Localization of Bence Jones Proteins in the Kidney of Myeloma Patients

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Six cases of multiple myeloma were studied regarding functions and pathology of the kidney. Glomerular changes were minimal, whereas tubular and interstitial changes were noted in all cases. Tubular cells were degenerative and atrophic in cases excreting more than 3 g BJP daily in the urine. Cases without BJP had only minor changes. The most severe impairment of renal function was found in a patient with Fanconi's syndrome, with hypercalcemia, calcium in the tubules, tubular atrophy and interstitial fibrosis. In attempting to correlate immunoglobulin abnormalities with renal histology, immunoperoxidase staining of immunoglobulin heavy and light chains were done on kidney sections. Glomerular and interstitial tissues gave a definitely negative reaction. In contrast, positive staining of light chain of corresponding type was found in the cytoplasm of the tubules and most tubular casts, with different degrees possibly in accord with the amounts of BJP.

Key Words: Myeloma, Renal pathology, BJP, Fanconi's syndrome, Tubular damage, Tubular casts, Immunoperoxidase.

Renal failure, a frequent complication of myeloma, is associated with decreased survival¹⁾²⁾. Several studies were conducted on renal functions and clinical manifestations, but, scarcely any attempts were made to correlate immunoglobulin abnormality with changes in renal histopathological findings. In relation to the clinical studies on Bence Jones protein, an immunohistochemical study was undertaken to demonstrate deposits of monoclonal immunoglobulins or Bence Jones proteins in the renal tissues of myeloma patients.

MATERIALS AND METHODS

Six cases of multiple myeloma were studied in the Kobe University Hospital. All cases were autopsied, and renal biopsy was

performed on one of them (Case A.T.). Major clinical and laboratory data are summarized in Table 1. As control subjects, 53 yo male of diabetic nephropathy and 52 yo female of nephrosis were also investigated. Histopathological examinations were done on specimens of the bilateral kidneys of 8 cases with hematoxylin-eosin stains, and Kossa stains for calcium deposits. Immunohistochemical examinations were done by peroxidase-labelled antibody method on the renal section films against all classes of immunoglobulin heavy and light chains. After washing in phosphate-buffered saline the sites of specific antibody binding were identified using a peroxidase-conjugated goat antibody (Fujizoki Co) directed against rabbit-IgG (Hoechst Co), followed by washing in PBS

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and staining with the diaminobenzine (DAB) reaction. Hematoxylin counterstain was followed. Black and white photomicrographs were obtained using a Tri-blue filter through microscope (Nikon HFM, Japan).

Monoclonal immunoglobulin or BJP was identified as described previously³⁾.

RESULTS

Table 1 summarizes the clinical data including age, sex, macroscopic autopsy findings, the type of M-protein abnormalities, the degree of bone changes and clinical complications. All cases were between 49 and 71 years of age and under the diagnosis of myeloma or plasma cell leukemia, exhibiting one type of M-proteins in the serum or in the urine. IgG (κ) was found 4 of 6 cases

in the serum. A prominent peak of Bence Jones protein (BJP) was noted in the serum in one case of Fanconi syndrome. In the urine, all but one excreted BJP. As to complications, Fanconi syndrome consisting of glycosuria without hyperglycemia, hyperphosphaturia, aminoaciduria was found in Case 4. Hypercalcemia subsequently developed in this case. Renal tubular acidosis in association with liver cirrhosis and hair apparatus carcinome was found in Case 5. Hepatoma with liver cirrhosis was found in Case 6.

Table 2 summarizes the results of renal function tests, hematological studies and immunoglobulin levels. Proteinuria of more than 3gm a day was noted with a major component of BJP in 3 cases, one of which

Table 1. Clinical data

Case	Age, sex	Macroscopic Autopsy findings	Serum	Urine	Bone destruction	Complications
1.	59 F	Plasma cell leukemia	IgG(K)	BJK	+	acute renal failure Hyperuricemia
2	49 M	Multiple myeloma (nodular)	(-)	BJλ	++	acute renal failure
3	51 M	Multiple myeloma (diffuse)	IgG	BJK	+	—
4	57 M	Multiple myeloma (diffuse)	BJλ	BJλ	++	Fanconi syndrome Hypercalcemia chronic renal failure
5	69 M	Multiple myeloma (diffuse)	IgG(K)	BJK	+	Renal tubular acidosis Liver apparatus Hair apparatus carcinoma
6	71 M	Multiple myeloma (diffuse)	IgG(K)	(-)	+	Liver cirrhosis Hepatoma

Table 2. Laboratory data

Case	urinary proteins (g/day)	BJ-P (g/day)	Renal functions						Peripheral blood										marrow	
			BUN (mg/dl)	S-Cr (mg/dl)	uric acid (mg/dl)	Cr (mg/min)	PSP 15' 120' (%)	TP (g/dl)	Ca (mg/dl)	IgG (mg/dl)	IgA (mg/dl)	IgM (mg/dl)	RBC (X10 ⁴ /mm ³)	WBC (/mm ³)	plasma cells (%)	plts (X10 ⁴ /mm ³)	NCC (X10 ⁴ /mm ³)	plasma (%)		
1.	4.5	4.0	35	1.5	10.8	72.1	9.2	42.5	9.2	8.5	6700	N.D.	N.D.	326	9,800	31	15.1	7.9	44.8	
2	0.6	0.3	16	0.8	5.0	117	31.9	75.2	5.6	9.3	1281	126	206	232	5,800	(-)	10.6	0.3	0.6	
3	0.5	0.3	20	0.7	8.3	105	45.3	98.4	11.8	8.8	12600	N.D.	N.D.	278	5,800	(-)	12.8	2.5	50.4	
4	4.2	4.0	128	10.9	12.2	16.2	3.6	13.6	8.2	13.0	800	30	35	181	3,700	(-)	5.9	3.0	24.8	
5	3.0	2.7	9	1.1	4.7	59.0	29.6	75.4	7.5	8.5	4158	56	47	338	2,700	(-)	11.4	12.5	12.4	
6	2.8	0	11	0.8	4.4	-	-		8.6	6.4	8000	42	40	339	5,600	(-)	8.3	15.3	48.8	

S-Cr: Serum creatinine, NCC: nuclear cell counts, ND: not detectable.

(Case 4) showed a highly impaired renal function. Low levels of proteinuria was associated with intact renal function. Hypercalcemia developed only in Case 4 of Fanconi syndrome, along with severe anemia, moderate leucopenia and thrombocytopenia. Only in Case 1, plasma cells were detected in the peripheral blood. The proportion of plasma cells in bone marrow aspirates were low in Case 2 with plasma cell proliferations of nodular type.

Table 3 shows histopathological findings in the kidneys. It is noteworthy that glomerular changes were minimal, whereas some tubular and interstitial changes were noted in all cases. Some renal tubular cells were degenerative and atrophic in Case 1 and 4 who excreted more than 3.0 g BJP daily in the urine. Case 6 without BJP in the urine had only minor changes of the tubules. In Cases 2 and 3 the degree of tubular damage was different, despite normal renal function and excretion of similar amount of BJP. BJP excretion thus varied widely. Casts were found in the tubules in all cases, and lamellar formation of the casts were noted only in case 4 with heavy cast deposits. Hypercalcemic case had a marked calcium deposits (Fig. 1A). Plasma cells infiltrated the renal interstitial tissue in non-leukemic Case 3, but not in leukemic Case 1. Inter-

Table 3. Histopathological findings

	Case 1	2	3	4	5	6
Glomerulus						
increase in mesangial matrix	-	-	-	-	-	-
thickening of tuft cap wall	-	-	-	-	-	-
amyloid deposits	-	+	-	-	-	-
Tubular						
tubular degeneration	+	-	+	-	+	-
proximal tubule dilation	-	-	-	-	+	±
tubular atrophy	-	-	-	-	-	-
casts	+	+	+	-	+	+
lamellar formation	-	-	-	-	-	-
calcium deposits	±	+	-	-	+	±
Interstitial						
plasma cell infiltration	-	-	+	-	-	-
fibrosis	+	+	-	-	+	-
Thickening of arterioles	-	-	-	-	-	-

Table 4. Positive staining for anti Ig-chains by immunoperoxidase method.

Case	1	2	3	4	5	6
Serum	IgG(K)	-	IgG(K)	BJλ	IgG(K)	IgG(K)
Urine	BJK	BJλ	BJK	BJλ	BJK	-
Glomerular						
capillary tuft	-	-	-	-	-	-
mesangial matrix	-	-	-	-	-	-
Tubular						
epithelial cells	K+	λ+	K+++	λ+++	K+++	-
casts	K+	λ+	K++	λ+++	K+	-
Interstitial	-	-	-	-	-	-

stitial fibrosis were seen in 4 cases.

Table 4 shows the positive staining for anti Ig-chains by immunoperoxidase method. Glomeruli and interstitial tissues gave a definitely negative reaction. In contrast, positive staining of light chain of the corresponding type was found in the cytoplasm of the tubules and most tubular casts (Fig. 1. B, C, D). Positive staining of all tubular cells were seen in some tubules, while other tubules were completely negative. Blocking and absorption experiments verified the specificity of these immunoperoxidase reactions. As to control cases without myeloma, there are no evidence of heavy or light chain deposits.

The most severe impairment of renal function was found in the patient with the lambda type myeloma associated with Fanconi's syndrome, with hypercalcemia, calcium deposits in the tubules, tubular atrophy and interstitial fibrosis. In attempting to correlate immunoglobulin abnormalities with renal histology by immunoperoxidase staining, all patients excreting BJP in urine showed positive light chain deposits of the corresponding type in the cytoplasm of tubule epithelial cells and intratubular casts with different degrees possibly in accord with the amounts of BJP, in contrast to negative stains in the glomeruli and the interstitium.

DISCUSSIONS

Renal failure of some degree is a common

manifestation among patients with myeloma^{1)~4)}. DeFronzo et al evaluated renal tubular and glomerular functions in 35 myeloma patients and correlated these with changes in renal histopathological findings and myeloma protein patterns¹⁾. The magnitude of BJP correlated well with the degree of renal insufficiency⁵⁾. Severely deranged renal histological findings seen only in patients with BJP consisted primarily of tubular atrophy and degeneration. Glomeruli appeared normal. These data suggest a direct nephrotoxic effect of BJP at the tubular level with resultant tubular dysfunction and tubular atrophy. Sanchez and Domz

showed similar findings in addition to the lamellated protein deposits in the tubules in myeloma patients²⁾. Zlotnick and Rosenmann pointed out casts in the distal and collecting tubules characterizing the myeloma kidney³⁾. Nephrocalcinosis and focal myeloma cell infiltration in the interstitium may be present. The findings in the present report are consistent with these reports.

BJP, homogeneous light chains of immunoglobulins, is excreted in the urine of approximately one-half of all myelomas. Myeloma, amyloidosis, Fanconi syndrome and distal tubular acidosis are occasionally associated with BJP⁴⁾⁵⁾, which were found

Fig. 1. Calcium deposits by Kossa stain ($\times 200$) (A), positive staining for anti lambda-chain (B), negative for anti kappa-chain (C) and negative for anti-gamma chain by immunoperoxidase method with hematoxylin counter stain ($\times 200$) in the kidney section of Case 4 of Fanconi's syndrome.

in one of 6 cases respectively in the present report. Case 4 with Fanconi syndrome excreted BJP λ , which is very unusual since predominance of BJP κ has been known in Fanconi syndrome⁴⁾.

The term light-chain nephropathy was proposed on a basis of a clinical observation in a patient with multiple abnormalities of proximal-tubule transport mechanisms, nephrogenic diabetes insipidus, and distal tubular acidosis⁶⁾. Direct confirmation of defective renal handling of light chains in primary tubular disease has been provided by the more rapid excretion or exogenously administered light chains in patients with Fanconi's syndrome more rapidly than in normal subjects^{7,8)}. Pieces of evidence for nephrotoxicity of BJP to the renal tubule and resultant tubular dysfunctions have been accumulated^{8,9)}. As they speculate in the paper, it seems reasonable to assume that the duration and magnitude of the light chain proteinuria of the different solubility property of different light chains are important determinants of the degree of toxic effect.

In this connection, immunofluorescent studies of the myeloma kidney were done with special reference to light chain disease by Levi et al⁹⁾. Renal tissue from nine patients with myeloma and five with renal failure secondary to other disease was studied. The most striking immunofluorescence was detected in tubular casts of myeloma patients. Predominant staining for kappa or lambda light chain components correlated with urinary light chain excretion data. Tubular cell localization of material reacting with specific anti-light chain conjugates was demonstrated as droplets within renal tubular cells⁹⁾.

In attempting to correlate immunoglobulin

abnormalities with renal histology, the present report identified the positive staining of tubular casts and cytoplasmic deposits of the tubules for BJ κ or BJ λ corresponding to urinary BJP excretion data with different degrees possibly in accord with the amounts of BJP. However, different BJP's may play different nephrotoxic roles, since Case 3, 4 and 5 are strongly stained despite the different concentrations of excretion in the urine. It is therefore worthy of pursuit whether the structural differences of BJP may explain the nephrotoxicity.

REFERENCES

- 1) DeFronzo RA, et al: Renal function in patients with multiple myeloma. *Medicine* 57: 151, 1978.
- 2) Sanckez LM, Domz CA: Renal patterns in myeloma *Ann Intern Med* 52: 44, 1960.
- 3) Zlotnick A, Rosenmann E: Renal pathologic findings associated with monoclonal gammopathies. *Arch Intern Med* 135: 40, 1975.
- 4) Maldonado JE, et al: Fanconi syndrome in adults. A manifestation of a latent form of myeloma. *Amer J Med* 58: 354, 1975.
- 5) Stone MJ, Frenkel ED: The clinical spectrum of light chain myeloma. *Amer J Med* 58: 601, 1975.
- 6) Smithline N, Kassirer JP, Cohen JJ: Light chain nephropathy. Renal tubular dysfunction associated with light-chain proteinuria. *New Engl J Med* 294: 71, 1976.
- 7) Waldmann TA, Strober W, Mogielnicki RP: The renal handling of low molecular weight proteins. *J Clin Invest* 51: 2162, 1972.
- 8) Wockner RD, Strober W, Waldmann TA: The role of the kidney in the catabolism of Bence Jones proteins and immunoglobulin fragments. *J Exp Med* 120: 207, 1967.
- 9) Levi DF, Williams RC, Lindstrom FD: Immunofluorescent studies of the myeloma kidney with special reference to light chain disease. *Amer J Med* 44: 922, 1968.