

# A Possible Clue for the Production of Anti-Glomerular Basement Membrane Antibody Associated with Ureteral Obstruction and Hydronephrosis

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## Key Words

Anti-glomerular basement membrane antibody · Anti-glomerular basement membrane glomerulonephritis · Ureteral obstruction

## Abstract

**Background:** Anti-glomerular basement membrane (anti-GBM) antibody-mediated glomerulonephritis (anti-GBM GN) is an autoimmune disease with rapidly progressive glomerulonephritis. Based on a case report of anti-GBM GN following hydronephrosis, we hypothesized that hydronephrosis may act as a trigger for the development of anti-GBM antibodies. **Patients and Methods:** We evaluated 11 patients who were diagnosed with hydronephrosis. It was measured with serum anti-GBM antibody. These patients' medical histories as well as risk factors for the development of anti-GBM antibodies and causes of hydronephrosis were reviewed. Renal function and hematuria were also considered. The serum anti-GBM antibody was measured with enzyme-linked immunosorbent assays (ELISA) or chemiluminescent enzyme immunoassays (CLEIA). Histopathological findings of renal biopsy specimens were also evaluated. **Results:** No patient had a medical history of renal disease. Five patients had a history of smoking. Ten of the 11 patients had renal dysfunction as evidenced by serum creatinine levels of 0.85–13.8 mg/dl, while 8 patients had RBCs in their urinary sediment at the time of diagnosis for hydronephrosis. Two of the patients assessed by ELISA and CLEIA were positive for anti-GBM antibodies. In 1 of these 3 patients, anti-GBM antibodies and renal dysfunction improved upon treatment for hydronephrosis. Another of the 3 patients devel-

oped anti-GBM GN, but anti-GBM antibodies and renal dysfunction improved dramatically upon treatment. In the 3rd patient without improved hydronephrosis, anti-GBM antibodies and renal dysfunction remained unchanged. **Conclusion:** Our results provide insights into the development of anti-GBM antibodies in patients with ureteral obstruction and hydronephrosis.

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## Introduction

Anti-glomerular basement membrane (GBM) antibody-mediated glomerulonephritis (anti-GBM GN) is an autoimmune disease consisting of rapidly progressive glomerulonephritis induced by anti-GBM antibodies. Although the pathological role of the anti-GBM antibodies has been demonstrated [1], the relevant immune responses and precise mechanisms involved have not yet been fully elucidated. GBM is produced by assembly through the interweaving of type IV collagen, while type IV collagen is composed of 6 different  $\alpha$ -chains,  $\alpha$ 1– $\alpha$ 6. The target of anti-GBM antibodies has been identified as the noncollagenous 1 domain (NC1), localized at the C-terminus of the  $\alpha$ 3-chain of the type IV collagen [ $\alpha$ 3(IV)NC1]. Since the native form of intact NC1 is composed of cross-linked hexamers of  $\alpha$ 3,  $\alpha$ 4 and  $\alpha$ 5 of each NC1 [2], the target of anti-GBM antibodies is present as a cryptic antigen. Considering the available evidence, it is hypothesized that a disruption of the stabilized NC1 hexamer and exposure of the cryptic antigen of the  $\alpha$ 3-chain to the host immune system are required for the development of anti-GBM antibodies [3]. Several studies have proposed causes for the modifications of intact NC1, namely smoking, infections, exposure to organic solvent and enforcement of extracorporeal shock wave lithotripsy (ESWL) [4–7].

A case was reported [8] in which the patient developed anti-GBM antibody production with a subsequent onset of anti-GBM GN followed by bilateral ureteral obstruction and hydronephrosis. In experimental animal models, ureteral obstruction induces the secretion of inflammatory cytokines and chemokines, the recruitment of inflammatory cells and the production of reactive oxygen; the glomeruli either remain normal or slightly change [9]. Thus, we are intrigued that the hidden antigen of  $\alpha$ 3(IV)NC1 can be exposed to the host immune system, leading to the development of anti-GBM antibodies within the process of hydronephrosis. The purpose of this clinical study is to clarify the pathogenic relevance of hydronephrosis to the development of anti-GBM antibodies leading to the onset of anti-GBM disease.

## Patients and Methods

In the records of patients with a definite diagnosis of hydronephrosis, we searched for patients in whom serum anti-GBM antibodies were measured at the time of diagnosis or after the treatment of hydronephrosis between 2003 and 2013 at Kitasato University Hospital. Eleven patients were found to participate in this study. For each of these patients, we retrospectively determined clinical features including age at diagnosis of hydronephrosis, sex, medical history of preexisting renal disease, especially membranous nephropathy, causes of hydronephrosis and the presence or absence of clinical risk factors for the development of anti-GBM antibodies such as smoking history, exposure to organic solvents, recent infection prior to the onset of renal impairment and enforcement of ESWL [4–7, 10]. We also investigated their serum creatinine (sCr mg/dl), the degree of hematuria shown as the number of urinary red blood cells (RBCs) per high-power field (HPF), the deformity of urinary RBCs

and RBC cast formation and titer of serum anti-GBM antibodies before and after treatment for hydronephrosis. The histopathological findings of renal biopsy specimens were evaluated for glomerulonephritis. The titer of serum anti-GBM antibodies was measured by outsourced enzyme linked immunosorbent assay (ELISA) until 2010. After 2010, a newly developed chemiluminescent enzyme immunoassay (CLEIA) was employed. The CLEIA method offered better sensitivity and specificity for the detection of anti-GBM antibodies compared to the ELISA method [11]. Titer values of anti-GBM antibodies greater than 10 EU in ELISA and 3 U/ml in CLEIA were regarded as positive. The research was compliant with the Declaration of Helsinki and was approved by the Institutional Review Board for Observation and Epidemiological Study of the Kitasato University School of Medicine (Approval Number: KME0:B14–12).

## Results

Table 1 presents the clinical characteristics of the 11 patients (6 males and 5 females). Their age at the discovery of hydronephrosis ranged from 19 to 75 years. Their medical history showed hypertension in 4 patients, depression treated with medication in 2, and long-term hospitalization for mental retardation in 2. One patient (No. 4) had a nephrotic syndrome and hydronephrosis suspicious of congenital ureteral stenosis of the left kidney. His diagnosis of nephrotic syndrome was compatible with his minimal change nephrotic syndrome. The remaining 2 patients had no relevant medical history. None of the 11 patients had any preexisting renal disease including membranous nephropathy. The causes of their hydronephrosis varied as follows: neurogenic bladder and advanced cervical cancer in 2 patients, ureteral stenosis after surgery, malignant lymphoma, cancer in the renal pelvis, bilateral staghorn calculus, peritoneal adhesion by salpingitis, possible congenital stenosis and nonspecific inflammatory stenosis of bilateral ureters in 1 patient. The only risk factor for producing anti-GBM antibodies was their smoking history, which was present in 5 of the 11 patients.

Table 2 shows the laboratory findings of all 11 patients. Ten patients had renal dysfunction at the discovery of hydronephrosis, with the sCr values ranging from 0.85 to 13.8 mg/dl. Their hematuria was positive as evaluated based on urinary sediments in 8 patients. Only 1 patient (No. 1) had a deformity of the urinary RBCs and cast formation. Three patients (No. 1, 2 and 11) showed increased levels of serum anti-GBM antibodies. Patients 1 and 2 were measured using the ELISA assay, while 11 was evaluated by using the CLEIA assay. Anti-GBM antibodies were found in patient 2 and 11 at the initial detection of hydronephrosis, while in patient 1, an increase in anti-GBM antibodies was found 12 days after treatment for hydronephrosis. Patient 1 developed a rapidly progressive glomerulonephritis in spite of a complete improvement of her bilateral hydronephrosis. She was diagnosed with anti-GBM GN based on a renal biopsy specimen. Patients 2 and 11 showed slight increases in sCr levels and microscopic hematuria. In patient 2, the renal dysfunction gradually improved, and hematuria disappeared after a complete improvement of his hydronephrosis. It is of note that his anti-GBM antibody level decreased to within the normal range upon improvement of hydronephrosis. In patient 11, whose hydronephrosis did not improve, anti-GBM antibodies and renal function likewise did not improve after treatment.

In the remaining 8 patients with negative serum anti-GBM antibodies, all except patient 4 were treated for hydronephrosis. Six out of these 7 patients showed a complete improvement of hydronephrosis, and all of these 6 patients simultaneously showed commensurate improvement in renal function.

### Case Presentation of Patient 1

Patient 1, a 26-year-old woman, had experienced a low-grade fever and abdominal pain for 1 month prior to admission. Macrohematuria also appeared 10 days before admission. She had no recent abdominal trauma. On admission, her body temperature was 37.5°C. No significant physical findings were observed in her chest and abdomen. Bilateral knocking dullness at a cost-vertebral angle was shown. The other examination was not significant, including skin lesions, lymphadenopathy, neurological findings and gynecological investigation. Urinalysis showed 0.2 g/day of protein and microscopic hematuria of 50–100 of RBCs/HPF with RBC deformity and RBC casts. A complete blood count in the peripheral blood revealed a white blood cell count of 10,800/μl. The laboratory data were as follows: serum urea nitrogen 13 mg/dl, and sCr 1.15 mg/dl. A chest X-ray did not show any abnormal findings. Meanwhile, an abdominal ultrasonography revealed bilateral hydronephrosis. An MRI study revealed bilateral hydronephrosis with focal stenosis of the right ureter at the pelvic-ureteral junction (fig. 1). Radiologists excluded retroperitoneal fibrosis, a tumor invasion and infection around the ureters, or prominent pelvic lesions. Retrograde pyelography and intravenous pyelography revealed a complete obstruction of the right ureter and an advanced constriction of the left ureter. Bacteriological examinations, including *Mycobacterium tuberculosis* in blood and urine, were negative. On the 9th hospital day, a stent graft was inserted in each ureter, leading to a complete improvement of the hydronephrosis. Nevertheless, the patient experienced a progressive renal dysfunction and deterioration in macrohematuria. Her sCr increased to 3.4 mg/dl, and her anti-GBM antibody count was 69 EU (normal value <10 EU) on the 21st hospital day. An open renal biopsy of the left kidney on the 25th hospital day revealed the presence of anti-GBM GN. Renal biopsy specimens revealed over 90% of glomeruli presenting with exuberant circumferential cellular crescents and the compression of the underlying tuft (fig. 2a). Immunofluorescent findings revealed intense and diffuse linear deposits of IgG and C3 (data not shown) along with GBM (fig. 2b). Her sCr and anti-GBM antibodies had worsened to 3.80 mg/dl and 112 EU, respectively, by the 26th hospital day. A plasma exchange and steroid pulse therapy followed by oral steroid treatment were administered, starting on the 28th hospital day. The patient's sCr and anti-GBM antibodies had decreased to 2.2 mg/dl and 28 EU by the 42nd hospital day. By the 80th hospital day, her anti-GBM antibodies had returned to normal, with a titer of <10 EU, and her sCr level had decreased to 1.7 mg/dl. The stenotic lesion of the right ureter was biopsied to investigate the cause of hydronephrosis. However, we were not able to determine the cause of her hydronephrosis.

### Case Presentation of Patient 2

Patient 2, a 60-year-old male, had been chronically hospitalized due to mental retardation and cervical spondylosis. A urinary balloon tube had previously been inserted because of a neurogenic bladder. At that time, the patient's renal function had been mostly stable, with an sCr of around 0.4 mg/dl and no abnormal urinary findings. He was referred to our hospital due to urinary retention. At the time of his referral to Nephrology, bilateral hydronephrosis was found, and sCr had worsened to 0.85 mg/dl, while his serum anti-GBM antibodies had increased to 32 EU. His urinary balloon tube was replaced on the day of admission, and bilateral hydronephrosis completely recovered shortly thereafter. His sCr level continued to fluctuate; however, it peaked at 1.74 mg/dl 30 days after treatment for his hydronephrosis. On the same day, however, his anti-GBM antibody level had decreased to with-

in the normal range (<10 EU). Deformity of urinary RBCs and RBC cast formation were not observed during the follow-up. Since the patient's sCr level remained at 1.5 mg/dl, he was discharged on the 37th hospital day.

## Discussion

To confirm the hypothesis that hydronephrosis serves as a trigger for the development of anti-GBM antibodies, we investigated the clinical features and laboratory data of 11 patients with a definite diagnosis of hydronephrosis and available data on serum anti-GBM antibody titers.

None of these 11 patients had a preexisting renal disease or any of the clinical risk factors known to be associated with the development of anti-GBM antibodies, namely recent infection, exposure to solvent or ESWL. Although 5 of the 11 patients did have a history of smoking, serum anti-GBM antibodies were not detected in any of these patients.

Three out of 11 patients were positive for anti-GBM antibodies at the discovery of hydronephrosis or after the treatment for it. Notably, in patient 2, deterioration of renal function and hematuria coincided with worsening serum levels of anti-GBM antibodies, whereas anti-GBM antibody levels improved to within the normal range as ureteral obstruction and hydronephrosis also improved. This indicates that exposure to autoantigens can be eliminated by the improvement of hydronephrosis. These results appear to support our hypothesis that ureteral obstruction and subsequent hydronephrosis is associated with the development of anti-GBM antibodies.

Patient 1 recovered completely from hydronephrosis, yet subsequently experienced severe anti-GBM GN. Urologists commented that bilateral ureteral obstruction and hydronephrosis were considered to have advanced in the course of at least monthly duration. Also, since renal dysfunction rapidly progressed after admission, we considered the development of anti-GBM antibodies and the onset of anti-GBM GN temporarily occurred following ureteral obstruction and hydronephrosis. Patient 11 showed an incomplete improvement of his hydronephrosis; accordingly, the renal function and hematuria remained unchanged. The clinical situations of these 2 patients are compatible with the hypothesis that ureteral obstruction and hydronephrosis are involved in anti-GBM antibody production.

On the other hand, the 8 patients without anti-GBM antibodies did not exhibit a worsening of their renal dysfunction after the treatment for hydronephrosis. There may be several explanations for the absence of anti-GBM antibodies in these patients. First, the methodology of measurement must be considered. ELISA is not as sensitive as CLEIA for detecting lower levels of anti-GBM antibodies. At present, since we used CLEIA rather than ELISA at our clinic, it is likely that additional anti-GBM antibody-positive patients like patient 11 would be found in the future. Secondly, appropriate human leukocyte antigen (HLA) predisposition is important to allow autoantigen presentation. HLA-DRB1\*1501 is a common allele for the development of anti-GBM disease in various populations [12]. Thus, HLA should be further evaluated in future studies.

Based on several studies, Weber et al. [8] have proposed the following mechanism for the development of anti-GBM antibodies in ureteral obstruction and hydronephrosis: (1) excreted urine NC1 may enter the renal interstitium under conditions of ureteral obstruction, whereupon (2) NC1 hexamer dissociates under acidic conditions induced by interstitial inflammation. In addition, appropriate HLA predisposition may be required for the development of autoantibodies.

Among the 3 patients who produced anti-GBM antibodies, only patient 1 developed severe anti-GBM GN. Two important factors are involved in determining the development and severity of anti-GBM GN: (1) anti-GBM IgG subclass, and (2) antigen epitope spreading. The IgG1 subclass was predominant in patients with impaired renal functions [13]. Also, epitope spreading, defined as the development of immune reactions to antigen epitopes distinct from and noncrossreactive with the original epitopes, amplifies the deleterious process of anti-GBM GN [14].

In conclusion, we have identified a possible clue for the induction of anti-GBM antibodies in patients with ureteral obstruction and hydronephrosis. Further studies are required to elucidate our hypothesis.

### Disclosure Statement

All authors declare that they have no financial relationship with any organization that might have an interest in the submitted work.

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**Table 1.** Characteristics of the patients with hydronephrosis and their risk factors for producing anti-GBM antibodies

Patient No.	Age at diagnosis, years	Sex	Medical history	Causes of hydronephrosis	Risk factors for producing anti-GBM antibodies			
					smoking (B.I.)	exposure to solvent	recent infection	ESWL
1	26	F	N	Bilateral ureteral stenosis due to inflammatory disease <sup>a</sup>	N	N	N	N
2	66	M	Long-term hospitalization	Neurogenic bladder	N	N	N	N
3	70	M	HT	Malignant lymphoma	N	N	N	N
4	19	M	Nephrotic syndrome at diagnosis	Congenital ureteral stenosis (suspected)	N	N	N	N
5	58	F	N	Cervix Ca (IVb)	N	N	N	N
6	30	F	Depression	Adhesion of ureter by salpingitis of ovary	20 × 11	N	N	N
7	70	M	HT	Renal pelvic cancer	20 × 13	N	N	N
8	75	M	HT	Postoperational stenosis	40 × 40	N	N	
9	69	M	HT	Staghorn calculus	20 × 48	N	N	N
10	50	F	Depression	Cervix Ca (IIIb)	10 × 30	N	N	N
11	28	F	Long-term hospitalization	Neurogenic bladder	N	N	N	N

F = Female; M = male; N = noncontributory; HT = hypertension; B.I. = Brinkman Index (number of cigarettes per day × duration of smoking in years).

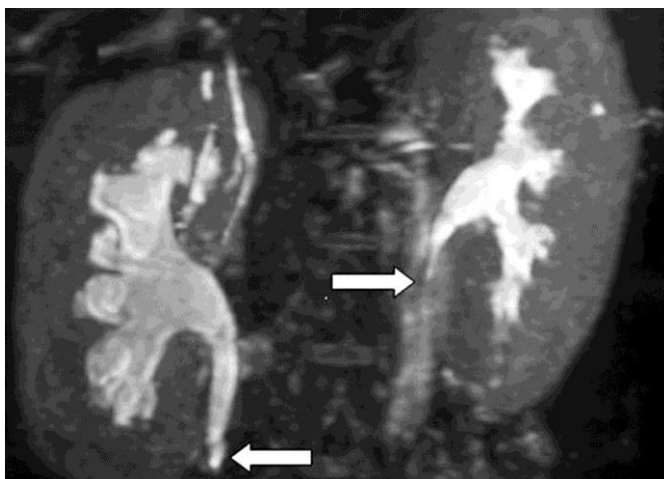
<sup>a</sup>Ureteral biopsy from a stenotic lesion showed fibrous tissue and slightly mononuclear cell infiltration after treatment.

**Table 2.** Laboratory findings of the patients

Patient No.	At diagnosis of hydronephrosis			Improvement of hydronephrosis	After treatment for hydronephrosis			Assay for a-GBM Ab	Diagnosis of renal biopsy specimen
	sCr mg/dl	hematuria RBCs/HPF	a-GBM Ab		sCr mg/dl	hematuria RBCs/HPF	a-GBM Ab		
1	1.15	50–100 <sup>1</sup>	N	Complete	3.4	>100 <sup>1</sup>	69 EU <sup>2</sup>	ELISA	a-GBM GN
					3.8	>100 <sup>1</sup>	112 EU <sup>3</sup>		
					1.7	20–30 <sup>1</sup>	<10 EU <sup>4</sup>		
2	0.85	>100	32 EU	Complete	1.7	10–20	12 EU <sup>5</sup>	ELISA	N
					1.5	1–4	<10 EU <sup>6</sup>		
3	9.8	5–8	<10 EU	Complete	1.5	1–4	N	ELISA	N
4	0.57	1–4	<10 EU	Incomplete (no treatment)	0.6	1–4	N	ELISA	N
5	7.8	5–9	<10 EU	Complete	1.6	1–4	N	ELISA	N
6	1.37	>100	<10 EU	Complete	0.7	>100	N	ELISA	N
7	2.8	1–4	<10 EU	Complete	1.6	1–4	N	ELISA	N
8	3.9	1–4	<10 EU	Complete	1.8	1–4	N	ELISA	N
			<3.5 IU/ml					CLEIA	
9	6.6	50–99	<10 EU	Incomplete	5.8	50–99	N	ELISA	N
10	13.8	>100	<3.5 IU/ml	Complete	0.84	5–8	N	CLEIA	N
11	1.18	10–19	6.5 IU/ml	Incomplete	1.2	5–8	6.7 IU/ml	CLEIA	N

N = Not tested; a-GBM Ab = anti-glomerular basement membrane antibody.

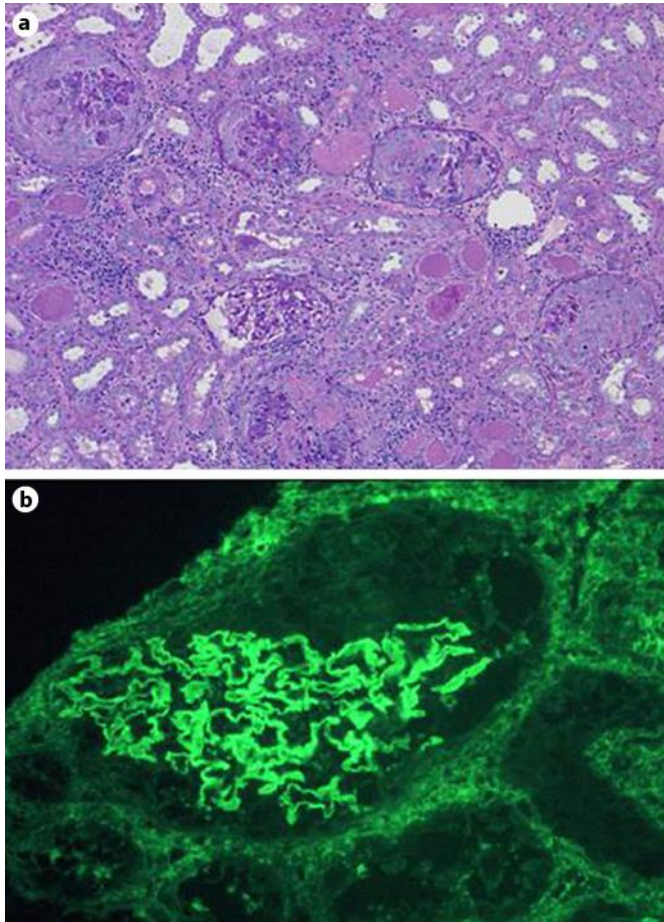
<sup>1</sup> Urinary RBC sediment with deformity and cast; <sup>2</sup> on hospital day 21, first-time measurement of a-GBM ab; <sup>3</sup> on hospital day 26, at renal biopsy; <sup>4</sup> on hospital day 80, at discharge; <sup>5</sup> on hospital day 30, at the peak of sCr; <sup>6</sup> on hospital day 37, at discharge.



**Fig. 1.** MRI study of the abdomen revealed bilateral hydronephrosis due to severe stenosis of both right and left ureters at the pelvic-ureteral junction level (arrows).



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**Fig. 2.** **a** Diffuse crescentic glomerulonephritis with large circumferential cellular crescents and severe compression of the glomerular tuft (periodic acid-Schiff stain; magnification  $\times 200$ ). **b** Direct immunofluorescence staining shows linear glomerular basement membrane deposits of IgG (magnification  $\times 400$ ).