

猫のモノクローナルIgAおよびBence Jones蛋白に関する免疫化学的研究

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Immunochemistry and Physicochemistry on a Feline Monoclonal IgA and Bence Jones Proteins

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ABSTRACT. From a feline patient, monoclonal-IgA (M-IgA) and Bence Jones (B-J) proteins were isolated and characterized for their biochemical properties. The molecular weight of the purified M-IgA was estimated to be approximately 230,000 daltons and the molecular weights of the B-J proteins were estimated to be 28,500 and 25,000 daltons, by SDS-polyacrylamide gel electrophoresis. The biochemical and immunochemical data support our earlier conclusion that the feline patients suffered from an M-IgA gammopathy associated with B-J proteinuria. Furthermore, B-J proteins in the patient's urine appeared to contain at least five B-J protein components, when analyzed by two-dimensional gel electrophoresis. These M-IgA and B-J have common antigenic determinants and identical polypeptide segment, as demonstrated by immunological tests and by the analysis of amino acid composition. The analysis of the amino acid composition of the feline B-J proteins also indicated that it is similar to that of the human B-J protein.—**KEY WORDS:** Bence Jones protein, feline, IgA-monoclonal gammopathy.

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Monoclonal gammopathy is a disease characterized by an increase of homogeneous immunoglobulin components in the serum and/or urine. M-IgA gammopathy associated with multiple myeloma and lymphocytic leukemia has been reported in dogs [2, 4, 10, 11, 16, 17] though monoclonal gammopathies are less frequently diagnosed in dogs and cats. However feline M-IgA gammopathy has not been reported precisely and B-J proteinuria in cats has also rarely been diagnosed [9].

In this paper, we describe the immunochemical and physicochemical properties of the M-IgA and B-J protein(s) from a feline case of M-IgA gammopathy which we previously reported briefly [19].

MATERIALS AND METHODS

Animals: The subject of this study was a 5-year-old, female Persian cat, which was

referred to Veterinary Hospital of Azabu University, because of a protrusion of the right eyeball and anorexia [19]. In this patient, a monoclonal peak (of 1.82 g/dl) was detected in the slow γ -globulin region by electrophoresis of serum protein and an abnormally high level of IgA (26.1 mg/ml) was also noted in the serum. In the urine, two narrow high peaks were detected in the fast γ -globulin region by electrophoresis. The concentration of protein in urine was 7.8 mg/ml, as determined by the biuret method; the urine protein aggregated upon heating at 55°C under acidic conditions (pH 5.0) and the aggregates redissolved upon heating to 100°C. While no excessive proliferation of bone marrow could be observed, many tumor nodules were detected on the peritoneum and omentum and the persistence of ascites was noted.

Purification of M-IgA from the ascites (Fig. 1(a)): Ascites (105 ml) from the

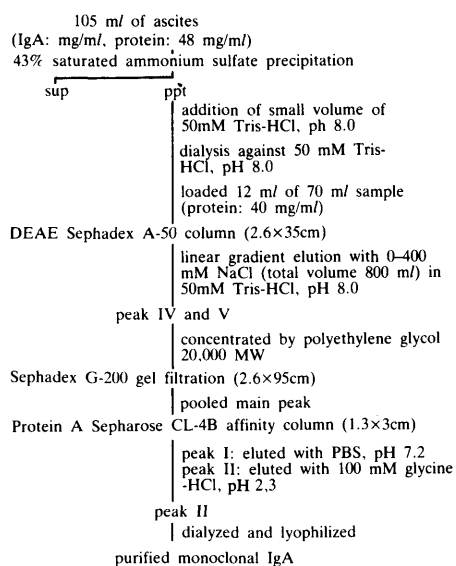


Fig. 1a.

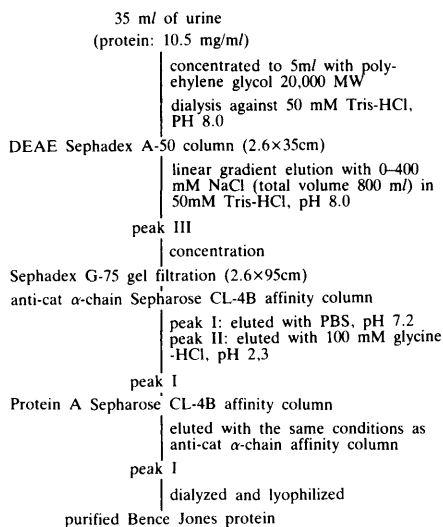


Fig. 1b.

Fig. 1. Schematic purification procedures for M-IgA (a) from the patient ascites and B-J proteins (b) from the patient urine.

patient were mixed with an equal volume of PBS, pH 7.2 and fractionated twice by precipitation with 43% saturation of $(\text{NH}_4)_2\text{SO}_4$ (final concentration). The precipitate was dissolved in a small amount of 50 mM Tris-HCl, pH 8.0 and dialyzed against the same buffer at 4°C. After centrifugation, a part of the supernatant (40 mg/ml, 12 ml) rich in IgA was applied to a DEAE-Sephadex A-50 column (2.6×35 cm) preequilibrated with 50 mM Tris-HCl, pH 8.0 and eluted with an 800 ml linear gradient of 0-0.3 M NaCl in 50 mM Tris-HCl, pH 8.0, as shown in Fig. 2 (a1). The fourth and fifth peaks (IV and V) from the DEAE chromatography were pooled and concentrated and approximate 3 ml of this materials (25 mg/ml) was subjected to gel-filtration on a Sephadex G-200 column (2.7×90 cm). Finally, the concentrated eluate of this column was applied to a protein A Sepharose CL-4B column (Fig. 2 (a2)). The fractions eluted from the column with 100 mM glycine-HCl (pH 2.3) were pooled, dialyzed, and lyophilized.

Purification of B-J protein(s) from the urine (Fig. 1(b)): Five ml of the concentrated urine (the protein concentration was approximately 7 g/dl) from the patient were applied to DEAE-Sephadex A-50 column and eluted as described above (Fig. 2(b)). The fractions from the third peak of the DEAE chromatography (III) were pooled and subjected to gel-filtration on a Sephadex G-75 column and then applied to two distinct affinity column: (1) anti-cat α chain and (2) protein A Sepharose CL-4B, in that order. Finally, the fraction eluted from the protein A column was dialyzed and lyophilized. We used a series of purification steps which consisted of ion-exchange, gel-filtration, and affinity chromatography.

Antisera: Anti-cat whole serum was prepared in a rabbit as described by Okoshi *et al.* [12]. Anti-cat γ , α and μ chain sera were prepared in rabbits [20]. Each rabbit received four injections of approximate 0.07 mg of each purified immunoglobulin with Freund's adjuvant. One injection (1 ml) was given every 10 days. Anti-cat B-J serum and

anti-patient's urine serum were also prepared as described above.

Gel electrophoresis: Immunoelectrophoresis was carried out according to the procedure of Scheidegger [15]. The purified M-IgA was analyzed by electrophoresis in 5% polyacrylamide gel (5%: 0.12%/acrylamide: bisacrylamide) in 2.5 mM Tris-HCl pH 8.0 and 25 mM EDTA, with the same buffer plus 0.1% SDS as the electrode buffer while B-J proteins were analyzed by 10% polyacrylamide gel electrophoresis, as described by Laemmli [8].

Immunodiffusion: Double diffusion was performed by the method of Ouchterlony [13].

Amino acid analysis: The purified protein was hydrolyzed in 6N HCl for 24 hours at 100°C under nitrogen. Amino acid analyses were performed on an ATTO MCL-703 S automatic amino acid analyzer (ATTO, Co., Japan).

RESULTS

Purification of the M-IgA and B-J proteins: Typical elution profiles for the column chromatography during purification are shown in Figs. 2 and 3. Final recoveries of M-IgA and B-J were estimated to be 29% and more than 95% of that in the starting materials, respectively.

Characterization: Results of the double immunodiffusion test on M-IgA protein fraction are shown in Figure 3(1). Anti-cat whole serum, anti- α chain serum and anti-cat B-J protein serum formed a single precipitin line with the purified M-IgA, respectively (Fig. 3(1) (M)). The molecular weight of M-IgA was estimated to be approximately 230,000 daltons by polyacrylamide gel electrophoresis (Fig. 4a). The purified B-J fraction was also characterized by double immunodiffusion tests (Fig. 3(1) (B)). Anti-cat γ , α and μ chain serum failed to produce a precipitin line with the purified

B-J protein(s) (Fig. 3(1) (B)).

Immunoelectrophoresis of the protein from urine with anti B-J serum showed a precipitin line, and the protein also reacted with anti-human λ -chain serum [19] (Fig. 3(2)). The purified B-J proteins were shown to contain two components of approximate molecular weights of 28,500 and 25,000 daltons by SDS-polyacrylamide gel electrophoresis (Fig. 4b(3)). This profile of protein components in the urine differs significantly from that of a reference cat with typical renal disease (Fig. 4b(5)), though the concentration of urine protein as determined by a strip method was the same in these two cats (data not shown). The cat with renal disease has a major protein of molecular weight of 68,000 daltons in the urine which is probably albumin (Fig. 4b(5)). By two dimensional electrophoresis combined of isoelectrophoretic focusing and SDS-polyacrylamide slab gel electrophoresis, approximately 3 major and 2 minor polypeptides, ranging in isoelectric point of pH from 5.3 to 7.0 were detected as B-J proteins in the urine protein (data not shown).

Amino acid analysis: The amino acid composition of the purified M-IgA and B-J proteins was analyzed and shown in Table 1 in comparisons with that of humans. A profile of the composition revealed to be similar between M-IgA and B-J proteins. Five major amino acid components, serine, glutamic acid, threonine, glycine and proline constituted about 52% of the total amino acids in the M-IgA fraction. These five amino acids constituted more than 59% of the total amino acids in the B-J protein fraction. However, some minor differences of amino acid composition, such as the ratio of His/Lys/Arg, were detected between these proteins (Table 1).

The amino acid composition of cat B-J proteins was found to be similar to that of human B-J protein reported [15] (Table 1).

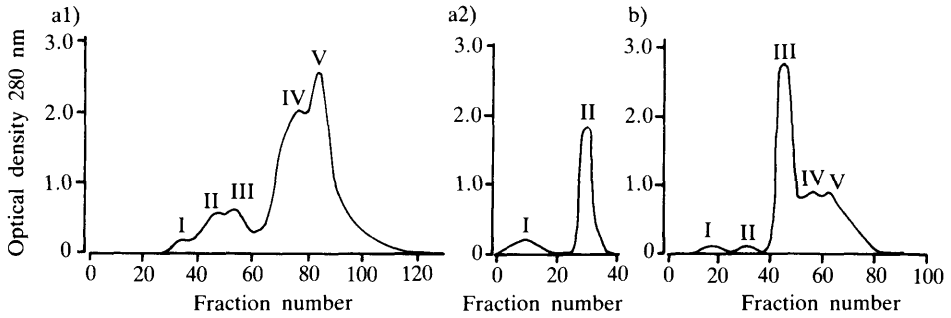


Fig. 2. Typical elution profiles from column chromatography during purifications of M-IgA (a) and B-J proteins (b). (a1): DEAE-Sephadex A-50 column chromatogram of fractionation of material precipitated with 43% saturated ammonium sulfate from ascites from the patient. Starting buffer: 50 mM Tris-HCl pH 8.0. Elution buffer: linear gradient of NaCl from 0 to 0.3 M. (a2): Protein A Sepharose CL-4B column chromatogram of the concentrate from a Sephadex G-200 column of the pooled fraction IV and V. After washing with PBS pH 7.2, M-IgA fraction was eluted with glycine-HCl pH 2.3. (b): DEAE-Sephadex A-50 column chromatogram of the concentrated urine from the patient.

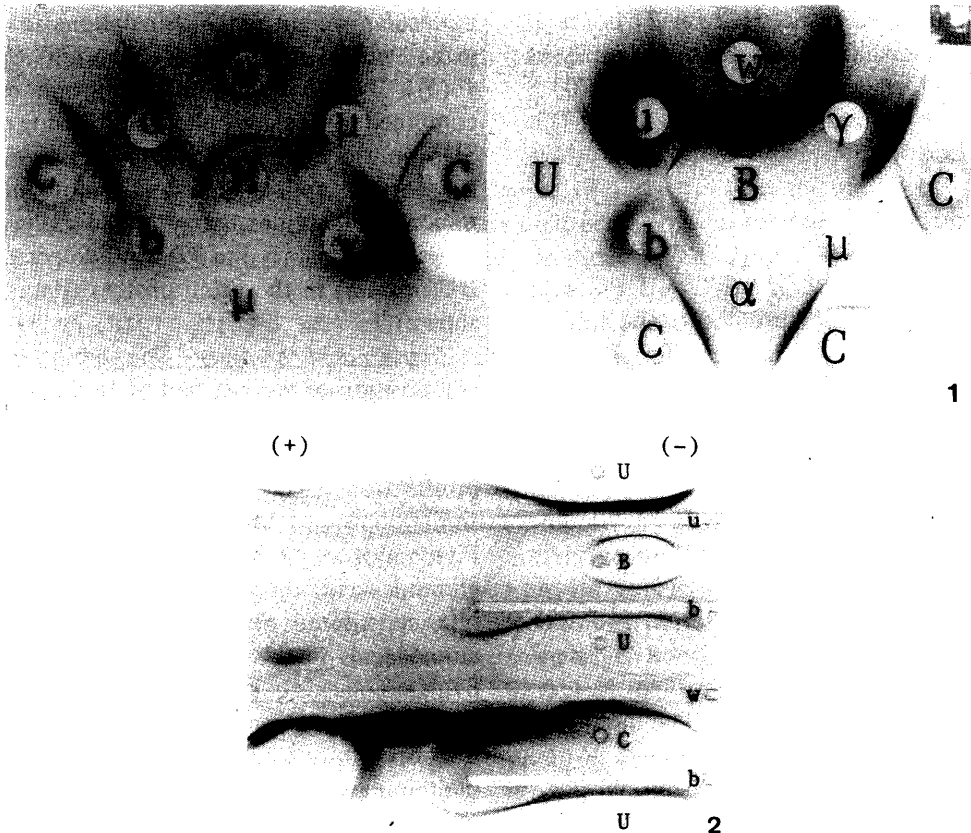


Fig. 3. Double immunodiffusion (1) and immunoelectrophoretic profiles (2) of M-IgA (M) and B-J proteins (B). C: control cat serum, U: patient urine, γ : anti-cat γ chain serum, α : anti-cat α chain serum, μ : anti-cat μ chain serum, b: anti-cat B-J protein serum, and w: anti-cat whole serum. In immunodiffusion analysis, the precipitating lines observed near the holes of α and μ may be those of albumin formed between w and C.

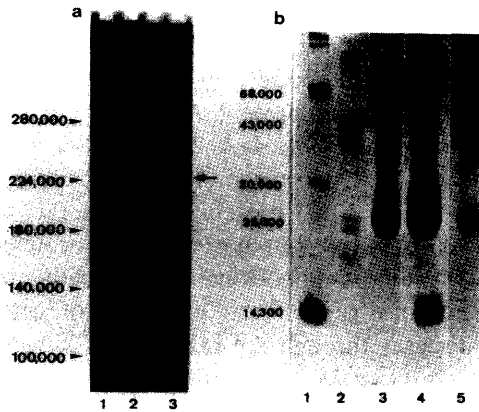


Fig. 4. SDS-polyacrylamide gel electrophoretogram of purified M-IgA (a) and B-J proteins (b); lane (1) of (a): patient serum, lane (2): patient ascites, lane (3): purified M-IgA. *Escherichia coli* RNA polymerase (180,000, 140,000, 100,000, 42,000 and 39,000; SDS- PAGE Marker I, Seikagaku Kogyo Co. LTD.) and protein-standard mixture III (280,000, 224,000, 168,000, 112,000 and 56,000; MERCK) were used as molecular weight standards. Some of them are shown in the Figure. Lane (1) of (b): bovine serum albumin (68,000), carbonic anhydrase (30,000) and lysozyme (14,300), lane (2): ovalbumin (43,000) and chymotrypsinogen (25,000), lane (3): purified B-J proteins, lane (4): the patient urine, lane (5): albuminuria demonstrated by use of urine from another cat.

Table 1. Amino acid composition of purified M-IgA and B-J proteins

	M-IgA	(mol %) B-J proteins	B-J protein (human) ^{a)}
Asp	8.53	7.20	8.7
Thr	8.66	8.89	7.8
Ser	12.65	17.44	14.3
Glu	11.68	9.91	11.8
Pro	9.11	7.04	7.0
Gly	10.28	11.26	9.4
Ala	4.78	6.43	5.8
Cys	1.41	1.13	1.5
Val	6.73	5.69	5.5
Met	0.99	0.60	0.7
Ile	1.95	2.41	3.7
Leu	8.37	7.25	5.6
Tyr	1.94	2.97	3.5
Phe	2.80	2.16	3.5
Lys	5.42	5.54	4.3
His	1.72	1.75	1.6
Arg	2.98	2.44	4.4
Acidic A.A.	20.21	17.11	20.5
Basic A.A.	10.12	9.73	10.3
Acidic/Basic	2.00	1.76	2.00
Asp/Glu	0.73	0.73	0.74
Ser/Thr	1.46	1.96	1.83
His/Lys/Arg	1/3.15/1.73	1/3.17/1.39	1/2.89/2.75

a) By Benditt and Eriksen

In particular, both human and cat proteins contained serine, glycine and glutamic acid as the three major amino acid components, which constituted about 35–38% of the total amino acid in the B-J proteins.

DISCUSSION

The establishment of simplified and reproducible biochemical purification methods for M-protein(s) is a very impor-

tant step in the biochemical and physicochemical characterization of M-IgA gammopathy, however little has been attempted along these lines with the cat, we happened to examine a feline case of M-IgA gammopathy associated with B-J proteinuria and succeeded in obtaining highly purified M-IgA and B-J proteins from the diseased cat.

The molecular characteristics of these two materials and their relationship(s) was investigated. As shown in Fig. 3(1), anti-cat B-J serum apparently formed a single precipitating line with the purified M-IgA. Furthermore, anti-cat γ , α and μ chain serum failed to show a precipitin line with the purified B-J proteins. These data strongly suggested that B-J proteins could be derived from feline lambda type light chains of IgA, IgG or IgM [19]. An SDS-polyacrylamide gel electrophoretic profile of the purified B-J proteins seemed to support this conclusion.

Three major and 2 minor polypeptides were detected in feline B-J proteins. Though these proteins seemed to have identical antigenicity, they might be heterogeneous, since some human heterogeneous B-J proteins, such as Sut, Mor, THO, etc, have been detected [5, 6, 18].

An abnormally high level of IgA (26.1 mg/ml; approximately 10 times of the normal amount) was noted in the serum from the patient, by quantitative assays with single radial immunodiffusion [19]. The molecular weight of the purified M-IgA from the patient's ascites was approximately 230,000 daltons. Thus, M-IgA, not α chain, may possibly be constituted by an intact IgA and other component(s).

Since a degree of similarity was found between the amino acid composition of M-IgA and B-J proteins, those two protein fractions may carry a common polypeptide segment.

In the present report, we used a protein A Sepharose CL-4B affinity column to purify

both M-IgA and B-J proteins as a final step. Protein A binds IgG derived from almost all mammalian species. In addition, it is also reported that protein A can interact with human myeloma IgA 2 [14] and colostrin IgA [7]. Our present research clearly demonstrates that protein A can interact with feline M-IgA but not with B-J proteins.

The IgA-monoclonal gammopathy in a cat may suggest the existence of certain diseases through the mammalian kingdom, as well as in dogs and humans.

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要 約

猫のモノクローナル IgA および Bence Jones 蛋白に関する免疫化学的研究：山田隆紹・松田基夫・佐俣哲郎・土屋 亮・小林好作（麻布大学）—— Bence Jones (B-J) 蛋白尿を伴った IgA モノクローナル γ -グロブリン血症の猫の腹水と尿から、モノクローナル IgA (M-IgA) と B-J 蛋白を各々精製し、その性状を検討した。これら 2 種の蛋白は、硫酸アンモニウム塩析法、DEAE Sephadex A-50 によるイオン交換法、Sephadex G-75 あるいは G-200 によるゲルろ過法、Protein A ならびに抗 α 鎖血清を用いたアフィニティー法を組み合わせ精製した。SDS-ポリアクリルアミドゲル電気泳動による分析で、M-IgA の分子量は約230,000であり、B-J 蛋白は 2 種の主要成分からなりその分子量は約28,500と25,000であった。B-J 蛋白は 2 次電気泳動による分析で少なくとも 5 種のポリペプチドで構成されていることが確認された。さらに免疫学的検索とアミノ酸組成の分析から、M-IgA と B-J 蛋白は抗原的に共通部分があり、かつ共通のポリペプチドから構成されていることが確認された。