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Primary cardiovascular amyloidosis with benign monoclonal gammopathy

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Summary. A 54-year-old woman is reported whose primary amyloidosis was diagnosed at autopsy. Amyloid deposits were found in the myocardium, the striated muscles, the smooth muscle layers of the gut and the wall of the blood vessels. The deposits showed resistance to induced proteolysis. A large number of mature plasma cells was demonstrated in the bone marrow, and immunocytochemical studies revealed a considerable increase in the proportion of plasma cells which were positive for kappa light chains of immunoglobulins, indicating a monoclonal gammopathy. This view was strongly supported by the unexpected finding that amyloid deposits were positive for kappa light chains. The relationship between the kappa positive reaction of amyloid and its resistance to induced proteolysis are discussed.

Key words: Primary amyloidosis – Monoclonal gammopathy – Kappa light chain positivity

Although amyloid was described by Virchow more than a hundred years ago, the pathogenesis of its formation and deposition has not been clearly defined. Recent investigations employing a wide variety of techniques (Cohen 1967; Pras and Gafni 1978; Terry et al. 1973) have differentiated various types of amyloid on the basis of the chemical nature of their fibrillary protein and enabled the introduction of a new terminology (AA-, and AL-amyloidosis; Glenner 1980). On the basis of this it is possible to identify the fibrillary proteins in different types of cardiac amyloidosis (Störkel et al. 1983).

In this paper we describe a case of primary amyloidosis with K light chain positive monoclonal bone marrow plasma cell population with a very

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low number of heavy chain positive cells. Furthermore, it was found that the amyloid deposits in this case were positive for K light chain only.

Case history

G.J., a 54-year-old woman was hospitalized for cardiac insufficiency several times in the last three years of life. Organic heart disease could not be demonstrated. Some weeks prior to death she was admitted with considerable loss of weight and therapy-resistant decompensation and after a few days she died of pulmonary oedema. No coronary sclerosis, hypoxic cardiac muscle damage, or valvular lesions was found at the autopsy. The heart was 640 g in weight. Considerable hypertrophy combined with dilatation of both ventricles and auricles was seen. Macroglossia was present. The myocardium was highly transparent and together with slices of the tongue and the skeletal muscles it turned black in Lugol's iodine solution.

Material and methods

Congo red staining following performic acid pretreatment and potassium permanganate-induced proteolysis, was performed on formol-fixed paraffin sections combined with mounting in gum arabic in order to differentiate between primary and secondary amyloidosis, (Romhányi 1971, 1972 and 1979). For polarization optical studies a Leitz Ortholux II polarizations microscope was used.

For electron microscopy small parts of the paraffin blocks of the heart and the tongue were rehydrated and then embedded in Durcupan (Fluka, Darmstadt, FRG). The ultrathin sections were examined under a JEOL 100 C electron microscope.

Bone marrow sections were stained with Giemsa solution following methylation (1% HCl in abs. methanol at 56° C for 20 min). 1,000 cells/section were counted and the ratio of plasma cells to other cells was calculated.

Bone marrow plasma cells were stained for heavy (α, γ, μ) and light chains (κ, λ) of immunoglobulins by the direct immunoperoxidase reaction and the ratio of kappa- and lambda-positive cells was determined. The antisera were purchased from Dacopatts (Copenhagen).

On the sections of the myocardium and tongue the following procedures were performed: (a) reactions for kappa and lambda chains, (b) preincubation of sections with unlabeled anti-kappa-serum (dilution 1:200, 1 h) and the direct immunoperoxidase reaction for kappa and lambda chains, (c) preincubation of the section with unlabeled antilambda serum (dilution 1:200, 1 h) and direct immunoperoxidase reaction for kappa and lambda chains. (d) Digestion with 0.2% trypsin (REANAL) in PBS, at pH 7.4, 37° C, for 20 min, then direct immunoperoxidase reaction for light chains. (e) overnight incubation with 0.1% horse-radish peroxidase (Reanal, RZ 0.6) in 0.2 M TRIS buffer, pH 7.4 and with 0.05% 3,3′-diaminobenzidine-HCl (DAB, Serva, Heidelberg, FRG) for 10 min. (f) Steps (a–e) were performed also on normal control myocardial tissue.

Results

Congo red induced birefringence revealed massive amyloid deposits in the heart in both ventricles and the atria, in the tongue, skeletal muscles, the smooth muscle layer of the gut and in blood vessel walls (Fig. 1a–d). Amyloid was seen only in vessels in the kidneys, liver or spleen. The birefringence of Congo red stained amyloid exhibited resistance to performic acid pretreatment and to induced proteolysis, i.e., it proved to be of the primary type. Electron microscopy demonstrated an irregular, non-branching, fibrillary material at the site of the Congo red positive deposits (Fig. 2a, b).

In the bone marrow a considerable increase in plasma cells was seen (17%) while myelo- and erythropoiesis appeared normal. The plasma cells

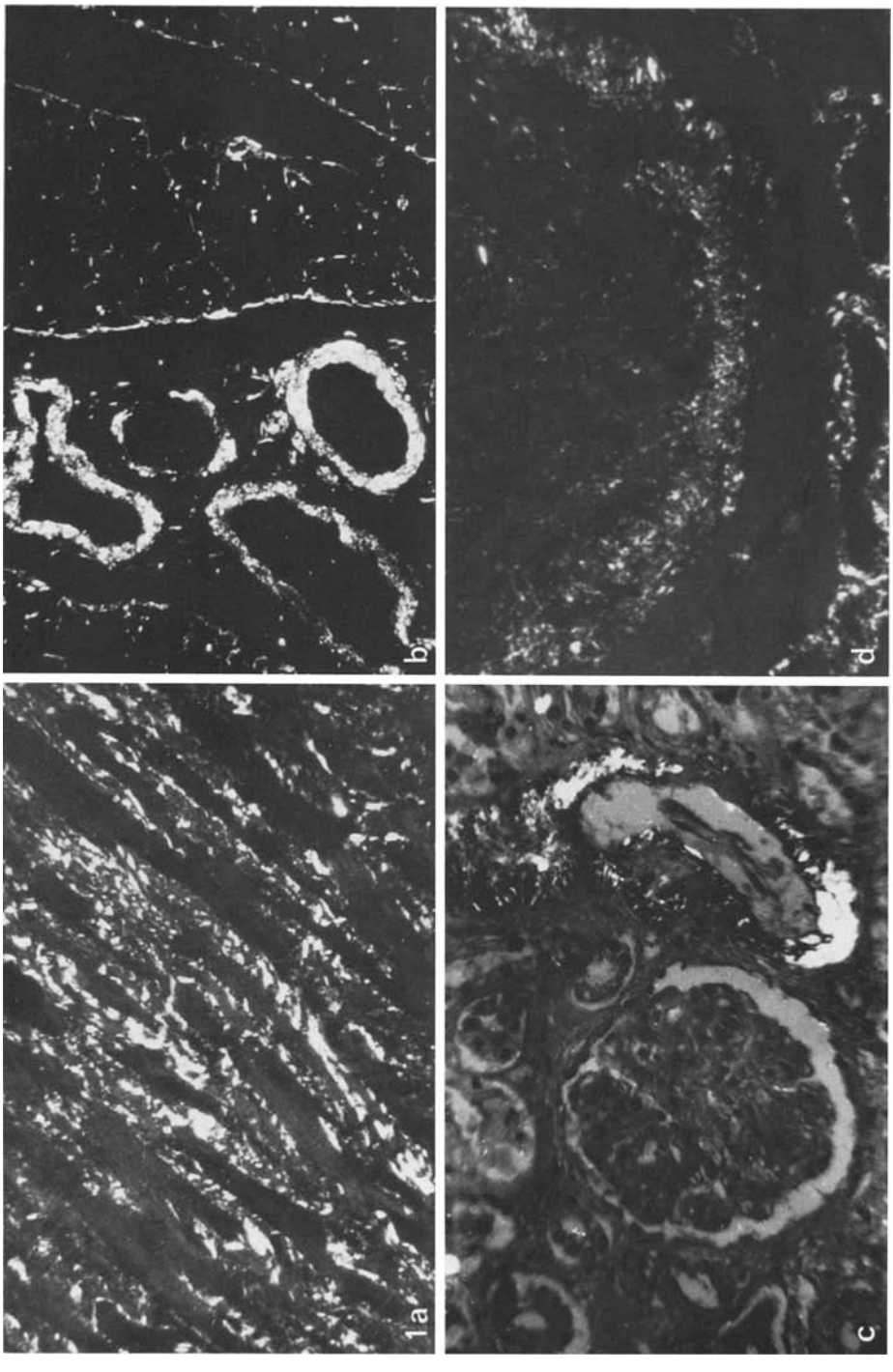

Fig. 1. Considerable amount of cardiac amyloid showing intensive birefringence (**a**, 200:1). Amyloid deposits in striated muscle cells of tongue and in wall of vessels (**b**, 64:1). Amyloid deposits are present in renal vessels, but not in the glomerulus (**c**, 320:1). Section from small bowel showing amyloid in the smooth muscle layer of the mucosa and in the vessels of the submucosa (**d**, 125:1). Congo red, × pol

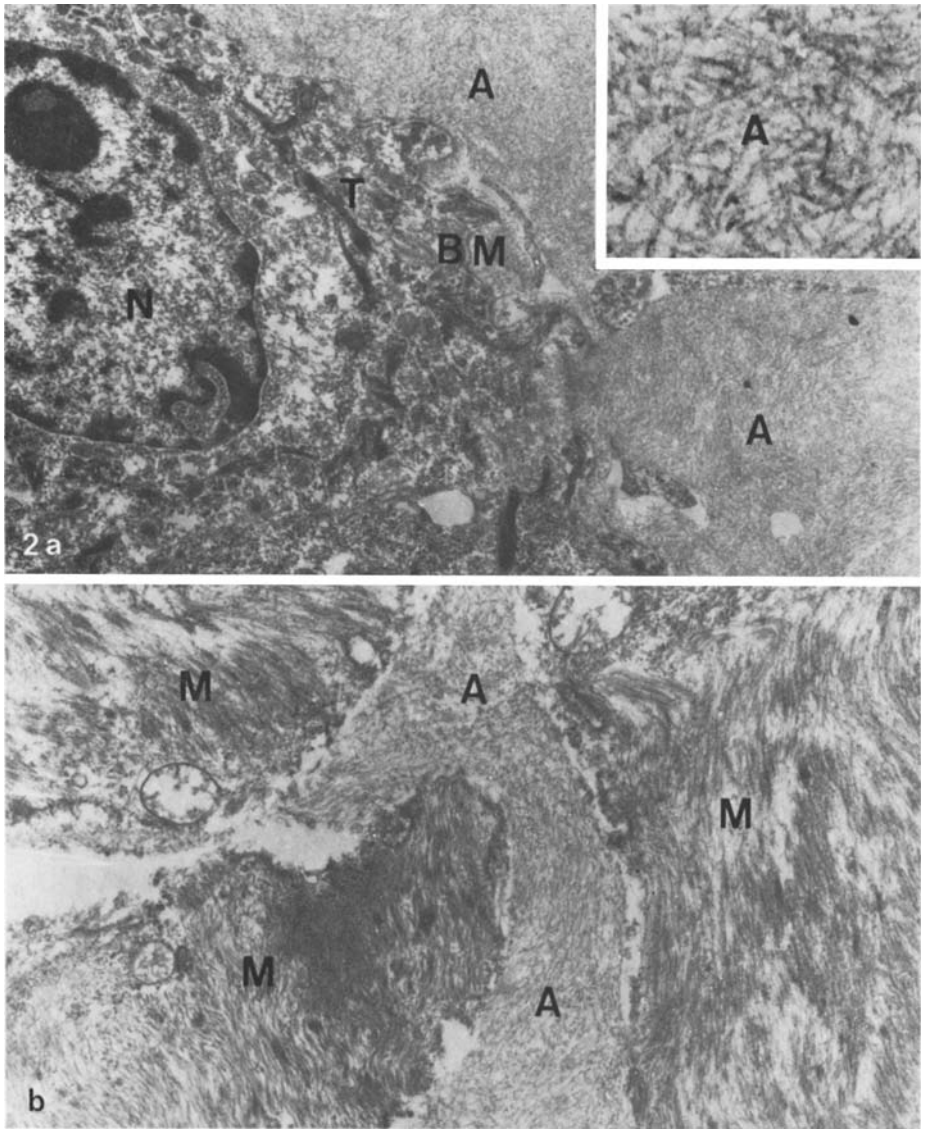

Fig. 2. a Part of an epithelial cell of the tongue embedded in a large amount of fibrillary material (*inset:* 45,000:1), 6,600:1. **b** Fibrillary amyloid deposits between myocardial cells (8,300:1). *N*, nucleus; *T*, tonofilament; *BM*, basal membrane, *A* = amyloid, *M* = myofilament

were typical mature cells. Table 1 summarises the distribution of plasma cells positive for different light and heavy chains of immunoglobulins. There was a considerable increase in the ratio of kappa/lambda chain positive cells (Fig. 3a, b), whereas the ratio of heavy/light chain positive plasma cells diminished.

In the amyloid deposits a relatively strong kappa light chain positivity was found, the reaction for lambda chain proved to be negative. In order

Table 1. The percentage distribution of bone marrow plasma cells positive for light and heavy chains

Light chain positive cells	16.5%
kappa	16.0%
lambda	0.5%
Heavy chain positive cells	0.6%
gamma	0.2%
alpha	0.2%
mu	0.1%
kappa/lambda	32
heavy chain/light chain	0.03

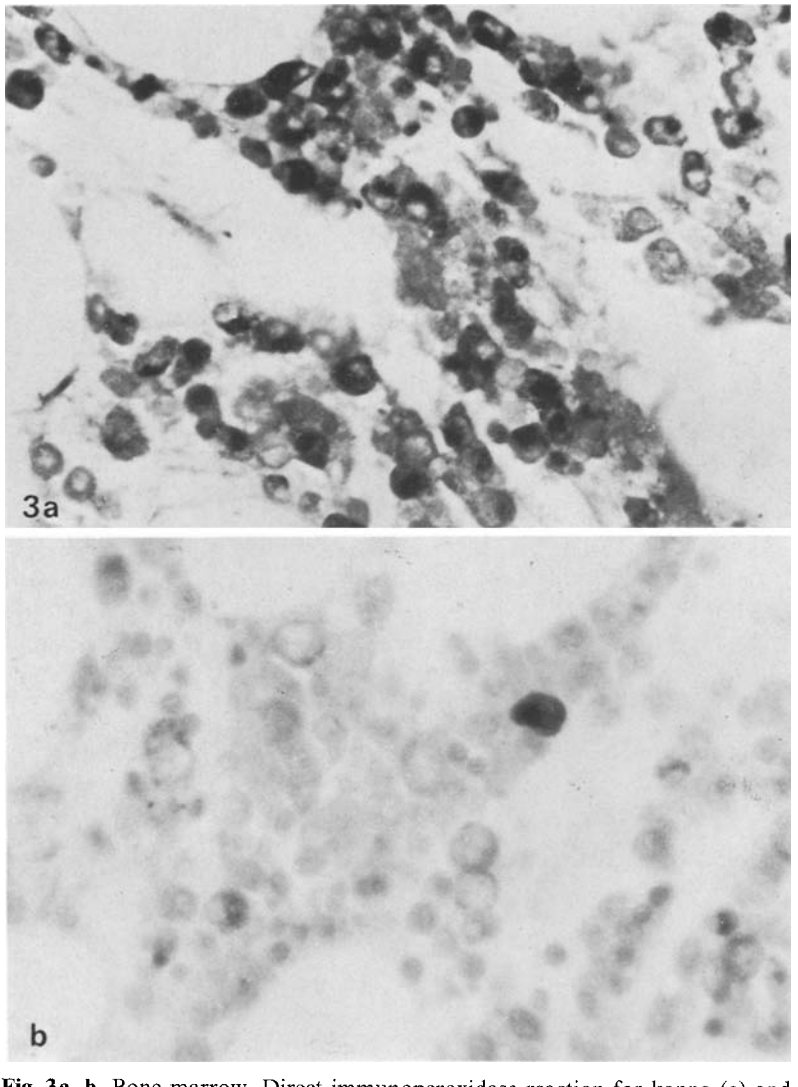

Fig. 3a, b. Bone marrow. Direct immunoperoxidase reaction for kappa (**a**) and lambda light chains (**b**), 450:1

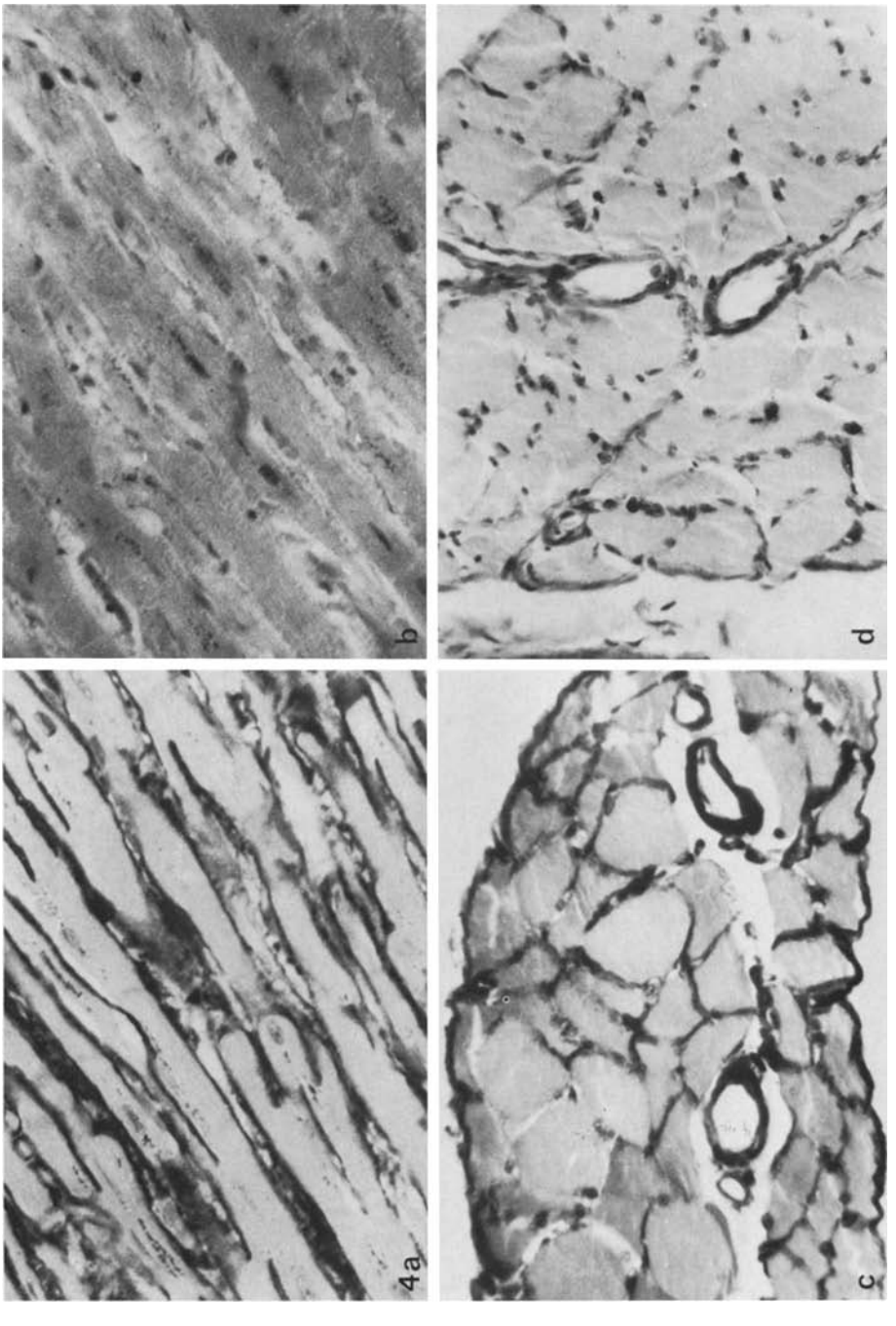

Fig. 4a, b Myocardium. Direct immunoperoxidase reaction for kappa (**a**) and lambda (**b**) light chains (320:1). **c, d** Tongue. Direct immunoperoxidase reaction for kappa (**c**) and lambda (**d**) light chains. 320:1

to avoid the possibility of non-specific staining, the sections were treated with unmarked sera prior to the direct immunoperoxidase reaction. We observed a strong positive reaction for kappa chain in sections pretreated with unmarked antilambda serum, but no staining in those pretreated with unmarked antikappa serum. The positivity for kappa chain was somewhat more intense in sections predigested with trypsin (Fig. 4a–d). In sections treated only with horseradish peroxidase and DAB the deposits were invariably negative.

Discussion

In systemic amyloidosis at least two chemical types of amyloid are known, depending on whether the major component of the amyloid is related to serum amyloid A protein (AA-amyloidosis, reactive amyloidosis), or to immunoglobulin light chains (AL-amyloidosis, immunocytic amyloidosis, previously called primary amyloidosis) (Glenner 1980). In the background of immunocytic amyloidosis many authors found some type of serum paraprotein (Cathcart et al. 1972; Pruzansky et al. 1976; Kyle and Bayrd 1975), which consisted of free light chains of immunoglobulins (Isobe and Osserman 1974).

In the normal bone marrow, plasma cells constitute only 2–5% of the total cell population, the distribution of positive cells is 7–12% for IgM, is 51–54% for IgG and is 35–37% for IgA, the ratio of heavy/light chain positive cells 1.1 and of kappa/lambda chain positive cells 1.3–2.2 (Thielemans et al. 1982; Hijmans et al 1971; Crocker and Curran 1981). In our case we observed 17% plasma cells in the bone marrow, the ratio of kappa/ lambda being 32:1, and that of the heavy/light chains 0.03. It is known (Chamotte 1977) that in reactive plasmocytosis e.g., in chronic inflammation the kappa/lambda ratio is fairly unaltered, however, in the cases of monoclonal plasmocytosis the ratio diminishes or increases depending on the type of light chains of proliferating cells. It was also found that in multiple myeloma the ratio of kappa/lambda is either less than 0.1 or more than 10.0 (Hijmans et al. 1971). In the present case serum and urine immunoelectrophoresis were not performed. Nevertheless, the bone marrow immunocytochemical data are indicative of a monoclonal plasma cell population. Since none of the clinicopathological findings were consistent with plasma cell myeloma, we assume a kappa positive benign monoclonal gammopathy in the background of the patients amyloidosis.

The strong positivity of the amyloid deposits for kappa light chain was an unexpected finding. On the basis of control experiments we were able to exclude the possibility of an non-specific staining reaction. Amyloid of the AL-type was found (Shirahama et al. 1981) to show a sporadic weak reaction to antihuman light chain sera. Jerath et al. (1980) reported 9 among 10 cases of renal amyloidosis, in which amyloid deposits were positive for light chains by immunofluorescence (six polyclonal, three monoclonal). Furthermore, non-fibrillary visceral deposits of free light chains of immunoglob-

ulins can also be demonstrated in some cases of monoclonal gammopathy or plasma cell myeloma (Preud'homme et al. 1980; Gallo et al. 1980; Seymour et al. 1980).

The different types of amyloid are divided into two groups on the basis of their stability of birefringence (Romhányi 1972). Wright et al. (1977) were able to discover a specific relationship between the different histochemical types of amyloid (sensitive or resistant to induced proteolysis) and the chemical nature of amyloid fibrillary protein.

In the present case the birefringence of Congo red positive amyloid deposits proved to be resistant to performic acid pretreatment and also to induced proteolysis. Furthermore, according to our immunohistochemical observations, these deposits contained kappa light chains or light chain fragments of immunoglobulin. These data seem to support the view that the deposits in immunocytic amyloidosis show the property of resistance to induced amyloidosis.

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