Table 1 The original disease in patients with RPGN studied

	Apheresis group (9)	Non-apheresis group (30)
MPO-ANCA associated vasculitis	3	18
MPO-ANCA associated nephritis	2	7
Pauci-immune glomerulonephritis without ANCA	1	0
Anti-GBM antibody nephritis	1	2
PR3-ANCA associated vasculitis	0	1
IgA nephropathy	2	2

RPGN: rapidly progressive glomerulonephritis, MPO-ANCA: myeloperoxidase-antineutrophil cytoplasmic antibody, GBM: glomerular basement membrane.

Table 2 Comparison of clinical parameters between apheresis group and non-apheresis group on admission

	Apheresis group	Non-apheresis group	
Number of patients	9	30	n.s.
Male/Female	6/3	11/19	n.s.
Age [year]	55.9±12.7	64.2±12.5	n.s.
RBC [high-power field]	77.2±31.5	54.4±38.6	n.s.
proteinuria [g/day]	3.4±2.5	1.5±1.4	p = 0.007
24hCcr [ml/min]	21.5±17.1	19.3±18.6	n.s.
Serum total protein [g/dl]	6.3±0.6	6.4±0.7	n.s.
Serum albumin [g/dl]	3.1±0.5	3.1±0.9	n.s.
BUN [mg/dl]	51.3±27.0	48.9±30.0	n.s.
Serum creatinine [mg/dl]	4.7±2.7	4.4±3.0	n.s.
CRP [mg/dl]	4.9±8.3	9.3±9.9	n.s.
WBC [/μl]	9,714.4±4,446.2	11,125.7±5,206.1	n.s.
MPO-ANCA [IU/ml]	368.6±315.9	388.8±2,971.0	n.s.

RBC: red blood cells, Ccr: creatinine clearance, BUN: blood urea nitrogen, CRP: C-reactive protein, WBC: white blood cells, MPO-ANCA: myeloperoxidase-antineutrophil cytoplasmic antibody.

change cases.

The original disease in RPGN was shown in Table 1. MPO-ANCA associated disease was seen in 5/9 (55.6%) in the apheresis group and 25/30 (83.3%) in the non-apheresis group. It accounted for the majority in both groups. Anti-GBM antibody nephritis was seen in 1 apheresis group case and 2 non-apheresis group cases. The patient with anti-GBM antibody nephritis in the apheresis group also had pulmonary hemorrhage at the time of admission, her serum Cr was 9.39 mg/dl, and hemodialysis was needed during therapy. Another patient in the non-apheresis group had a serum Cr of 1.73 mg/dl. According to our RPGN criteria, each group included 2 IgA nephritis cases, while others had only vasculitis.

Table 2 shows the characteristics of each group on admission. No difference in age or sex was observed in the either group. On blood tests, the average serum Cr level was 4.7±2.7 mg/dl in the apheresis group and 4.4±3.0 mg/dl in the non-apheresis group. CRP was 4.9±8.3 mg/dl in the former, and 9.3±9.9 mg/dl in the latter, and there were no statistically significant differences in renal function or inflammatory reactions. As for proteinuria, on admission 4/9 were in a nephritic state in the former, 4/30 in the latter, and the average amount was 3.4±2.5 g/day (0.38-6.44 g/day), with only the 1.5±1.4 g/day (0.12-5.58 g/day) difference being significant (p=0.007). Concerning MPO-ANCA positive patients, the titer was 368.6±315.9 IU/ml in the former, 388.8±297.0 IU/ml in the latter, and the difference was not significant. In treatment regimen, cyclophosphamide therapy was added to 2 patients in apheresis group and 1 patient in non-aphersis group.

Fig. 1 shows proteinuria reduction rate 8 weeks after starting treatments. As compared with the

Short-Term Effects of Apheresis on Renal Function and Proteinuria in the Treatment of Rapidly Progressive Glomerulonephritis

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Table 3 Renal prognosis 8 weeks after starting treatments

a: Outcome of renal death after 8 weeks			
	Renal death	Survival	Total
Apheresis group	0	9	9
Non-apheresis group	11	19	30
	11	28	39
b: Outcome of death after 8 weeks			
	Death	Survival	Total
Apheresis group	0	9	9
Non-apheresis group	3	27	30
	3	36	39

sis, but all later withdrew. In the non-apheresis group, 3 died and their initial diagnosis was MPO-ANCA associated disease. The causes of the death were 2 infections, and 1 gastrointestinal hemorrhage. None of the apheresis group patients died.

As for histological examination, the renal biopsy rate was 7/9 in the apheresis group (77.8%) and 22/30 (66.7%) in the non-apheresis group. The number of glomeruli was 20.6±14.7 in the former and 25.8±18.4 in the latter group, and neither the rate of crescent formation (73.7±16.7% in the former and 63.7±31.1% in the latter) not that of glomerulosclerosis (28.8±26.3% in the former, and 33.2±29.5% in the latter) differed significantly the between the two.

Furthermore, among the ANCA positive patients in the apheresis group, the ANCA titer after conducting apheresis was 116.8±137.7 IU/I, resulting in a rapid reduction in comparison to the amount on admission (368.6±315.9 IU/I). As compared to the non-apheresis group 8 weeks after starting treatment, it was 106.6±155.4 IU/I, p=0.89, not a statistically significant difference.

Multivariate analyses were performed to examine if apheresis was a useful predictor for improvement of serum Cr as selecting dependent variables for age, sex, proteinuria, MPO-ANCA titer, CRP, steroid pulse therapy and total steroid doses. Plasma exchange was a significant predictor for improvement of serum Cr (p=0.0068), while not in age (p=0.855), sex (p=0.915), proteinuria (p=0.1335), MPO-ANCA titer (p=0.680), CRP (p=0.245), steroid pulse therapy (p=0.478) and total steroid doses (p=0.5774).

The final outcome of RPGN was judged at 6 months after starting treatment. There were 11 of 30 patients in the non-apheresis group needed hemodialysis. On the other hand, no patients in the apheresis group needed on going hemodialysis.

Discussion

RPGN, if not treated, will proceed to end-stage renal disease in several weeks or months[2]. In an attempt to improve RPGN prognosis, it is important to diagnose the types of renal dysfunction immediately and to start prompt treatment. However, vasculitis, which accounts for most RPGN may progress rapidly and there is not enough time to wait for the results of serological tests or renal biopsy in many cases. Furthermore, many patients are elderly, and there are dangers such as fatal complications involving infections with immunosuppression. We should thus be cautions in starting this therapy. In case given steroids, it is advisable to start this treatment after diagnosing the clinical state including histological findings and serological tests.

Although apheresis is associated with, such complications as blood pressure depression accompanied by extracorporeal circulation or allergy to blood preparations used for exchanging with plasma and anti-coagulation, there is little risk of side effects as compared to using steroids[4]. There are various opinions on treatment effects for RPGN. In anti-GBM antibody-nephritis, apheresis efficacy has been reported based on reductions in serum anti-GBM antibodies and the serum Cr level, and even reduction of progression to terminal renal insufficiency, etc., as shown by randomized control trials (RCT) conducted by Johnson et al[5] and several other studies. The treatment guidelines of crescentic nephritis by the European Vasculitis Study Group recommend plasma exchange for cases with serum Cr 600 μmol/L or accompanying lung lesions. In Japan's RPGN treatment guidelines, immunosuppressant treatment is adopted according to the clinical state for ANCA-associated disease, and apheresis is not included therein.

Table 4 shows studies of the efficacy of apheresis therapy in RPGN identified by a through literature

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ORIGINAL ARTICLE

Tubulointerstitial nephritis without glomerular lesions in three patients with myeloperoxidase-ANCA-associated vasculitis

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Abstract
Background Myeloperoxidase–antineutrophil cytoplasmic antibody (MPO–ANCA)-associated vasculitis frequently induces crescentic glomerulonephritis. However, a few cases have so far been reported to have only tubulointerstitial (TI) nephritis without any apparent glomerular lesions. We recently treated three similar cases. Therefore, their pathological features as well as clinical manifestations were studied in detail.
Methods The pathological study was performed with immunohistochemical staining using various antibodies to the vascular endothelial cell surface markers, von Willebrand factor, type IV collagen, cytokeratin, E-cadherin, and MPO in addition to the routine histochemical examination.
Results The study disclosed the loss of CD34 endothelial cell surface markers with and without the destruction of type IV collagen (capillary basement membrane) in the peritubular capillaries, even though the glomeruli showed good staining of these factors. Electron microscopy showed breaks in the capillary basement membrane. The loss of CD34 staining was associated with the infiltration of a few mononuclear cells and neutrophils in the lumen of peritubular capillaries and the surrounding interstitial tissues. The cytokeratin staining in the tubular epithelial cells was also diminished around these areas. Tubulitis was demonstrated with or without the destruction of the tubular basement membrane. The clinical manifestations of these three cases were only a few red blood cells and granular casts in the urinary sediment as well as slightly increased β2-microglobulin in the urine, but no proteinuria.
Conclusion Based on these findings, the loss of CD34 vascular endothelial markers occurs in the early phase of the disease because of the MPO, which is presumed to have burst out from the infiltrated, activated neutrophils. This MPO, which releases proteolytic enzymes and radical oxygen species, acts on tissue destruction, namely the lysis of endothelial cell membranes as well as vascular basement membranes in the peritubular capillary. This mechanism eventually proceeds to the destruction of the peritubular capillary walls (vasculitis). This pathogenesis is thought to play an important role in the pathogenesis of TI nephritis, which is associated with MPO–ANCA vasculitis.

Keywords Tubulointerstitial nephritis · MPO–ANCA · Peritubular capillaritis · Tubulitis

These cases were presented at the 2006 American Society of Nephrology meeting, San Diego, 15 November 2006, and at the 12th Tubulointerstitial Diseases Meeting, Tokyo, 13 September 2008.

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Introduction

Myeloperoxidase–antineutrophil cytoplasmic antibody (MPO–ANCA)-associated vasculitis is a disease that mainly affects the capillaries and commonly induces necrotizing vasculitis in the lung and kidney. The typical histopathology of the kidney is crescentic glomerulonephritis and is usually associated with the tubulointerstitial (TI) lesions to some extent. Most of these TI lesions in this

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proteinuria level was higher in the apheresis group. If proteinuria is considered to be an index of renal dysfunction, the apheresis group has more aggressive renal injury than the non-apheresis group. Reduction rates of proteinuria after 8 weeks were evaluated,as shown in Fig. 1. Reduction of proteinuria was to 40.6±27.7% in the apheresis group vs 30.3±44.0% in the non-apheresis group, not a significant difference. We compared therapy results based on total steroid doses for 8 weeks (Fig. 2). The apheresis group showed significantly lower amount, i.e. had the same reduction in proteinuria as the non-apheresis group receiving lower steroid doses.

Furthermore, there were no renal deaths or any other deaths in the apheresis group (Table 3a, b). Although the two groups did not differ in serum Cr level or creatinine clearance on admission, all 3 HD patients in the apheresis group were weaned from dialysis, while 11 of 17 patients in the non-apheresis group were maintained on dialysis. There were 3 deaths in the non-apheresis group, of whom 2 (due to infection) were considered to have been affected by immunosuppressant side effects. Steroid therapy plays an important role in RPGN, but its side effects are often fatal and severe, e.g gastrointestinal hemorrhage, infection, etc. Lower total steroid doses are considered to be a reason for the improved prognosis in the apheresis group. Our present study is the first to show that both renal and life prognoses are better in RPGN patients receiving apheresis combination therapy.

The principle of apheresis utility has yet to be resolved, and there are reports describing depletion of immune complexes or inflammatory mediators[17], i.e immunoreactive modifications[18][19]. Herein, we cannot rule out the possibility of rapid and considerable reductions in ANCA titers with apheresis. We considered apheresis to decrease immune complexes, the inflammatory factors determining treatment efficacy in ANCA negative RPGN patients. Therefore, apheresis was effective in cases with and without ANCA.

As to the primary diseases, ANCA rates were higher in both groups (Table 1). The primary disease causing the 3 deaths was ANCA-associated disease. There were no deaths in the apheresis group probably because of the low number of ANCA-associated disease cases. However, there was no difference in systemic state on admission and progress after admission between the two groups (Table 2). This result is considered to be valuable, though BVAS scores were not compared due to the retrospective nature of the study[20]. Comparing only ANCA-associated disease cases, rapid and remarkable ANCA titer reductions after apheresis were seen in our study as well. The evaluation at 2 months after starting therapy showed no difference in ANCA titers between the two groups, and that each group showed significant reductions. There are no reports indicating that lowering ANCA in blood may rapidly lead to the improvements in disease activity and the relation between the absolute ANCA titer and disease conditionsis not clear. Even though ANCA is the first sign of vascular endothelial dysfunction, reducting ANCA in the early stage of disease is very meaningful. Apheresis can directly eliminate pathogenic factors in plasma until the efficacy of immunosuppressants, including steroids, manifests during the acute phase. Immunosuppressive therapy is greatly enhanced by apheresis. Certain reports have recommended combined use of apheresis for cases with high serum Cr levels or for RPGN cases on dialysis. In our study, there were no significant differences in serum Cr level on the whole, and 3/9 patients in the apheresis group needed dialysis on admission, while 17/30 in the non-apheresis group required dialysis. The results indicate that apheresis in the early phase is effective, depending on individual patient status, even for cases in whom renal function had not deteriorated at the beginning of the therapy.

There were a few limitations in our study. The study was a retrospective analysis with a short term observation period. Therefore, we should be careful to interpret the efficacy of apheresis on the clinical outcomes in patients with RPGN. However, the final outcome of RPGN patients in apheresis group needed on going hemodialysis 6 months after starting treatment.

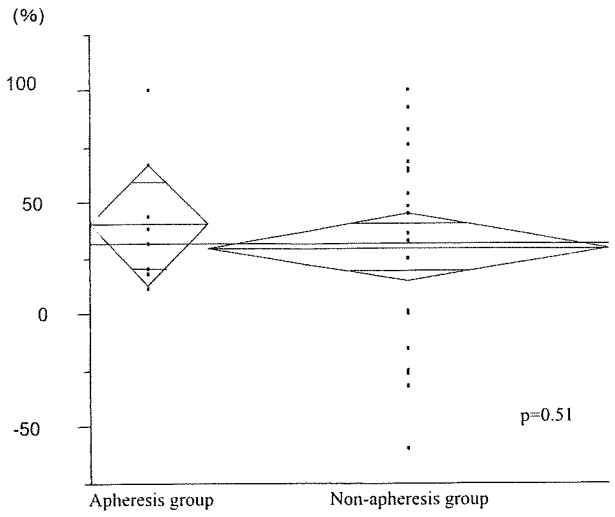

Fig. 1 Proteinuria reduction rate 8 weeks after starting treatments
As compared with the time of admission, the apheresis and non-apheresis groups demonstrated reductions in proteinuria levels, with no significant difference in those rates (p=0.51).

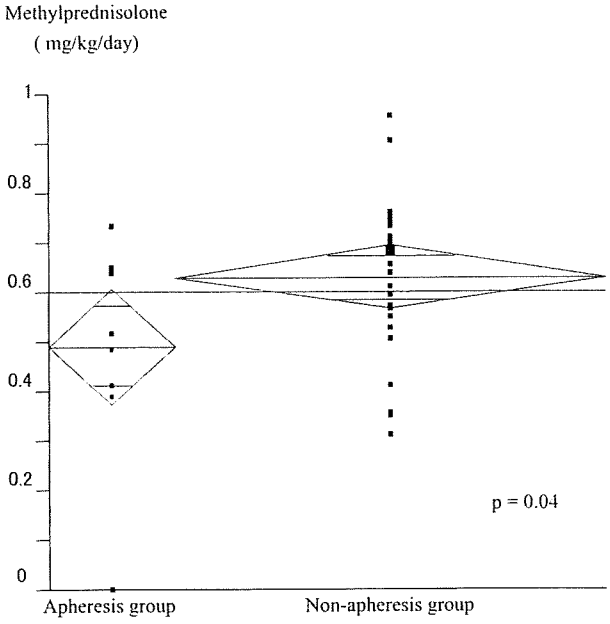

Fig. 2 Total steroid dosage for 8 weeks per 1 kg per day
In each group, the rate of patients who received steroid pulse therapy against those who did not, did not differ significantly. The dose was evaluated with the amount excluding the pulse.

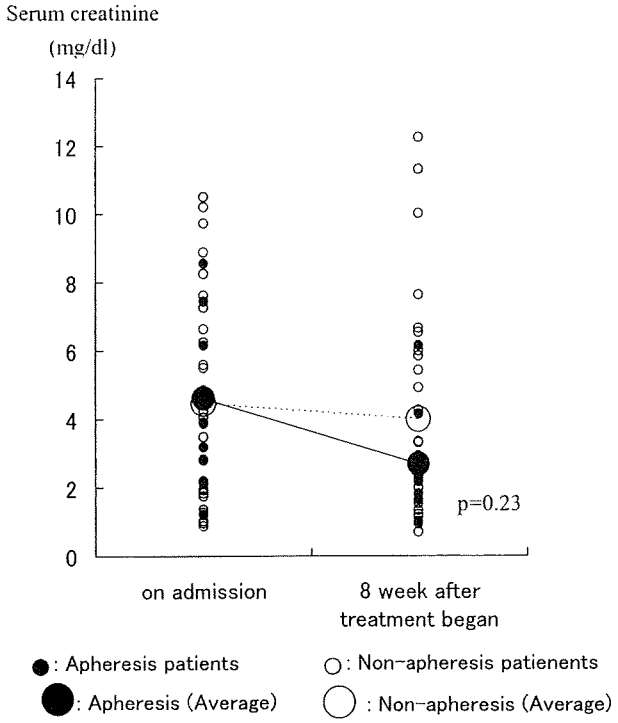

Fig. 3 Serial changes of serum creatinine levels 8 weeks after treatment
Large circles show average of serum creatinine in each group. Small circles show individual serum creatinine level. Large black circle shows that the average serum creatinine level tends to be decreased (p=0.23).

time of admission, the apheresis and non-apheresis groups demonstrated reductions in proteinuria levels, i.e. 40.6±27.7% and 30.3±44.0% with no significant difference in those rates (p=0.51). Total steroid dosage for 8 weeks per 1 kg per day is provided in Fig. 2. In each group, the rate of patients who received steroid pulse therapy against those who did not, did not differ significantly (6/9 patients of the former group, 17/30 patients of the latter it ; p=0.59, χ^2 test). The dose was evaluated with the amount excluding the pulse. As shown in Fig. 2, it was 0.49±0.22 mg/kg/day in the former, and 0.63±0.16 mg/kg/day in the latter, indicating a significantly smaller steroid dose in the apheresis group (p= 0.04). Fig. 3 shows serial changes of serum Cr levels. In the apheresis group, serum Cr tended to improve more than in the non-aphresis group (p=0.23).

Table 3a shows renal prognosis 8 weeks after starting treatments, and Table 3b life prognosis. There were 17/30 patients who needed hemodialysis during the process in the non-apheresis group. Of these 6 withdrew from hemodialysis, but 11 needed on going treatment. On the other hand, in the apheresis group, 3/9 initially needed hemodialy-

急速性進行性糸球体腎炎の治療における血漿交換療法

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急速進行性糸球体腎炎（rapidly progressive glomerulonephritis；RPGN）の治療において，副腎皮質ステロイド薬などの免疫抑制薬が第一選択であるが，血漿交換療法（plasma exchange；PE）も治療の一環として施行されることがある．本研究の目的は，RPGNの治療におけるPEの有効性を検討することである．我々は1995～2005年までの10年間で，39例のRPGNに対する治療法に関して，PE施行群とPE未施行群に分けて，後ろ向きに検討した．1例を除けば，全例で副腎皮質ステロイド薬の投与を受けていた．PE施行群の9例では，二重膜濾過あるいは単一膜血漿交換法による治療を，2～7回に渡って施行されていた．両群間における腎機能と炎症マーカーには有意差を認めなかった．治療開始時の尿蛋白量は，PE施行群で3.4±2.5 mg/dl(0.38～6.44 g/日)，PE未施行群では1.5±1.4 mg/dl (0.12～5.58 g/日）で有意差を認めた（p＝0.007）．しかし，治療期間中の総ステロイド投与量は，PE未施行群の0.63±0.16 mg/kg/日に対して，PE施行群では0.49±0.22 mg/kg/日と有意に少なかった（p＝0.04）．両群の腎機能と予後は，PE未施行群に比しPE施行群で良好であった．これらの結果から，RPGNの治療においては，免疫抑制薬とPEの併用により，腎保護と予後の改善が期待できると考えられた．

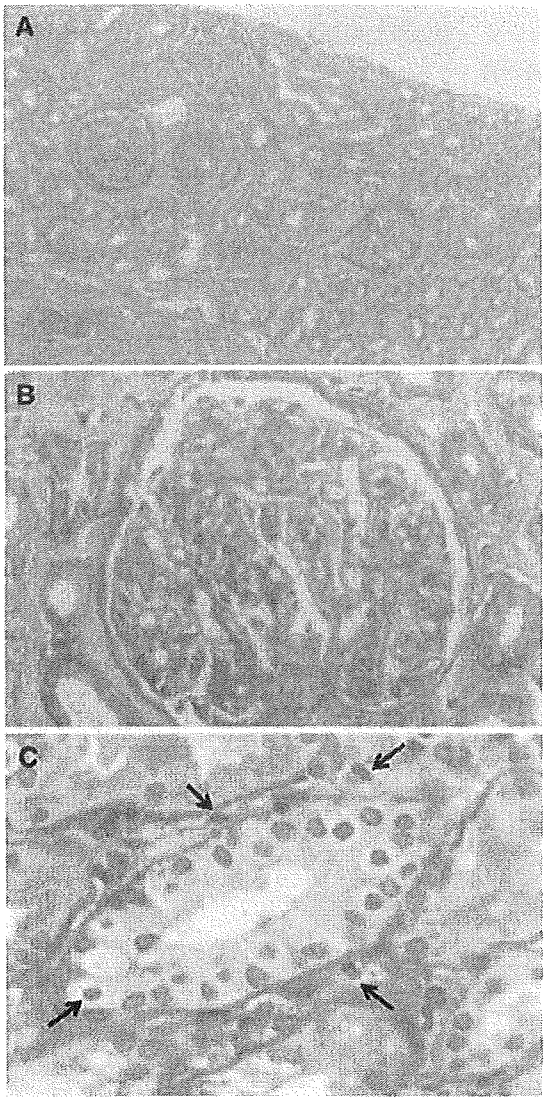

Fig. 1 Case 1: Light microscopy shows an almost normal appearance of glomeruli and slight infiltrations of cells in the interstitium. High magnification demonstrates the infiltration of a few neutrophils and/or mononuclear cells in the peritubular capillaries as well as tubular epithelial cells (*arrows*) (PAS staining, **a** ×100, **b** ×400, and **c** ×600)

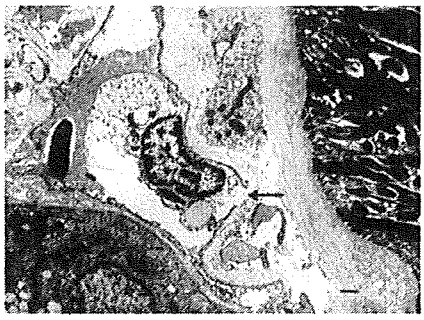

Fig. 2 Case 1: Electron microscopy reveals the rupture of peritubular capillary basement membrane (an *arrow*, ×8,000). A *bar* indicates 1 μm in length

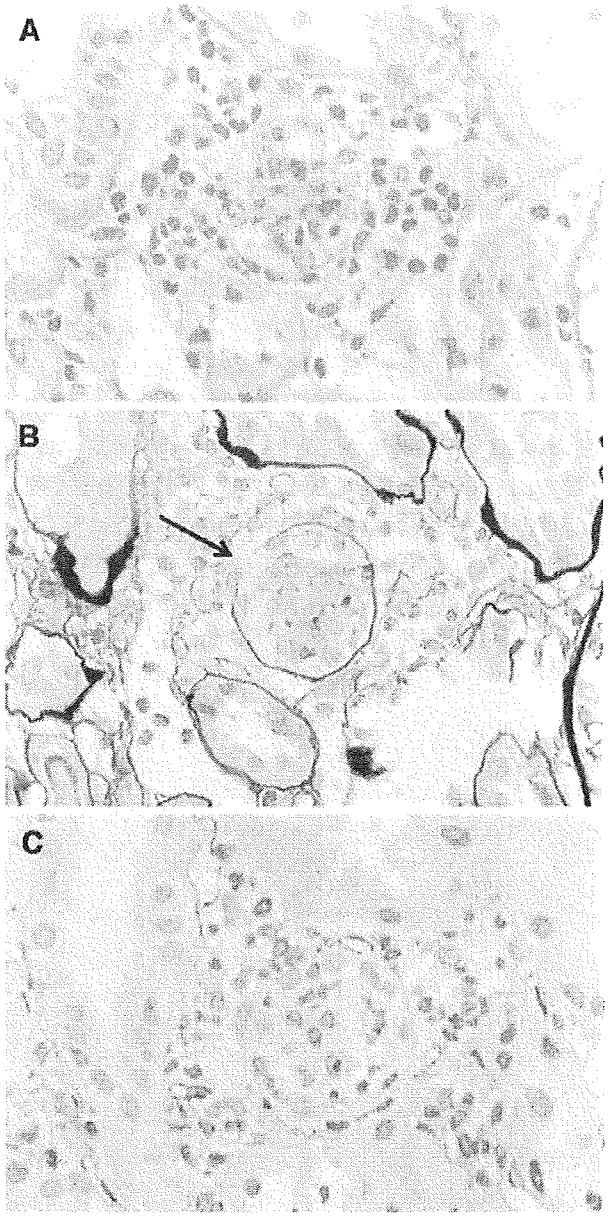

Fig. 3 Case 2: Serial sections. Tubulitis is associated with the desquamation of the tubular epithelial cells and infiltrating neutrophils as well as mononuclear cells, and shows partial lysis of the tubular basement membrane (*arrow*) (PAS staining, **a** ×600; PAM staining, **b** ×600). Anti-CD34 Ab staining shows good preservation of its positive finding on the surrounding peritubular capillary even in the area of tubulitis (**c** ×600)

or decreased staining along the damaged or non-damaged peritubular capillary walls, depending on the areas (data not shown). Accordingly, only supportive findings were obtained with this antibody in this study. In all three cases, some peritubular capillaries demonstrated increased positive staining, whereas most of them showed no staining of this factor (data not shown).

disease are thought to be due to secondary phenomena resulting from the rupture of Bowman's capsule associated with crescent formation, arteriolitis/arteritis, and venulitis in the interstitium, tubulitis, or the release of cytokines by the infiltrated inflammatory cells in the interstitium [1, 2]. However, the exact role of vasculitis in the peritubular capillaries, which is presumed to occur in MPO-ANCA-associated vasculitis and to play an important role in the pathogenesis of TI lesions, has not been described in the literature outside of several cases complicated both by possible drug-induced MPO–ANCA positivity and TI nephritis [3–6]. These reports as well as the other presentations still did not describe the exact existence of peritubular capillaritis and its role in the pathogenesis of TI nephritis [1–8]. In addition, the lysis of the tubular basement membrane in the place of tubulitis, which is not accompanied by secondary TI nephritis due to the above-mentioned causes (the rupture of Bowman's capsule, etc.) in this disease, also has not been previously reported in the literature, except for one article [9]. Therefore, three cases that showed TI nephritis without any apparent glomemlar lesions in patients with MPO-ANCA positivity were studied. The tissue specimens obtained from kidney biopsies were investigated by immunohistochemical staining using antibodies (Abs) to the endothelial cell surface markers, von Willebrand factor, type IV collagen, cytokeratin, E-cadherin, and MPO in addition to the routine histochemical staining. The results suggest that peritubular capillaritis as well as tubulitis plays an important role in the pathogenesis of TI nephritis due to MPO-ANCA-associated kidney disease.

Case reports

Case 1

An 83-year-old female developed anorexia and low grade fever in late May 2006 and received antibiotics from her physician, but there was no improvement of the symptoms. Thereafter, increased CRP 10.2 mg/dl and leukocytosis 14,500/μl were found, and the patient was eventually admitted to our university hospital on 29 June 2006. A physical examination on admission showed her BP to be 159/60 mmHg, but there were no other positive findings. The laboratory data were as follows: hemoglobin (Hb) 7.9 g/dl, white blood cells (WBC) 22,800/μl, platelets 36.7 × 10^4/μl, C-reactive protein (CRP) 10.2 mg/dl, total protein 5.0 g/dl, serum albumin 2.0 g/dl, blood urea nitrogen 28.6 mg/dl, serum creatinine 1.6 mg/dl, MPO–ANCA 65 EU, ANA 320 × homogeneous pattern, CH_{50} 54.0 U/ml, and rheumatoid factor 550 IU/ml. Urinalysis showed negative proteinuria and red blood cells (RBCs) 0–1/high power field (HPF) in the sediment, but was accompanied by 30–49 granular casts in the whole fields. In addition, the urinary β_2-microglobulin at urine pH 7.0 was increased to 809 μg/l (<300 μg/l). The patient underwent a renal biopsy based on these abnormal urinary findings.

Case 2

A 73-year-old male noticed fever (38.6°C), cough, and sputum, and was diagnosed to have pneumonia by his family physician in February 2006. He received a bactericidal drug that relieved the symptoms. However, he developed anorexia, myalgia in his thighs and calves, ankle arthralgia, pyrexia (37–38°C), and weight loss (7.0 kg) in March 2006. These symptoms persisted, and at this time he was diagnosed to have polymyalgia rheumatica based on the above-described symptoms as well as increased CRP 8.2 mg/dl and negative ANA. Therefore, 20 mg per day of prednisolone was prescribed on 4 July 2006, but the patient showed positivity for MPO–ANCA 289 EU. He was admitted to our university hospital on 31 July 2006, because MPO-ANCA-associated vasculitis was suspected. A physical examination on admission showed his BP was 163/94 mmHg and body weight 54.0 kg (previously 61.0 kg), but there was no muscle tenderness or leg edema. His laboratory data were as follows: urinalysis was negative for proteinuria, RBCs 1–4/HPF and granular casts 1–4/HPF in the sediment, and β_2-microglobulin at urine pH 6.5 was 231 mg/l at 32 days after prednisolone therapy. The other blood tests revealed WBC 11,600/μl, serum creatinine 0.7 mg/dl, CRP 3.9 mg/dl, MPO–ANCA 140 EU, negative ANA, negative anti-Jo-1 antibody, CH_{50} 74.7 U/ml, rheumatoid factor 62 IU/ml, and KL-6 272 U/ml. The patient underwent a renal biopsy based on these abnormal urinary findings and the history of present illness.

Case 3

A 62-year-old female had been treated by her family physician with 1.25 mg/day glibenclamide for non-insulin-dependent diabetes mellitus since 1995. In August 2000, she developed a cough and low grade fever, and received medication from her physician, but had no improvement of these symptoms. Thereafter, she visited another hospital and was found to have leukocytosis 10,200/μl and CRP 15.8 mg/dl. Subsequently, she was admitted to this hospital, and several antibiotics were prescribed, but no improvement was observed in her symptoms or laboratory data. Her body temperature increased to 38–39°C and persisted. Therefore, she was transferred to our university hospital on 10 November 2000 for further evaluation. A physical examination on admission showed weight loss

Conclusions

Both renal and life prognoses were better in the apheresis group in treating RPGN patients. The total steroid dose for 2 months after admission was lower in the apheresis group, and the improvements in proteinuria were similar to those of the non-apheresis group. As there are fewer cases in the apheresis group, we should accumulate more cases and repeat the evaluation.

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0.01% hydrogen peroxide was used for enzymatic visualization. These stained tissue specimens were then examined. For the staining of the serial sections using six different antibodies, the serial sections were successively applied to each staining in the arrangement of CD34, von Willebrand factor, type IV collagen, MPO, cytokeratin AE1/AE3, and E-cadherin according to the above-mentioned methods. An immunofluorescence study using frozen tissues was performed for the detection of immunoglobulin and complement deposits. An extensive electron microscopic examination of the peritubular capillaries was done to assess basement membrane breaks in addition to the usual observation.

Results

The pathological findings in each case

Routine study

Case 1: One kidney tissue specimen was obtained, and it contained six glomeruli that showed an almost normal appearance except for some infiltration of neutrophils in the lumens of the capillary loop. In the interstitium, only focal and minor infiltrations of neutrophils and mononuclear cells in the peritubular spaces as well as tubulitis were noted (Fig. 1a, b, c). However, no infiltration of eosinophils and plasma cells was found. High power magnification showed a few neutrophils and mononuclear cells in the peritubular capillaries with blurred, thickened, or disappearing capillary walls (Fig. 1c). The tubulitis was demonstrated by the infiltration of mononuclear cells in the tubular epithelium associated with blurred and thickened tubular basement membranes (Fig. 1c). No arteritis or arteriolitis was found. Immunofluorescence revealed no immunoglobulin or complement deposits. Electron microscopy showed that there were no dense deposits in the glomeruli or the capillaries. Extensive observation demonstrated breaks in the peritubular capillary basement membrane (Fig. 2).

Case 2: Two kidney specimens were obtained; one had six glomeruli, and the other had only medulla. All six glomeruli showed almost normal appearance, and only a few neutrophils were observed in their capillary lumens. In the interstitium, slight and focal infiltrations of neutrophils and mononuclear cells among peritubular tissues were noted in addition to tubulitis. High power magnification revealed few neutrophils and mononuclear cells in the peritubular capillaries with blurred capillary walls. Tubulitis was also noted around the peritubular capillaries, which were accompanied by inflammatory cells. It showed the partial disappearance of tubular basement membrane in addition to desquamating tubular epithelial cells, and this lesion was associated with the infiltration of neutrophils and mononuclear cells (Fig. 3a, b). Immunofluorescence showed no deposits of immunoglobulins and complements. No tissue was available for electron microscopic evaluations.

Case 3: Two kidney specimens were obtained, and they contained 15 glomeruli in total. Three glomeruli showed global sclerosis, and two glomeruli revealed fibrous thickening of Bowman's epithelial cells, with both slight wrinkling of the capillary loops and some widening of the mesangial matrix. None of these glomeruli showed any rupture of Bowman's capsule. The remaining ten glomeruli demonstrated almost normal appearance. In the interstitium, there were focal and slight infiltrations of neutrophils and mononuclear cells in the peritubular tissues except for the surrounding areas of sclerotic glomeruli, where a considerable number of mononuclear cells were noted. Scattered tubulitis was found, but it was rarely associated with the destruction of tubular basement membranes outside of the areas of sclerotic glomeruli. High power magnification revealed the infiltration of a few mononuclear cells in the lumens of the peritubular capillaries, and this was accompanied by the destruction of the capillary walls. Arterio-arteriolosclerosis was noted, but without fibrinoid degeneration. Immunofluorescence demonstrated faint linear staining with anti-IgG and IgA antibodies on the glomerular capillary walls. Electron microscopy showed both a slightly thickened glomerular basement membrane and some increased mesangial matrix, but no apparent breaks of the peritubular capillary basement membranes as far as we could determine based on our findings.

Immunohistochemical study

Vascular endothelial cell findings with anti-CD34 antibody The antibody to CD34 marker, which binds to the cell surface of vascular endothelial cells [10], was used for the detection of the vascular wall. In the preliminary staining, the antibody showed good visualization of the vascular walls on the peritubular capillary and glomerulus in normal subjects (Fig. 4a, inset) as well as the renal tissues of several glomerulonephritides (data not shown). All three cases showed an almost similar appearance. Namely, the glomeruli revealed good staining along the capillary walls (Fig. 4b). In contrast, a loss of staining was focally observed on the peritubular capillaries in the interstitium where the capillary walls were either destroyed or evenly preserved (Fig. 4a, b).

Vascular endothelial cell findings with anti-von Willebrand factor antibody The von Willebrand factor, some of which is known to be secreted from the endothelial cells, is restored beneath the vascular endothelial cells [11]. The preliminary study on the other glomerulonephritides showed increased

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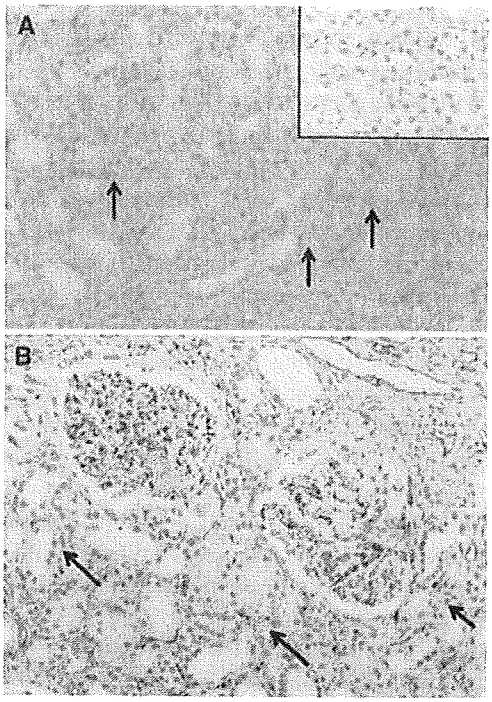

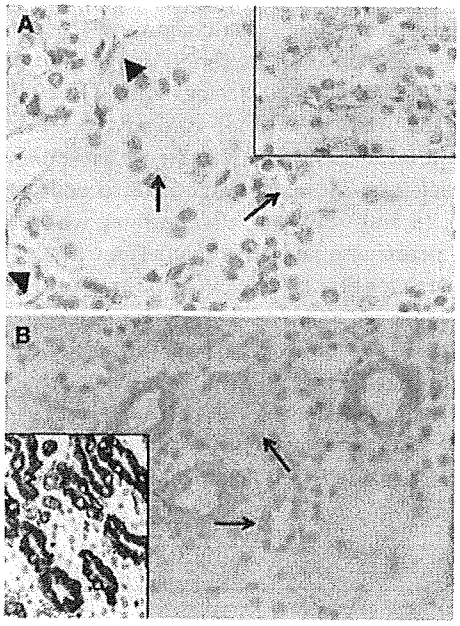

Fig. 5 Case 3: Anti-type IV collagen Ab staining of the renal tissue from a 65-year-old healthy male shows positive findings on tubular basement membrane and peritubular capillary walls (**a**, *inset* ×400). However, some tubular basement membranes and peritubular capillary walls of the patient show decreased staining (*arrows*), whereas the other area reveals its increased intensity with wrinkling or duplicating of the tissue (*arrowheads*) (**a**, ×600). Anti-cytokeratin AE1/AE3 Ab staining of the renal tissue from a 65-year-old healthy male shows homogenous positive findings of tubular epithelial cells. However, two tubuli probably belonging to the thick limb of Henle show negative staining (**b**, *inset* ×400). In contrast, the tubular epithelial cells of the patient show the loss of positivity to some extent, but not all of them (*arrows*) (**b**, ×400)

Fig. 4 Case 1: Anti-CD34 Ab staining of the renal tissue from a 65-year-old healthy male shows a positive finding of the peritubular capillary walls in the interstitium (**a**, *inset* ×400). In contrast, some peritubular capillary walls of the patients lose this marker (*arrows*), whereas the other area keeps the positivity of this maker (**a** ×400). The glomeruli and periglomerular capillary walls show positive findings, but some capillary walls in the interstitium lose this marker, too (*arrows*) (**b** ×400)

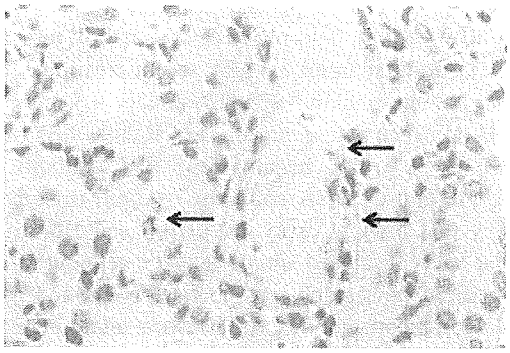

Fig. 6 Case 1: Anti-MPO Ab staining demonstrates trace amounts on a peritubular capillary wall and on the infiltrating neutrophils in the tubular lumen as well as a probable neutrophil in a peritubular capillary (*arrows*) (×600)

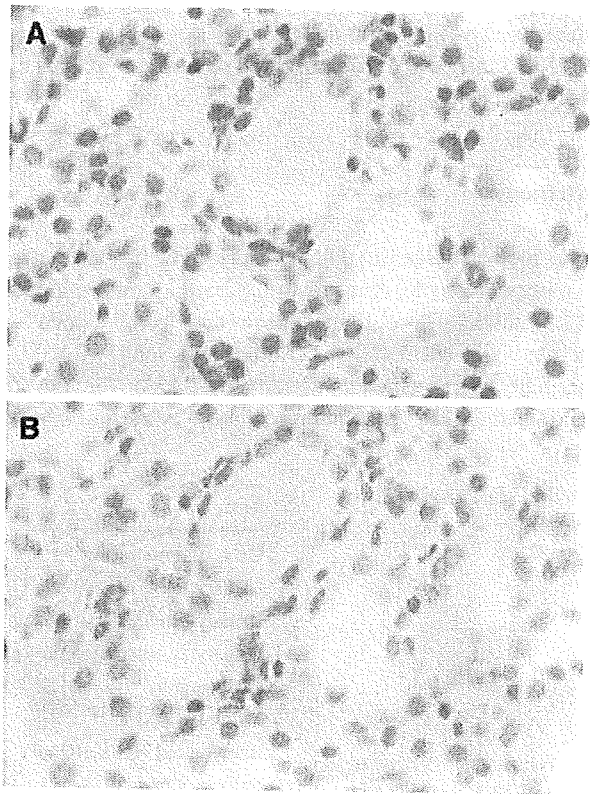

Fig. 7 Case 2: Serial sections. Anti-CD34 Ab and anti-type IV collagen Ab stainings demonstrate the apparent loss of CD34-positive findings, whereas type IV collagen staining is noted (anti-CD34 Ab staining, **a** ×600; anti-type IV collagen staining, **b** ×600)

from 59.0 to 53.0 kg, muscle tenderness in both the upper arms and shoulder areas as well as the calves, motion arthralgia in both elbow and shoulder joints, crackles in the bilateral chest bases, hypoparesthesia in both legs and the radial side of the right forearm, and leg edema. Laboratory data revealed the following: urinalysis showed negative proteinuria, (+) glucosuria, RBCs 1–4/HPF and granular casts 1–4/HPF in the sediment, and urinary β_2-microglobulin 407 μg/l at urine pH 6.5. The blood tests were Hb 7.7 g/dl, WBC 9,400/μl, platelet 30 × 10^4/μl, total protein 6.3 g/dl, serum albumin 2.3 g/dl, serum creatinine 0.7 mg/dl, creatine phosphokinase 32 IU/ml, CRP 9.4 mg/dl, CH_{50} 43.3 U/ml, rheumatoid factor 422 IU/ml, MPO–ANCA 43 EU, ANA ×320, negative anti-Jo-1 antibody, KL-6 257 U/ml, anti-ds DNA antibody 7 IU/ml, immune complex by C_1q binding assay 3.0 μg/ml, and HbA1c 4.7%. Further study disclosed simple diabetic retinopathy, negative gallium scintigraphy of the total body, and negative results for blood cultures for bacteria and fungi. MPO–ANCA-associated vasculitis was highly suspected because of her myalgia, arthralgia, weight loss, fever, peripheral neuropathy, increased CRP and MPO–ANCA, and negative findings for blood cultures and gallium scintigraphy. Therefore, the patient was persuaded to undergo a renal biopsy for evaluation of vasculitic manifestations as well as the severity of diabetic nephropathy.

The clinical data from all three patients are presented in Table 1.

Methods

The biopsy tissues of kidney were fixed in 4% buffered paraformaldehyde, embedded in paraffin, and sectioned 3–4 μm thick. These tissue specimens were stained with hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and periodic acid-methenamine silver (PAM) for the routine histological examination. These paraffin-embedded tissues were also used for the immunohistochemical stainings, which were performed with antibodies to vascular endothelial cell surface markers (CD34: Nichirei, Tokyo), von Willebrand factor (Dako, Denmark), type IV collagen (Dako, Denmark), cytokeratin AE1/AE3 (Dako, Denmark), E-cadherin (BD Transduction Lab, California), and myeloperoxidase (Dako, Denmark). The antibodies to CD34, von Willebrand factor, type IV collagen, cytokeratin AE1/AE3, and E-cadherin are monoclonal antibodies to each human element, respectively. The antibody to myeloperoxidase (MPO) is a polyclonal antibody to human leukocyte MPO. The staining procedure was as follows: each mouse monoclonal antibody was diluted to CD34, ×50; von Willebrand factor, ×100; type IV collagen, ×100; cytokeratin AE1/AE3, ×100; E-cadherin, ×400, respectively. Polyclonal antibody to MPO was diluted to ×10,000. Antigen retrieval was performed by protease in type IV collagen, MPO, and cytokeratin AE1/AE3, but was by citrate buffer (pH 6.0) in von Willebrand factor and E-cadherin. No retrieval was done in CD34. Next, the diluted antibodies were applied to the renal tissue specimens for the first antibody under 4°C and an overnight reaction. After washing with phosphate-buffered saline (PBS), biotinylated goat anti-mouse antibody was reacted on the renal tissue as a second antibody for 60 min in the antibodies of CD34, von Willebrand factor, type IV collagen, and cytokeratin AE1/AE3. In the antibody of MPO, which is produced in rabbits, biotinylated goat anti-rabbit antibody was reacted on the renal tissue as a second antibody for 60 min. In both stainings, after washing with PBS, horseradish peroxidase-labeled streptoavidin was applied to these specimens for 60 min (Ventana system, Tucsan, AZ). However, in the antibody of E-cadherin, biotinylated goat anti-mouse IgG antibody labeled with horseradish peroxidase was reacted on the renal tissue as a second antibody for 60 min (Envision System, Dako, Kyoto). After performing all of these reactions and post-washing with PBS, 3-diaminobenzidine tetrahydrochloride (DAB) including

Table 1 Laboratory data on admission

Test	Normal value	Case 1	Case 2	Case 3
Urinary protein (mg/dl)	(–)	(–)	(–)	(–)
Urinary RBC in sediment (/HPF)	0–1	0–1	1–4	1–4
Urinary granular cast in sediment (/HPF)	0	30–49[a]	1–4	1–4
Urinary β_2-microglobulin (μg/l)	20–300	809	231[b]	407
CRP (mg/dl)	<0.4	10.2	3.9	12.0
MPO–ANCA (EU)	<20	65	140	43
Rheumatoid factor (IU/ml)	<20	550	62	422
Hemoglobin (g/dl)	11.5–15.5	7.9	13.9	7.7
WBC (/μl)	3,500–9,000	22,800	11,600	9,400
Platelet (×10^4/μl)	15.0–38.0	36.7	19.9	30.2
Serum creatinine (mg/dl)	0.2–0.8	1.6	0.9	0.7

[a] Means the numbers of urinary granular casts over the whole field in the sediment

[b] The value of 32 days after the treatment of prednisolone 20 mg/day

glomerular lesions, and this incidence is almost consistent with previous reports [1, 17]. TI nephritis in our three cases was presumed to result from peritubular capillaritis and tubulitis, because the kidney specimens showed almost normal glomeruli and no existence of arteritis, arteriolitis, or venulitis except in case 3. Case 3 had some sclerotic glomeruli and fibrous thickening of Bowman's epithelial cells and showed the infiltration of inflammatory cells around them, but this pathology was only secondary TI nephritis. The remaining tissue demonstrated identical features to cases 1 and 2. Peritubular capillaritis in the interstitium was diagnosed by loss of CD34 staining, type IV collagen wrinkling or loss of staining, and basement membrane breaks in the peritubular capillary walls as well as the infiltration of neutrophils and mononuclear cells in the peritubular capillary lumens and in the surrounding interstitium. Tubulitis was also diagnosed by the standard definition and based on inflammatory cell infiltration into the tubular epithelial cells [18]. Furthermore, tubulitis was associated with TBM lysis and desquamating tubular epithelial cells, which were observed with the infiltration of neutrophils and mononuclear cells into the tubular epithelial cells. The tubulitis was also accompanied by the infiltration of neutrophils and mononuclear cells in the surrounding peritubular tissue. However, the area of TI nephritis did not show the presence of eosinophils or plasma cells. Close observation of the peritubular capillaries revealed the loss of CD34 staining even with continued staining of type IV collagen and the intact appearance of the capillary wall on PAS staining. These lesions disclosed the loss of cytokeratin AE1/AE3 staining and diminished expression of E-cadherin staining in the surrounding tubular epithelial cells. Based upon these pathological findings, TI nephritis associated with peritubular capillaritis and tubulitis was diagnosed in the three cases. When the pathogenesis of peritubular capillaritis and tubulitis is considered, MPO-ANCA-associated vasculitis is highly suspected, but a few other pathogenetic mechanisms must be addressed. First, drug-induced TI nephritis associated with MPO–ANCA positivity must be suspected. However, most cases in the literature report the infiltration of mononuclear cells, including eosinophils and plasma cells, thus suggesting that the origin of TI nephritis is drug-induced [3–6]. The three current cases showed none of these cells. Second, TI nephritis associated with virus infections shows similar pathological features and the infiltration of mononuclear cells of lymphocytes, plasma cells, and histiocytes, but not neutrophils [19]. Third, autoimmune TI nephritis, such as Sjögren syndrome, systemic lupus erythematosus (SLE), tubulointerstitial nephritis–uveitis syndrome, rheumatoid arthritis with arteritis (RAA), and essential cryoglobulinemia should also be considered [20–22]. These types of autoimmune TI nephritis are reported to have the infiltration of mononuclear cells, but not neutrophils, in the interstitium (Sjögren syndrome) or depositions of immunoglobulins and complements on the capillary walls as well as the TBM (essential cryoglobulinemia, SLE, etc.), except for RAA. In RAA, the patients who manifest the necrotizing type of vasculitis usually show low levels of serum CH_{50} in addition to apparent joint manifestations of arthritis, such as swollen and deformed joints [22]. These findings were not observed in our three cases. In addition, capillaritis due to the necrotizing type of vasculitis has never been described until today [22]. Therefore, these pathogeneses were not considered in the three cases presented here. When the pathogenic processes are considered in regard to the present cases, the loss of CD34 cells in the peritubular capillaries occurred initially and relatively diffusely in the interstitium. This staining loss was noted in the areas of TI nephritis, but was also observed even in the areas with good type IV collagen staining. This observation suggests that MPO, which is included in the infiltrating neutrophils, bursts out from these cells, and releases proteolytic enzymes and radical oxygen species, induces the antigenic loss of vascular endothelial cells, and results in the initial damage of the peritubular capillary walls [1]. This process progresses to peritubular capillaritis and the infiltration of neutrophils and mononuclear cells in the surrounding interstitium [23]. However, similar processes were not observed in the glomeruli, which showed both preservation of CD34 and type IV collagen staining in all three cases. The discrepancy between these two findings in this study is not easily explained, but might be due to MPO epitope differences or different MPO affinities [24, 25]. In tubulitis, TBM lysis was noted with the infiltration of neutrophils and mononuclear cells into the tubular epithelial cells. This lesion was accompanied by the infiltration of these cells in the surrounding interstitium. This observation implies that tubulitis itself could induce TBM lysis as described by Akikusa et al. [9] in which the tubulorrhexis in systemic vasculitis was described.

This study described three cases with TI nephritis but without glomerular lesions in MPO-ANCA-associated vasculitis and early loss of CD34 antigenicity in the peritubular capillary. This early loss of CD34 antigenicity due to MPO and tubulitis played important roles in the pathogenesis of peritubular capillaritis and the lysis of tubular basement membrane, and induced TI nephritis in the present cases. These observations and these pathogenetic processes have not previously been described in MPO-ANCA-associated vasculitis as far as we could determine in the literature.

Table 1 Classification of the initial clinical subtypes and their subsequent development during the observation period (observation more than 2 years, average 4.3 years)

Initial clinical subtype (n = 30)	Subsequent clinical subtype during the observation period [development n = 9, nondevelopment n = 21]
Pulmonary n = 12	Pulmonary n = 5
	Pulmo-renal n = 5
	Systemic n = 2
Renal n = 5	Renal n = 4
	Pulmo-renal n = 1
Pulmo-renal n = 7	Pulmo-renal n = 7
Peripheral neuritis + α n = 3	Peripheral neuritis + α n = 3
Musculo-articular n = 1	Musculo-articular n = 1
ENTE n = 1	*ENTE plus pulmo-renal n = 1*
Systemic n = 1	Systemic n = 1

Patients with nondevelopment n = 21 (70%)

Patients with development (italicized) n = 9 (30%)

α, musculo-articular or mild nephritis

musculo-articular symptoms and 1 patient with nephritic urinalysis), 1 patient with musculo-articular type, and 1 patient with systemic type. In contrast, the group demonstrating new organ symptoms included five patients with initial pulmonary type changing into pulmo-renal type, two patients with initial pulmonary type changing into systemic type, one patient with renal type changing into pulmo-renal type, and one patient with ENTE type changing into ENTE plus pulmo-renal type. The time of development ranged from 2 to 14 years and the average time was 5.1 years.

Classification of clinical subtypes, numbers of patients, and involved organs on admission (Fig. 1)

Our patients consisted of 5 patients with pulmonary type, 4 patients with renal type, 13 patients with pulmo-renal type, 3 patients with peripheral neuritis type (2 patients with additional musculo-articular symptoms and 1 patient with nephritic urinalysis), 1 patient with musculo-articular type, 1 patient with ENTE plus pulmo-renal type, and 3 patients with systemic type. These data and the total involved organs of all patients are shown in Fig. 1.

Patient survival (Figs. 2, 3)

Death occurred in 13 patients. The causes of death, as well as the incidence and time of death, are shown in Figs. 2 and 3. Of these patients, three were diagnosed as having died of vasculitis, of whom two had systemic type transformed from pulmonary type. Those two patients died due to either sigmoid bleeding or pulmonary hemorrhage. The third patient died of pulmonary hemorrhage from systemic type. The observed bleeding and hemorrhaging were due to the vasculitis itself with the following evidence. The sigmoid bleeding was confirmed by colon fiberscope which demonstrated fresh hemorrhaging from multiple deep small ulcers in the intestinal wall. Pulmonary hemorrhaging was diagnosed with hemoptysis as well as the findings of chest X-ray and computed tomography, which revealed tiny alveoli-filling high-density shadows. These findings are quite compatible with vasculitis in nature. These deaths occurred, respectively, at 2, 2, and 6 months after the diagnosis of vasculitis, even though steroid treatment was

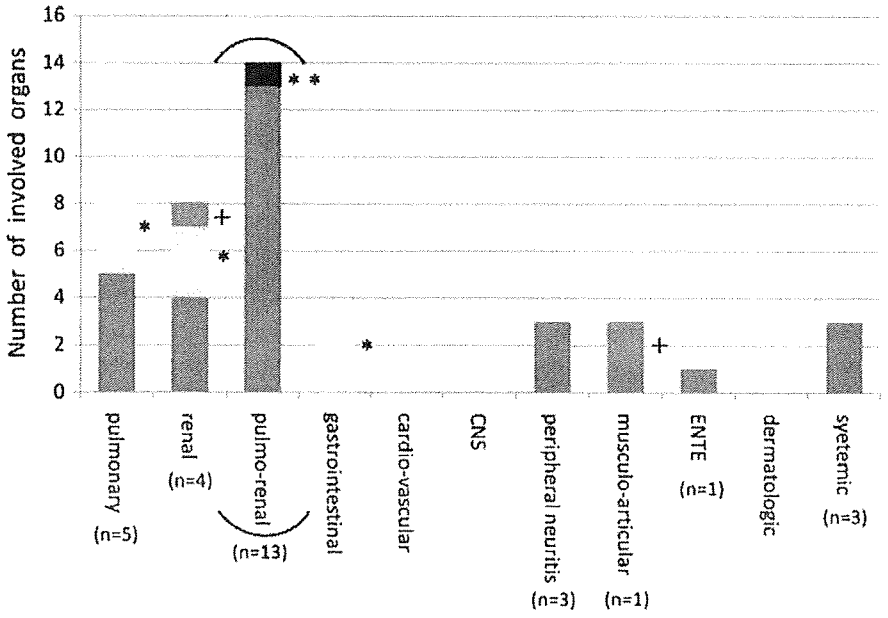

Fig. 1 Classification of clinical subtypes, number of patients, and the involved organs on admission. *Red colored columns* indicate the number of patients with classification of clinical subtypes. *Yellow colored columns* (*single asterisk*) mean the involved organs in systemic type. A *black colored column* (*double asterisks*) shows an additional organ involvement of pulmo-renal tissues in addition to ENTE type. *Green colored columns* (*plus symbols*) are an additional organ involvements of musculo-articular or nephritic symptoms in addition to peripheral neuritis type

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ORIGINAL ARTICLE

Classification of clinical subtypes, patient survival, kidney prognosis, and relapse in patients with MPO-ANCA-associated vasculitis: a single-center experience

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Abstract Myeloperoxidase-type antineutrophil cytoplasmic antibody (MPO-ANCA)-associated vasculitis may manifest various organ symptoms. Treatment allows recovery from early, but severe, organ involvement. However, the relationship between the initial organ involvement and the eventual clinical course has not been studied in this disease. Therefore, the current study evaluated 30 patients who were hospitalized and then categorized into ten clinical subtypes based on organ involvement. The relationship of these subtypes to development of clinical features, patient survival, kidney prognosis, and relapse were evaluated over an average observation period of 4.3 years. During this study, the most common clinical features were lung and kidney involvement. Twenty-one patients already manifested clinical features around the time of admission and did not commonly present new symptoms as long as they were receiving the treatment for vasculitis. In contrast, as far as pulmonary involvement type at the initial time was concerned and in those not being treated for vasculitis, 7 of the 12 patients progressed to pulmo-renal involvement and 5 of them went onto renal failure. Progression to renal failure also occurred frequently in patients with pulmo-renal type manifesting at the initial time. Thirteen patients died, including three patients due to vasculitis of systemic type, seven due to infections, and three due to malignancy. Death due to vasculitis occurred in the early phase of treatment and was associated with either pulmonary hemorrhage or gastrointestinal bleeding. Infectious death occurred throughout the entire course of treatment, mostly in patients with pulmo-renal or pulmonary type, and tended to be associated with opportunistic organisms. Death with malignancy was observed after several years of treatment. Regarding renal prognosis, ten patients underwent hemodialysis. At initiation of hemodialysis, nine patients had pulmo-renal type and only one had renal type. A relapse was observed in ten patients, mainly in patients with pulmo-renal or pulmonary type, and it occurred after about 2.7 years, even with treatment. Such relapses manifested in a similar manner to their initial clinical subtypes. These results suggest that pulmo-renal type as well as pulmonary type have a high chance to progress to renal failure or systemic type, and they were fairly commonly associated with vasculitic or infectious death. Therefore, classification of clinical subtypes at the initial time and on admission is meaningful to some extent for predicting patient survival, kidney prognosis, and relapse, in addition to indicating the appropriate treatment regimen.

Keywords MPO-ANCA · Vasculitis · Prognosis · Relapse · Clinical subtype

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Introduction

MPO-ANCA-associated vasculitis mainly affects capillary and induces necrotizing capillaritis in lung and kidney involvement with high frequency [1, 2]. However, capillaries exist throughout all human organs, and capillaritis demonstrates various organ manifestations such as cardiac, gastrointestinal, ears, nose, throat, eyes, muscle/joint, cutaneous, and central nervous system (CNS) features as

Table 2 Tubulointerstitial nephritis without glomerular lesions in the three cases associated with MPO-ANCA-positive vasculitis

	Glomerular lesions				Tubulointerstitial lesions				
	Crescent	Fibrin in Bowman's space	Neutrophils in glomerular capillary loops	Increased cells in glomeruli	Peritubular capillaritis[a] (PTCitis)	TBM lysis	Tubulitis[b]	CD34 positivity on PTC	CD34 positivity on glomeruli
Case 1	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)
Case 2	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)
Case 3	(−)	(−)	(+)	(−)	(+)	(+)	(+)	(−)	(+)

[a] The infiltration of neutrophils in the peritubular capillary lumens, breaks of peritubular capillary walls, and the infiltration of inflammatory cells around the peritubular capillary

[b] The infiltration of mononuclear cells and neutrophils into the tubular epithelial cells with or without the lysis of tubular basement membrane

Type IV collagen finding with this antibody This antibody, as is known, shows positive staining of the peritubular capillary walls as well as the glomerular capillary loops, Bowman's capsules, and tubular basement membranes insofar as they are not damaged [12] (Fig. 5a inset). Damage was associated with a loss of staining in the complete destruction or increased staining with the wrinkling of tissues in the early phase of destruction. All three cases showed positive type IV collagen staining in the glomeruli and Bowman's capsules (data not shown). In the interstitium, some peritubular capillaries revealed loss of type IV collagen, but the others had increased staining with wrinkling or duplicating of the tissues (Fig. 5a). Similar findings were observed on the tubular basement membranes as well.

Cytokeratin AE1/AE3 finding with this antibody The antibody to cytoskeleton AE1/AE3 is an antibody to the cytokeratin of human epithelial cells [13]. In the preliminary study, it stained the tubular epithelial cells belonging to the thin limb of Henle, distal collecting duct, cortical collecting duct, and medullary collecting duct in the normal kidneys as described in the textbook [14]. However, the staining was lost when those tissues were damaged for different reasons. All three cases showed some areas without staining for this antibody (Fig. 5b).

E-cadherin finding with this antibody E-cadherin is a transmembrane glycoprotein that is localized in the adherent regions of epithelial cells [15]. In normal kidneys, E-cadherin is found in the epithelial cells of the distal tubules, but not in the proximal tubules [16]. The loss of this glycoprotein in the kidney indicates a decreased adherent junction between tubular epithelial cells, implying the loss of normal tubular cell functions. All three cases showed some areas where the staining with antibody was diminished or absent (data not shown).

Localization of myeloperoxidase with anti-MPO antibody In the experimental model and hypothesis of MPO-ANCA-associated vasculitis, MPO-ANCA exists on the vessel walls and induces the destruction of vessel walls [1]. MPO staining on the vessel walls was observed only in limited areas in the three patients, although the neutrophils were routinely stained positive for MPO (Fig. 6).

Immunohistochemistry in serial sections stained with the six above-mentioned antibodies Serial sections were observed to clarify the relationship between the loss of CD34 antigenicity and the destruction of vessel walls in the peritubular capillaries. The destruction of vessel walls was evaluated by the preservation of type IV collagen. The alteration of both cytokeratin AE1/AE3 and E-cadherin glycoprotein in the surrounding tubular epithelial cells was also examined. In specimens from all three patients, most areas that showed disappearance of CD34 antigenicity were associated with diminished staining or increased wrinkled staining of type IV collagen. However, some areas showed the loss of CD34 antigenicity, even though type IV collagen staining appeared normal (Fig. 7a, b), and the peritubular capillary wall seemed almost intact without a blurred appearance by PAS and PAM staining. Only a few areas showed remarkable tubulitis with the infiltration of a small number of neutrophil and mononuclear cells and lysis of the tubular basement membrane (Fig. 3a, b). However, these observations were not accompanied by the loss of CD34 positivity of the surrounding peritubular capillaries (Fig. 3c). The surrounding tubular epithelial cells diminished or lost cytokeratin AE1/AE3 and E-cadherin staining as well (Fig. 5b). The total immunohistochemical findings are summarized in Table 2.

Discussion

A total of 66 renal biopsy specimens were obtained from patients with MPO-ANCA-associated nephritis from 1998 to 2007 in our department. However, only three cases (4.5%) showed TI nephritis without any apparent

peripheral neuritis, musculo-articular, ear-nose-throat-eye (ENTE), dermatologic or systemic type. Systemic type included patients who had lesions in at least three vital organs. The vital organs included the lung, heart, kidney, gastrointestinal tract including the pancreas, gallbladder as well as liver, and CNS. As patients with both pulmonary and kidney involvement were frequently observed, the pulmo-renal type was tentatively added to the original ten clinical subtypes. If the patient manifested new clinical subtypes during the observation period, then this was defined as having developed a new type of disease. Clinical subtypes on admission were also evaluated and recorded for the study.

Patient survival and kidney prognosis

Patient survival was determined by the death of patients, and causes of death were analyzed by clinical features and laboratory data. Kidney prognosis was determined by reaching end-stage renal disease necessitating hemodialysis. The data regarding the patient survival are shown in the life table analysis.

Relapse

A relapse was defined as when at least one of the following occurred after the subsidence of initial organ symptoms with the treatment [5, 11, 12]:

(1) Repetitive urinalyses showing proteinuria and/or red blood cells (RBCs) in the sediment increased by more than two times, (2) a rapid rise in the serum creatinine level without any other cause for the deterioration of renal function, (3) hemoptysis, pulmonary hemorrhage or new expanding shadows by X-ray and/or computed tomography or (4) new appearance or reappearance of symptoms belonging to the remaining other organs described in the Appendix.

These symptoms were mostly accompanied by elevated C-reactive protein (CRP), but not always in the study. Infection-associated similar these symptoms were ruled out by the refractory findings to the treatment with various antibiotics, antifungal drugs, and antiviral agents.

Treatment

The recommended regimen was introduced to the patients based on the Research Committee of Refractory Vasculitis Syndrome sponsored by the Ministry of Health, Welfare, and Labor of Japan [9]. Specifically, steroid pulse therapy was introduced to patients complicated by either pulmonary hemorrhage, rapidly progressive glomerulonephritis, gastrointestinal bleeding (not occult bleeding), progressive pulmonary fibrosis or CNS involvement. Other patients were treated with 0.6–0.8 mg/kg body weight oral prednisolone per day. In addition, patients treated with steroid pulse therapy first were subsequently treated with the same dosage of oral prednisolone (PSL) as the other patients. The oral PSL dosage was gradually tapered off according to improvement of clinical symptoms and laboratory data. Oral immunosuppressants such as cyclophosphamide (CY), azathioprine (AZP), mizoribine (MZB), and methotrexate (MTX) were added according to the severity of the disease. However, none of these agents were intravenously administered during this study.

Results

The time interval for diagnosis

The time interval for diagnosis was 0 months for 12 patients, 0.5 years for 9 patients, and more than 2 years for 9 patients. The time intervals ranged from 0 months to 11 years, and the average interval was 2.6 years. The patients diagnosed to have MPO-ANCA-associated vasculitis more than 2 years after the initial organ involvement included eight with pulmonary fibrosis and one with nephritic urinalysis. These patients were observed for several years without a diagnosis of vasculitis.

Initial organ involvement and the observation period

Initial organ involvement was pulmonary type in 12 patients, renal type in 5 patients, pulmo-renal type in 7 patients, peripheral neuritis type in 3 patients (2 associated with additional slight musculo-articular symptoms and 1 associated with additional trace proteinuria and hematuria), musculo-articular type in 1 patient, ENTE type in 1 patient, and systemic type (lung, kidney, and gastrointestinal involvement) in 1 patient. No patients with dermatologic type, CNS type or cardiovascular type were observed in this study. The observation periods of the study patients ranged from 2 to 14 years and the average observation years was 4.3 years.

Development of clinical subtypes during the observation period and time of development (Table 1)

Two groups were observed in the study. One group continued to demonstrate the same clinical subtype whereas the other group showed new organ symptoms. The group demonstrating the same clinical subtype included 21 patients in total: 7 patients with pulmo-renal type, 5 patients with pulmonary type, 4 patient with renal type, 3 patients with peripheral neuritis type (2 patients with additional

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特集：急速進行性糸球体腎炎

RPGN，ANCA 関連血管炎の疫学

藤元昭一

はじめに

われわれが経験する急速進行性糸球体腎炎(RPGN)の多くは myeloperoxidase(MPO)に対する MPO-ANCA が陽性である。また，腎症を伴う血管炎のなかでは顕微鏡的多発血管炎(MPA)をしばしば経験し，その多くは MPO-ANCA が陽性である。一方，proteinase-3(PR3)に対する PR3-ANCA が陽性の患者や腎症を伴う Wegener 肉芽腫症(WG)を経験することは少ない。しかし，海外の論文をみると，RPGN や血管炎のなかでは PR3-ANCA 陽性や WG の患者の比率が本邦と比べてかなり高い。本稿では，本邦の RPGN や ANCA 関連血管炎の発症率や罹患率，特徴を中心に，欧米と比較して述べる。

疾患概念

RPGN には，その原因疾患として抗糸球体基底膜抗体腎炎，免疫複合体腎炎，間接蛍光抗体法にて免疫グロブリンの沈着を欠く pauci-immune 型腎炎(多くは ANCA 陽性)が含まれる。一方，ANCA 関連血管炎(ANCA-associated vasculitits，あるいは small vessel vasculitis と呼ばれている範疇)には，主として MPA，WG，Churg-Strauss 症候群(CSS)が含まれ，臓器障害としてしばしば腎症を伴う。腎以外の他臓器障害を伴わない ANCA 陽性の腎症は，腎限局型血管炎(RLV)として扱われる場合もある。

本邦では，ANCA が陽性で，組織学的に pauci-immune 型半月体形成性腎炎を呈する場合に ANCA 関連腎炎と呼称されている。欧州の血管炎グループ(EUVAS)は ANCA 陰性の pauci-immune 型半月体形成性腎炎も同じ範疇の疾患として取り扱っている[1,2]。また RLV は，広い意味では MPA に含まれるとの考え方もある。現在，世界的に用いられている血管炎の分類[3]，定義[4]には ANCA に関する事項は考慮されておらず，本邦で用いられている診断基準[5]は必ずしも世界的に認められているわけではない。現在，欧州や米国のリウマチ学会を中心に，新たな血管炎の分類，定義，診断基準の作成が進められている。

疫学調査の差異

疫学調査報告を比較して考える場合に，発症率(incidence)と罹患率(prevalence)を混同しないこと，どの範疇の疾患群を扱っているかを明らかにしておくことが重要である。調査方法も異なっており，英国を主体とした血管炎の疫学研究は，住民の移動が非常に小さく，疾患の発症が必ず把握できる医療システムが確立されている地域で，前向きに発症率を調査している(a prospective, population-based survey)。一方本邦などでは，全国の血管炎診療に関連する施設へのアンケート調査の集計に基づいて，罹患率の概数を調査している(a nationwide, retrospective, hospital-based survey)。

欧州を中心とした血管炎の疫学調査の多くは rheumatologists により行われており，primary systemic vasculitides (PSV)として MPA，WG，CSS の 3 疾患の初発症例を集めた発症率の報告が多い(ときには結節性多発動脈炎を含んだ 4 疾患)。PSV の 3 疾患の年間 100 万人当たりの発症率は 10～20 例であり，その内訳は WG 3～11 例，MPA 3～8 例，CSS 1 例前後と報告されている[6~9](**表 1**)。発症時の平均年齢は約 60 歳だが，ピークは 65～74 歳にある。性差は明らかではない。なお，これらの報告では腎症を伴わない血管炎も含まれている。米国では nephrologists と pathologists により ANCA-associated small vessel vasculitis として MPA，WG，CSS，およびしばしば RLV を含めた報告が多

Epidemiology of rapidly progressive glomerulonephritis (RPGN) and ANCA-associated vasculitis

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