipients (Fig. 2).

Discussion

West and Murphy (1965) analyzed the distribution of amyloid in various organs of A/SN mice at different ages. According to their report, in mice over 6-months of age there was a gradual increase in the number of organs involved by amyloid deposits, as well as in the amount of the deposits. In fact, we observed that spontaneous murine amy-

loidosis of the AA type increased with age (unpublished data). Spontaneous age-associated amyloidosis occurred in SJL/J (Scheinberg et al. 1976) and SAM (Matsumura et al. 1982) strains, but their amyloid proteins were different in AA protein.

It is now accepted that AA fibril is formed in involved tissues by degradation of its precursor protein, serum amyloid A protein (SAA). SAA is an acute phase reactant protein synthesized mainly by hepatocytes (Takahashi et al. 1985, 1986). Although the nature of AEF has not been clarified, its appearance may be stimulated by a condition that facilitates the transformation of SAA to AA. AEF activity is lacking in the A/J mouse strain, which is highly resistant to the development of experimental amyloidosis (Gervais et al. 1988). The experimental murine amyloidosis induced by administration of AEF, followed by injections of casein, is of the AA type. AEF has no immunological relationship to AA, SAA, amyloid P component (AP), serum AP, or the other normal serum components (Keizman et al. 1972; Axelrad et al. 1982). Baltz et al. (1986) reported that AEF activity existed in the spleen, liver, kidney, and heart of the normal mouse. Axelrad et al. (1982) reported that 1×10^8 spleen cells from normal mouse reduced the amyloid induction time in recipients to approximately 4 days, and an equivalent result was obtained with 2×10^4 cells from the amyloidotic mouse. These authors therefore, suggested that AEF in the amyloidotic animals is increased in amount by around 5000 times over the level in normal mouse. In the present study, administration of homogenates of the livers, hearts and kidneys from all of the mice and of the spleens from young mice (9 weeks) did not have the ability to accelerate amyloid deposition in the recipients. Weak AEF activity was detected in the spleen homogenates from the 28-week-old mice but strong activity was found in the spleens from old mice (52 weeks) – clearly AEF activity increases with advancing age.

Many investigators have reported on the characteristics of AEF extracted from the organs of several animals. A variety of different results have been demonstrated with regard to the materials and molecular weights related with AEF (Janigan 1969; Hardt and Hellung-Larsen 1972; Keizman et al. 1972; Axelrad et al. 1982; Baltz et al. 1986; Hol et al. 1985; Niewold et al. 1987; Varga et al. 1986). Hol et al. (1985) reported that Sepharose 4B gel filtration of the AEF-containing spleen extract revealed four major fractions corresponding to molecular weights of about 12000, 59000, 280000 and above 10^7 (Vo), and AEF activity was present in each fraction. They suggested that AEF was probably a low molecular weight substance that aggregated or associated with other components present in the extract. In our study, AEF activity was predominantly limited to the first peak (Vo fraction), of which the molecular weight was more than 100 million daltons by Sephacryl S-300 gel filtration, and was weakly present in the second peak. AEF activity was not detected in the fraction of the lower molecular weights examined.

It is reported that aging mice are more susceptible to experimental amyloidosis than younger mice (Toivanen et al. 1972), and the occurrence of spontaneous murine amyloidosis increases with advancing age (West et al. 1965). Although the reason for the increase of AEF activity in aging mice is not clarified by the present study, high AEF activity was confirmed in the spleen of the 52-week-old mice. The high AEF activity explains the susceptibility to experimental amyloidosis and the increase in spontaneous amyloidosis in aging mice very well. Furthermore, it indicates that when aging mice are exposed to particular stimuli (acute inflammation etc.) sharing an ability to raise SAA, amyloidosis will occur more easily and highly in aging mice than in young mice.

Acknowledgements. This study was supported by a Grant-in-Aid for Scientific Research (62570164) from the Ministry of Education, Science and Culture, and a research grant from the Primary Amyloidosis Research Committe, the Ministry of Health and Welfare.

References

Axelrad MA, Kisilevsky R (1980) Biological characterizations of amyloid enhancing factor. In: Glenner GG, Costa PP, Freitas AF (eds) Amyloid and amyloidosis. Proceedings of the third international symposium on amyloidosis. Amsterdam-Oxford-Princeton, Excerpta Medica, pp 527–533

Axelrad MA, Kisilevsky R, Willmer J, Chen SJ, Skinner M (1982) Further characterization of amyloid-enhancing factor. Lab Invest 47:139–146

Baltz ML, Caspi D, Hind CRK, Feinstein A, Pepys MB (1986) Isolation and characterization of amyloid enhancing factor (AEF). In: Glenner GG, Osserman EF, Benditt EP, Calkins E, Cohen AS, Zucker-Franklin D (eds) Amyloidosis. New York, Plenum Press, pp 115–121

Gervais F, Hebert L, Skamene E (1988) Amyloid-enhancing factor: Production and response in amyloidosis-susceptible and -resistant mouse strains. J Leukocyte Biol 43:311–316

Hardt F, Hellung-Larsen P (1972) Transfer amyloidosis. Studies on the nature of the amyloid inducing factor in a murine transfer system. Clin Exp Immunol 10:487–492

Hol PR, van Andel ACJ, van Ederen AM, Draayer J, Gruys E (1985) Amyloid enhancing factor in hamster. Br J Exp Pathol 66:689–697

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Janigan DT (1969) Pathogenetic mechanisms in protein-induced amyloidosis. Am J Pathol 55:379–393

Janigan DT, Druet RL (1968) Experimental murine amyloidosis in X-irradiated recipients of spleen homogenates or serum from sensitized donors. Am J Pathol 52:381–390

Keizman I, Rimon A, Sohar E, Gafni J (1972) Amyloid accelerating factor. Purification of a substance from human amyloidotic spleen that accelerates the formation of casein-induced murine amyloid. Acta Pathol Microbiol Scand (Section A) 80, Suppl 233:172–177

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Matsumura A, Higuchi K, Shimizu K, Hosokawa M, Hashimoto K, Yasuhira K, Takeda T (1982) A novel amyloid fibril protein isolated from senescence-accelerated mice. Lab Invest 47:270–275

Niewold ThA, Hol PR, van Andel ACJ, Lutz ETG, Gruys E (1987) Enhancement of amyloid induction by amyloid fibril fragments in hamster. Lab Invest 56:544–549

Scheinberg MA, Cathcart ES, Eastcott JW, Skinner M, Benson M, Shirahama T, Bennett M (1976) The SJL/J mouse: A new model for spontaneous age-associated amyloidosis. I. Morphologic and immunochemical aspects. Lab Invest 35:47–54

Takahashi M, Yokota T, Ishihara T, Uchino F (1986) Immunoelectron microscopic study of liver in experimental murine amyloidosis using anti-mouse AA antiserum. In: Glenner GG, Osserman EF, Benditt EP, Calkins E, Cohen AS, Zucker-Franklin D (eds), Amyloidosis. New York, Plenum Press, pp 193–199

Takahashi M, Yokota T, Yamashita Y, Ishihara T, Uchino F (1985) Ultrastructural evidence for the synthesis of serum amyloid A protein by murine hepatocytes. Lab Invest 52:220–223

Toivanen A, Toivanen P, Yunis EJ, Rodey G, Good RA (1972) Inconsistency of experimental amyloidosis in mice. Int Arch Allergy 43:683–690

Varga J, Flinn MSM, Shirahama T, Rodgers OG, Cohen AS (1986) The induction of accelerated murine amyloid with human splenic extract. Probable role of amyloid enhancing factor. Virchows Arch (B) 51:177–185

West WT, Murphy ED (1965) Sequence of deposition of amyloid in strain A mice and relationship to renal disease. J Natl Cancer Inst 35:167–174

Yokota T, Ishihara T, Takahashi M, Yamashita Y, Kawano H, Fujinaga Y, Uchino F, Hanai N, Yoshida H, Honda S, Sato M, Matsumoto N (1987) Monoclonal antibodies against synthesized short peptides corresponding to human AA amyloid protein. Acta Pathol Jpn 37:1135–1142

Received October 11, 1988 / Accepted February 1, 1989