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Immunohistochemical Localization of Apolipoprotein E in Renal Amyloidosis

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ABSTRACT

To clarify the role of apolipoprotein E (apo E) in the formation of amyloid deposits, we examined specimens from 11 patients with renal amyloidosis who underwent renal biopsy by an immunohistochemical method using a monoclonal antibody (murine IgG_1). Apo E was distributed in the amyloid deposits of all patients in a pattern similar to that obtained with Congo red staining. Strong positive staining for apo E was found on the amyloid deposits in the glomeruli. These results suggest that apo E is a common constituent of amyloid fibrils and that it may be a useful marker for immunohistochemical studies of systemic amyloidosis including renal amyloidosis.

Key words: *Apolipoprotein E, Pathological molecular chaperones, Amyloid deposits*

Amyloidosis is a systemic disease which shows various symptoms because of the distribution of amyloid in various tissues[3,5,6]. Of the organs affected, the kidney is injured the most frequently which is why this condition is often called renal amyloidosis. Nephrotic syndrome is the most frequent condition clinically and the prognosis is generally poor because it ultimately leads to renal failure[4,9].

Recently, it was reported that amyloid deposits stained positively for apolipoprotein E (apo E) and that the staining pattern was similar to that obtained with Congo red staining in tissues from patients with systemic amyloidosis. It was also suggested that fragments of apo E are a common constituent of amyloid fibrils of diverse origin, and that it may be a new marker for immunohistochemical studies of systemic amyloidosis[16].

In this study, we describe 11 patients with renal amyloidosis who were diagnosed in our hospital and examined immunohistochemically using a monoclonal antibody (anti-apo E antibody).

MATERIALS AND METHODS

Tissue samples and classifications:

Eleven patients who underwent renal biopsy at our depertment between 1983 and 1994 were included in this study. These patients were initially diagnosed with renal amyloidosis by Congo red staining. There were 2 males and 9 females with a mean age of 56.5 ± 10.2 years. Of these 11 patients, the first symptoms were proteinuria and edema in 8 patients, edema in 2, and renal dysfunction in 1, while 9 patients out of the 11 showed nephrotic syndrome. Cases 1–6 were of primary amyloidosis and cases 7–11 were of secondary amyloidosis (Table 1). Chemical types of amyloid were immunohistochemically defined using IgG antibody against amyloid A (AA) component (Biomeda Corp., USA).

Immunohistochemistry:

Apo E was stained as follows: paraffin-embedded sections were deparaffinized, and antigen was unmasked by treatment with 0.01% trypsin diluted with 0.06 M phosphate-buffered saline (pH 7.2) for 30 minutes at room temperature and incubated with 3% hydrogen peroxide in methanol for 20 minutes at room temperature to block intrinsic peroxidase activity. They were incubated in a primary antibody (murine IgG_1, MON 5029, SANBIO BV, Holland) overnight at 4°C and stained immunohistochemically using an avidin-peroxidase/biotin staining kit (Vector Laboratories, USA). The immunoproducts were visualized by the application of diaminobenzidine, and the nuclei were stained with methyl green.

RESULTS

Clinical findings at biopsy:

Total daily urinary protein was 0.3–9.4 (mean ± SD; 5.5 ± 3.2) g/day, serum total protein was 4.7–7.4 (5.5 ± 0.7) g/dl, and serum albumin was 1.4–3.9 (2.5 ± 0.9) g/dl. Serum creatinine was 0.49–3.27 (1.43 ± 1.06) mg/dl, and creatinine clearance was 14.8–93.4 (51.9 ± 28.1) ml/min (Table 2). Based on these findings, almost all patients with amyloidosis had nephrotic syndrome

Table 1. Background characteristics of patients with renal amyloidosis

Case	Name	Age	Sex	First symptoms	Nephrotic syndrome	Diagnosis
1	S.M.	55	female	proteinuria, edema	+	primary
2	H.H.	59	female	edema	–	primary
3	T.Y.	67	female	proteinuria, edema	+	primary
4	M.N.	58	female	proteinuria, edema	+	primary
5	K.M.	49	male	proteinuria, edema	+	primary
6	K.I.	69	female	edema	+	primary
7	H.Y.	32	male	proteinuria, edema	+	secondary (RA)
8	C.O.	66	female	proteinuria, edema	+	secondary (RA)
9	T.O.	53	female	renal dysfunction	–	secondary (RA)
10	O.M.	59	female	proteinuria, edema	+	secondary (RA)
11	S.H.	55	female	proteinuria, edema	+	secondary (RA)

Table 2. Clinical findings at biopsy

Case	Name	TUP (g/day)	TP (g/dl)	Alb. (g/dl)	Cr (mg/dl)	Ccr (ml/min)
1	S.M.	5.8	5.2	2.7	3.27	15.2
2	H.H.	2.1	5.9	3.8	0.90	42.9
3	T.Y.	5.9	4.7	1.4	0.49	53.2
4	M.N.	9.4	5.4	2.5	0.70	66.9
5	K.M.	7.2	4.9	2.2	1.39	93.4
6	K.I.	9.0	4.9	2.1	0.66	50.6
7	H.Y.	7.4	5.1	2.9	3.20	84.5
8	C.O.	8.4	5.3	2.8	0.80	56.3
9	T.O.	0.3	7.4	3.9	1.10	14.8
10	O.M.	1.2	5.5	3.0	0.62	78.6
11	S.H.	3.6	6.3	2.6	2.54	15.5

TUP, total urinary protein; TP, total protein; alb., albumin; Cr, creatinine; Ccr, creatinine clearance.

and exhibited renal dysfunction.

Distribution of amyloid deposits by Congo red staining:

Congo red staining showed that amyloid deposits were distributed in the glomeruli of all patients, and 10 of 11 patients were positive in the vascular walls. Moreover, the epithelial cells in the tubules and interstitial area were positive in all patients except for cases 1 and 3.

Chemical types of amyloid using by AA peptides:

Five patients showed positive staining for AA peptides and 6 were negative. The five patients positive for AA peptides were all diagnosed with rheumatic arthritis (RA) associated with secondary amyloidosis.

Distribution of apo E:

Apo E was distributed in the amyloid deposits of all patients in a pattern similar to that obtained with Congo red staining. Strong positive staining for apo E was found on the amyloid deposits in the glomeruli (Fig. 1).

DISCUSSION

Apo E is a plasma protein of 299 amino acids with a relative molecular mass of 34,000, which is involved in cholesterol transort[14]. Apo E has a wide variety of functions including a structural role in a number of lipoprotein particles, as well as that of regulating their metabolism. Moreover, apo E is produced by most organs (the largest amount of apo E mRNA is found in the brain, second only to the liver), and its synthesis is increased following injury and is implicated in the

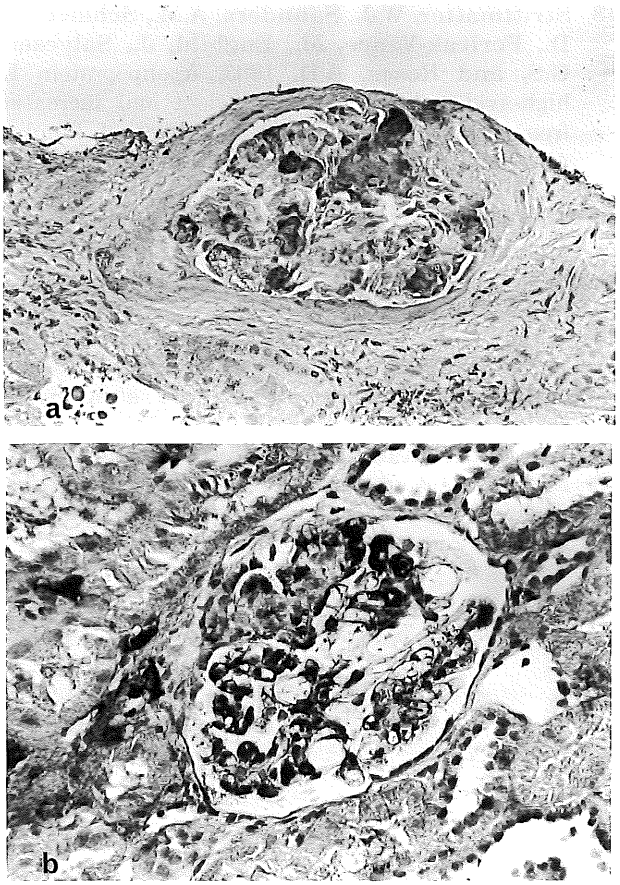

Fig. 1. The distribution of apolipoprotein E: strong staining for apo E was observed on amyloid deposits in the glomeruli. (×350)
a) Immunohistochemistry with Apo E antibody for a biopsy specimen with the patient in primary amyloidosis.
b) Immunohistochemistry with Apo E antibody for a biopsy specimen with the patient in secondary amyloidosis.

growth and repair of the nervous system during development or after injury[13,14]. Recently, it was reported that apo E may be an essential cofactor for amyloid fibril formation and that it plays an important role in the distribution of amyloid deposits in various tissues[1,7,11,13,16]. This idea is supported by recent immunohistochemical and molecular biological findings which showed positive immunostaining of amyloid deposits or expression of apo E mRNA in Alzheimer's disease and Down's syndrome associated amyloid, primary systemic amyloidosis and secondary amyloidosis coexistant with dialysis or RA[1,7,11,13,15,16]. In this study, we examined 11 patients with renal amyloidosis immunohistochemically. The present results provide further confirmation that apo E is distributed in the glomeruli, vascular wall and/or the interstitial area, and suggest that apo E staining correlates highly with the presence of amyloid deposits. It is speculated that amyloid-associated proteins such as P component, glucosaminoglycans and α_1-anti-chymotrypsin reduce the solubility and stabilize the β-pleated structure in amyloid fibrils or their precursor proteins, and so play an important role in the formation of amyloid deposits[8]. Our results suggest that apo E, in addition to the above proteins, may function as 'pathological molecular chaperones'[14]. Further studies using models of amyloid formation or in situ hybridization are needed to clarify the interactions between pathological chaperones and amyloid deposits.

At present, patients with renal amyloidosis have been treated by controlling precursor proteins or by the prevention and removal of amyloid deposits[2,10,12], but these therapies are not very effective. If the functions of pathological chaperones such as apo E, P component and glucosaminoglycans can be clarified, anti-pathological chaperones may be of therapeutic benefit.

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