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NOTE

Pathology

Changes in lectin-binding patterns in the kidneys of canines with immune-complex mediated glomerulonephritis

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ABSTRACT. We surveyed the kidneys of dogs with immune-complex mediated glomerulonephritis (ICGN) by lectin histochemistry using seven lectins—namely WGA, RCA-I, ConA, PNA, SBA, DBA, and UEA-I. Their binding patterns were compared with those from normal dogs. RCA-I signals became weak in the brush borders of the proximal tubules, whereas DBA signals became positive in Bowman's capsules. Also, varying intensity of the UEA-I signal was noted in the distal tubules, especially in the macula densa. The binding pattern profiles varied among the cases; this diversity in the lectin-binding patterns might be induced as a result of the diverse pathologies seen in canine ICGN.

KEY WORDS: dog, immune-complex mediated glomerulonephritis, kidney, lectin histochemistry

Carbohydrates play important physiological roles in several biological processes, including pinocytosis, cellular differentiation, and intercellular recognition and adhesion [11]. In kidney disease, changes in cellular carbohydrates have been investigated using the lectin histochemistry, and changes in lectin-binding patterns have been documented in human glomerulonephritis [2, 5, 9, 17]. Immune-complex mediated glomerulonephritis (ICGN) is known as a major cause of protein-losing nephropathy in dogs [14], but the changes in cellular carbohydrates in this disease have not been described. The aim of the present study was to evaluate the changes in lectin-binding patterns in the kidneys of dogs affected with canine ICGN.

Tissue samples were obtained from the renal biopsies of dogs (n=12) who had been clinically diagnosed with protein-losing nephropathy. The diagnosis of ICGN was made by light microscopy, transmission electron microscopy, and immunofluorescence according to the established guidelines for pathologic evaluation of canine renal biopsies [3]. The signalment, symptoms, clinicopathological data, and pathological diagnoses of the ICGN cases are shown in Table 1. The experiments in this study were performed in accordance with the Guidelines for Animal Experimentation of Kagoshima University, Japan (VM15020), and the dog owners provided informed consent for the renal biopsies to be used for the definitive diagnosis of glomerular disease and research after the diagnosis. Kidney samples were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin blocks of kidneys from healthy beagles (n=3) were used as normal controls. The paraffin blocks were cut into 3 μm-thick sections and used for lectin histochemistry. Seven commercially available biotinylated lectins, namely WGA, RCA-I, ConA, PNA, SBA, DBA, and UEA-I, were used in the present study (Lectin Kit I; Vector Laboratories, Burlingame, CA, U.S.A.). Staining was performed as described in our previous report [16]. Abbreviations, concentrations, specificities, and inhibitory sugars of the lectins are shown in Table 2.

Lectin binding patterns in the ICGN kidneys are summarized in Table 3. In glomeruli, capillary wall binding was observed for WGA, RCA-I, and Con A. In some cases, the glomerular capillary walls were weakly stained with RCA-I; however, no other remarkable changes were found in the present study.

Detection of immune complex deposits in the glomeruli is a necessary requirement for the diagnosis of ICGN. A study of human type II membranoproliferative glomerulopathy reported that WGA lectin could detect glomerular immune complex deposits [10]. However, in the present study, none of the lectins (including WGA) could detect glomerular deposits that indicate ICGN immune complexes.

In other nephron segments, staining patterns for ConA, WGA, PNA, and SBA lectins in ICGN kidneys were similar in ICGN and normal kidneys. However, staining patterns of RCA-I, DBA, and UEA-I lectins in the ICGN kidneys were different from those seen in normal kidneys.

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Table 1. Signalment, symptoms, clinicopathological data, and pathological diagnoses of the ICGN cases

Cases	Breed	Sex	Age (years)	NS	Pathological diagnosis	Blood chemical analysis				UP/C	SBP (mmHg)
						BUN (mg/dl)	Cre (mg/dl)	Alb (g/dl)	TP (g/dl)		
1	Welsh Corgi	F	4	-	MG	>140.0	2.8	ND	7.4	20.9	175
2	Yorkshire Terrier	F	8	-	MG	42.0	1.3	2.4	ND	13.6	ND
3	Yorkshire Terrier	M	2	+	MPGN	17.3	0.6	2.3	4.4	4.9	156
4	Papillon	NM	5	+	MPGN	29.0	0.7	1.5	3.4	4.5	133
5	Shetland Sheepdog	M	7	-	MG	48.1	3.5	ND	7.0	11.6	214
6	Yorkshire Terrier	NM	7	-	MG	14.8	0.7	2.2	4.9	4.84	140
7	Welsh Corgi	NF	9	-	MPGN	31.5	1.1	2.9	7.4	9.1	178
8	Welsh Corgi	NM	10	-	MPGN	6.0	0.8	1.9	5.6	5.0	ND
9	Papillon	NM	7	+	Undetermined	63.0	1.0	1.7	4.0	6.1	ND
10	Miniature Dachshund	NF	8	-	IgA nephropathy	8.5	0.3	3.4	9.6	4.3	152
11	Toy poodle	F	5	-	IgA nephropathy	31.9	0.2	2.1	ND	37.3	ND
12	Pomeranian	M	4	-	MPGN	33.0	0.6	2.0	5.8	20.9	ND
13	Chihuahua	M	8	+	MG	47.6	1.5	1.8	4.8	5.4	155

F: female, M: male, NF: neutered female: NM: neutered male, NS: nephrotic syndrome, MG: membranous glomerulopathy, MPGN: membranoproloferative glomerulonephritis, BUN: blood urea nitrogen, Cre: creatinine, Alb: albumine, TP: total protein, UP/C: urinary protein/creatinine ratio, SBP: systolic blood pressure, ND: not determined.

Table 2. Binding specificities of lectin used

Abbreviation	Lectin	Specificity	Hapten sugar	Concentration (μg/ml)
WGA	Wheat germ agglutinin	GlcNAc, sialic acid	360 mM GlcNAc	4
RCA-I	*Ricinus communis* agglutinin I	Galactose, GalNAc	180 mM galactose	4
ConA	Concanavalin A	Alpha-linked mannose	180 mM alpha-methyl mannoside/ 180 mM alpha-methyl glucoside mixture	4
PNA	Peanut agglutinin	Galactosyl (beta-1,3) GalNAc	180 mM galactose	8
SBA	*Soybean* agglutinin	Alpha- or beta-linked GalNAc	90 mM GalNAc	8
DBA	*Dolichos biflorus* agglutinin	Alpha-linked GalNAc	90 mM GalNAc	8
UEA-I	*Ulex europaeus* agglutinin I	Alpha 1,2-linked fucose	90 mM fucose	8

GlcNAc: N-acetylglucosamine, GalNAc: N-acetylgalactosamine.

Table 3. Changes of the lectin binding patterns in the kidneys of ICGN cases

	WGA		RCA-I		ConA		PNA		SBA		DBA		UEA-I	
	Gl	Tubules	Gl	Tubules	Gl	Tubules	Gl	Tubules	Gl	Tubules	Gl	Tubules	Gl	Tubules
Normal	Cap	PT, TAL, DT, TL, CD	Cap	PT	Cap	Gl, PT, TAL, DT, TL, CD	Nega	TL, CD	Nega	TL, CD	Nega	TL, CD	Nega	TAL, DT, MD
Case 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Case 2	-	-	-	-	-	-	-	-	-	-	Up (BC)	Up	-	Up
Case 3	-	-	-	Down	-	-		-	-	-	Up (BC)		-	Down
Case 4	-	-	-	Down	-	-	-	-	-	-	-	-	-	-
Case 5	-	-	-	-	-	-	-	-	-	-	Up (BC)		-	Up
Case 6	-	-	-	Down	-	-	-	-	-	-	-	-	-	-
Case 7	-	-	Down	-	-	-		-	-	-	-	-	-	Up
Case 8	-	-	Down	Down	-	-	-	-	-	-	-	-	-	Up
Case 9	-	-	Down	-	-	-	-	-	-	-	-	-	-	-
Case 10	-	-	Down	Down	-	-	-	-	-	-	-	-	-	-
Case 11	-	-	-	-	-	-	-	-	-	-	Up (BC)	Up	-	Down
Case 12	-	-	-	-	-	-	-	-	-	-	Up (BC)	-	-	-
Case 13	-	-	Down	Down	-	-	-	-	-	-	-	-	-	-

-: unremarkable changes compared from normal kidneys, Up: increased staining intensity, Down: decreasing the staing intensity, AA19Gl: glomeruli, Cap: capillary walls, Me: mesangium cells, Po: podocytes, BC: Bowman's capsules, PT: proximal tubules, TAL: thick ascending limbs, DT: distal tubules, TL: thin limbs, CD: collecting ducts: MD: macula densa.

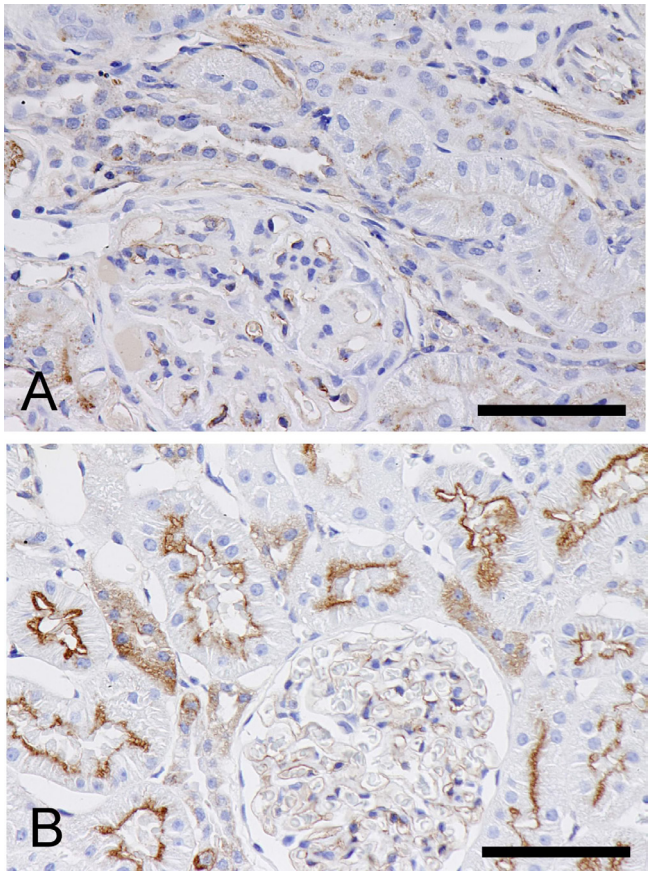

Fig. 1. Histochemical staining for RCA-I lectin. A: a case of ICGN. B: a normal dog. Staining intensity in the brush border of the proximal tubules in the ICGN kidney was weak compared to that in the normal kidney (B). Bars: 80 μm.

RCA-I: In normal kidneys, positive signals were present largely in nephron segments, and the brush border of the proximal tubules (PTs) was especially well-stained with this lectin. In the ICGN kidneys, six out of 12 cases showed weaker staining in the brush border compared with that seen in normal kidneys (Fig. 1). The intensity of the staining signal of the remaining 6 cases was similar to that of the normal kidneys. Brush border of the PT epithelium is composed of microvilli which serves to increase the apical cell surface, and is coated with glycocalyx that is composed of mucins, glycoproteins, and glycolipids. The apical microvilli of the PT are an important structure and function as a sensor of the luminal urine [15]. The glycocalyx covering the microvilli of the PT plays a critical role in sensing and transmitting the force of flowing urine [13]. The decrease of the signal intensity for RCA-I could reflect on the changes in the glycocalyx of the PT, and this change might alter the mechanosensing property of the PT.

DBA: Five out of 12 cases of ICGN showed positive signals on the Bowman's capsules, while normal kidneys were negative for DBA (Fig. 2). Two of these 5 cases also showed an increase of the positive tubules, especially in the thin limbs of the loop of Henle (Fig. 2). The remaining 7 cases had staining characteristics similar to those of the normal kidneys. DBA lectin not only distinguishes alpha-linked N-acetylgalactosamine residues, but is also known as an indicator of cell differentiation [12] and morphogenesis [8]. The Bowman's capsule is an initial segment of the tubule which receives filtrate directly from the glomeruli. Since the glomerular filtration barrier system is known to be collapsed in most of the ICGN cases, initial filtrate which flows into the Bowman's capsule contains a large amount of protein. It is well known that proteins in the filtrate, especially albumin, lead to the overwork of PT epithelium and induce pathological events which lead to progressive tubulointerstitial damage [1]. Although the Bowman's capsule might be considered as a simple cup which receives the filtrate, our results suggest that the protein-rich filtrate may cause functional stress on the capsule, and may induce transformation of the epithelium. DBA lectin may detect this pathological event of ICGN; however, the staining pattern in Bowman's capsule was not related to the severity of proteinuria (Tables 1 and 3).

UEA-I: In normal kidneys, positive signals were observed in the apical surfaces of the thick ascending limbs and distal tubules. These signals were restricted to segments that are close to the glomeruli, and signals in the the macula densa were markedly strong. The binding pattern of UEA-I in normal canine kidney replicates what we described in our previous report [16]. In the ICGN kidneys, expansion of the positive area was observed in four out of 12 cases, and staining signals were not restricted to the segments close to the glomeruli (Fig. 3). Two cases showed weak signals in general, and particularly in the region of the macula

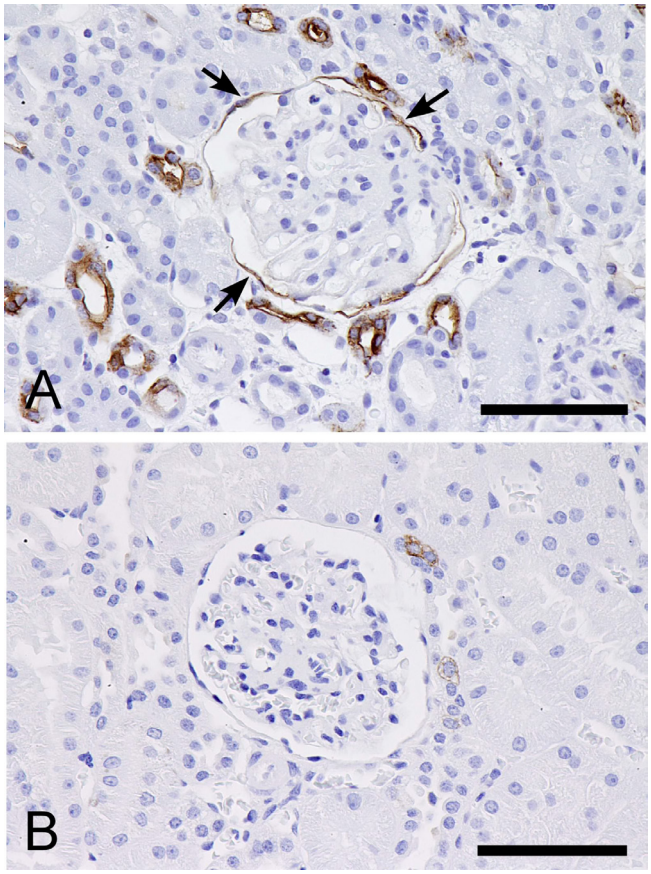

Fig. 2. Histochemical staining for DBA lectin. A: a case of ICGN. B: a normal dog. Bowman's capsule shows positive signal in the ICGN kidney and negative signal in the normal kidney. The thin limbs of the loop of Henle also showed positive signals in ICGN kidneys. Arrows indicate positive signals in Bowman's capsule. Bars: 80 μm.

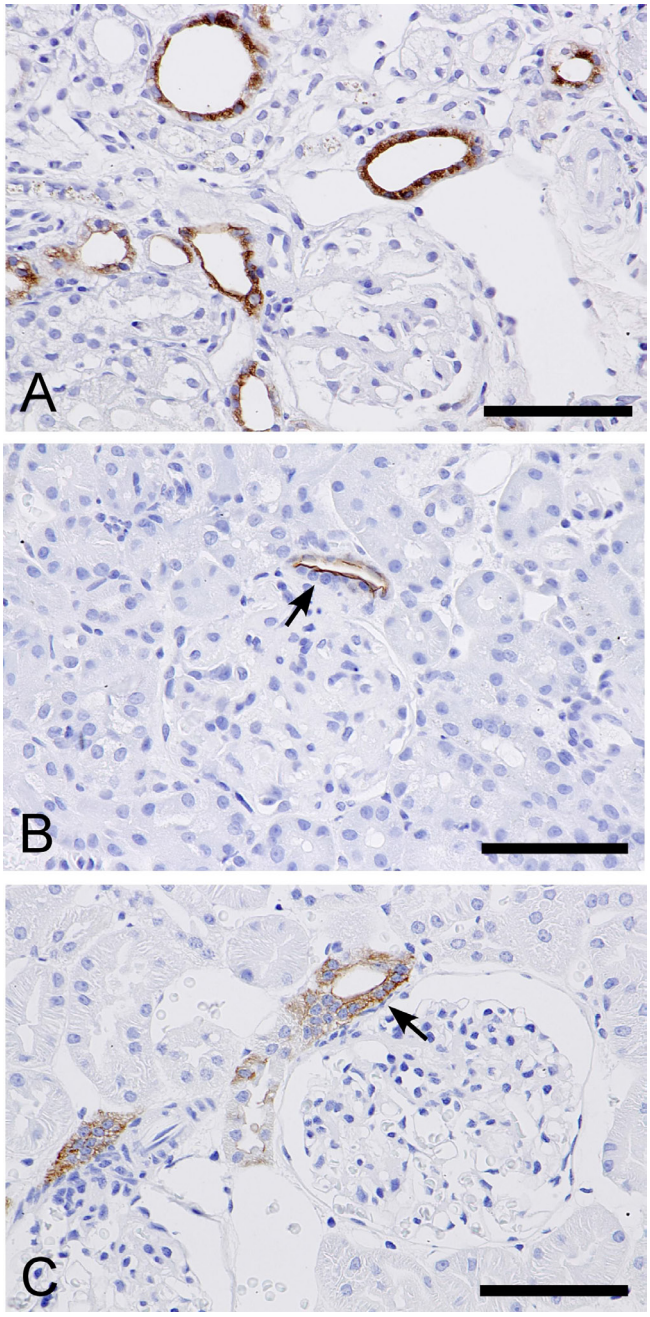

Fig. 3. Histochemical staining for UEA-I lectin. A & B: cases of ICGN. A: positive signals are observed in many of the distal tubules. B: positive signals are restricted to the surface area of the macula densa. C: a normal dog. Arrows indicate the macula densa. Bars: 80 μm.

densa (Fig. 3). The remaining 6 cases showed staining patterns similar to normal kidneys. The macula densa is a specialized region of the thick ascending limb adjacent to the hilum of the glomerulus, and plays an important role in the control of body fluid balance and blood pressure as a sensor of the tubuloglomerular feedback system and the renin-angiotensin system. Changes of the signal intensity of UEA-I might reflect upon the functional changes of the macula densa in the kidneys of ICGN dogs. For example, in human ICGN, upregulation of cyclooxygenase-2, which is constitutively expressed in the macula densa and stimulates the renin-angiotensin system and tubuloglomerular feedback [4, 6], was reported in membranoproliferative glomerulonephritis [7]. However, the present study found no relationship between the staining pattern for UEA-I in the macula densa and the clinical and clinicopathological profiles of ICGN cases (Tables 1 and 3).

The present study documented lectin-binding patterns in the kidneys of canine ICGN. The profiles of binding patterns varied

among the cases, and there was no specific pattern which correlated with the type of ICGN (membranous glomerulopathy, membranoproliferative glomerulonephritis, and others) or its respective clinical features (nephrotic syndrome, hypertension, and stage of chronic kidney disease). The diversity of lectin-binding patterns may reflect the diversity of pathologies among cases, as well as the complicated pathophysiological status of the nephron in canine ICGN.

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