Case Report

Tubulointerstitial Nephritis and Uveitis Syndrome Associated with Renal Tryptase- and Chymase-positive Mast Cell Infiltration

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We report the clinical course and immunohistochemical analysis of a patient who presented with tubulointerstitial nephritis and uveitis syndrome (TINU syndrome). The patient, a 40-year-old woman, was referred to our hospital with general fatigue and a slight fever from another hospital. Mast cells are closely related to the development of renal interstitial fibrosis in patients with glomerulonephritis. To determine the role of mast cells in renal interstitial injury in TINU patients, we performed immunohistochemical studies on renal biopsy specimens using anti-human tryptase and anti-human chymase antibodies specific for mast cells. Double immunostaining of tryptase and chymase was also performed in renal tissues. In double immunofluorescence, cells with both chymase and tryptase (MCtc) were marked in the regions of interstitial fibrosis in this patient. It appears that mast cells are one of the constitutive cells of interstitial fibrosis in patients with TINU syndrome. [*Hong Kong J Nephrol* 2007;9(1):50–4]

Key words: chymase, interstitial nephritis, mast cell, TINU, tryptase

本文描述一位腎小管間質性腎炎 — 葡萄膜炎 (TINU) 綜合症患者的臨床歷程及相關的免疫組織化學分析結果。病人是一位 40 歲的女性，症狀包括疲勞及低燒，從別院轉介至本院作進一步處置。基於在腎小球腎炎患者中，肥大細胞與腎臟間質性纖維化的發展密切相關，我們利用特定於肥大細胞的抗體，包括類胰蛋白酶抗體 (anti-human tryptase) 及糜蛋白酶抗體 (anti-human chymase)，對病人的腎臟活組織樣本作出免疫組織化學分析，包括雙重免疫染色，以調查在 TINU 綜合症患者的腎間質損傷中，肥大細胞的可能角色。雙重免疫熒光檢測法顯示，在本病人的腎臟活組織樣本中，具類胰蛋白酶及糜蛋白酶表現的肥大細胞 (MCtc) 明顯出現於間質性纖維化的區域內，這意味著肥大細胞在 TINU 綜合症患者的間質性纖維化過程中，可能擔當著若干的角色。

Introduction

In 1975, Dobrin et al first reported tubulointerstitial nephritis in two adolescent girls who demonstrated granulomatous syndrome characterized by reversible acute renal failure with eosinophilic interstitial nephritis, bilateral anterior uveitis, bone marrow and lymph node granulomas, hypergammaglobulinemia and an increase in erythrocyte sedimentation rate (ESR) [1]. Both patients showed improvement of renal function and bone marrow granulomas. However, bone marrow granuloma and interstitial eosinophilia were not observed in many previous reports. This condition has been called tubulointerstitial nephritis and uveitis (TINU) syndrome by Vanhaesebrouck et al [2]. Although the etiology of TINU syndrome is unclear, it is assumed to be an autoimmune disease [2]. Kondo et al found that both mast cells and macrophages infiltrate into fibrotic areas in TINU patients [3]. Repeated renal biopsies showed that the number of infiltrating mast cells and macrophages in the interstitium decreased with improvement of clinical symptoms and pathologic lesions.

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In this paper, we report the clinical course and immunohistochemical approach in a 40-year-old woman with a comparatively typical course of TINU syndrome. Chemical mediators released from granules in the mast cells, i.e. chymase and tryptase, may play an important role in the development of renal interstitial fibrosis [4–6]. For instance, the number of infiltrating tryptase-positive mast cells was well correlated with the degree of interstitial scarring [4] and creatinine clearance [3]. Immunocytochemical studies have shown the presence in the tissues of two mast cell phenotypes distinguishable by their neutral protease content: the MCt phenotype contains only tryptase and the MCtc phenotype contains both tryptase and chymase [7].

To examine the role of mast cells (MCtc and MCt) in renal fibrosis, we performed an immunohistochemical study using anti-chymase and anti-tryptase antibodies specific for mast cells in a patient with TINU syndrome.

Case Report

In April 2002, a 40-year-old woman was referred to the Juntendo University Hospital because of a 2-month history of low grade fever and general fatigue. She was first diagnosed as having a common cold and treated with antibiotics and nonsteroidal anti-inflammatory drugs. She had a history of allergic disease such as bronchial asthma and nettle rash, and had an allergic shock to the antibiotic Cephaclor.

On admission, her temperature was 37.2°C. Initial laboratory findings showed mild renal insufficiency and anemia. Serum creatinine was 2.27 mg/dL, serum urea nitrogen was 23 mg/dL, hemoglobin was 9.1 g/dL, hematocrit was 28.8%, and ESR was 102 mm/hour. Total serum protein, albumin, immunoglobulins complement and anti-nuclear antibody were normal. Urinalysis showed glucosuria but no proteinuria, while urinary excretion of β_2-microglobulin was 29,750 ng/dL (Table). Blood gas analysis showed slight acidosis (pH 7.339, HCO_3 16.9, BE –8.9) caused by tubular dysfunction. Ultrasonography and computed tomography of the abdomen showed normal findings. She was diagnosed with anterior uveitis by an ophthalmologist, but the cause of the uveitis could not be determined at that time. Toxoplasmosis, tuberculosis and sarcoidosis were ruled out.

Percutaneous renal biopsy was performed on June 4, 2002. Marked mononuclear cell infiltration and interstitial fibrosis were observed in the interstitium on light microscopy. No glomerular or granulomatous changes, or vascular alterations were observed (Figure 1). In immunofluorescence, there was no positive staining of immunoglobulins (IgG, IgM, IgA) and complement (C3, C4) in the renal tissues. Three-micron sections from the formalin-fixed and paraffin-embedded renal tissues were stained with human monoclonal antibodies to human mast cell chymase (Serotec, Raleigh, NC, USA) and human mast cell tryptase (Dako, Glostrup, Denmark) using an autoclave-based antigen retrieval technique and a modified peroxidase anti-peroxidase method. Briefly, after autoclaving (121°C, 5 minutes) 0.01 M citric buffer (pH 6.0) and inactivating endogenous peroxidase 0.3% in H_2O_2 in methanol, the renal sections were blocked with blocking

Table. Laboratory findings on admission and after treatment

	Admission	After treatment (1 yr after)
WBC (× 10^3/mm^3)	10,500	5,500
RBC (× 10^6/mm^3)	3.41	4.07
Hemoglobin (g/dL)	9.1	12.4
Hematocrit (%)	28.8	36.8
ESR (mm/hr)	102	18
CRP (mg/dL)	1.7	0.1
SUN (mg/dL)	23	14
Creatinine (mg/dL)	2.27	0.82
Total protein (g/dL)	9.1	8.0
IgG (mg/dL)	2,603	1,584
IgA (mg/dL)	612	452
IgM (mg/dL)	382	236
Complement level	67.4	42.3
Antinuclear antibodies	× 20	× 20
Proteinuria	Negative	Negative
Glucosuria	(++)	Negative
Urinary β_2-microglobulin (ng/dL)	29,750	1,040
N-acetyl-β-glucosaminidase (U/L)	7.4	2.1
Creatinine clearance (mL/min)	19	81

WBC = white blood cell count; RBC = red blood cell count; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; SUN = serum urea nitrogen.

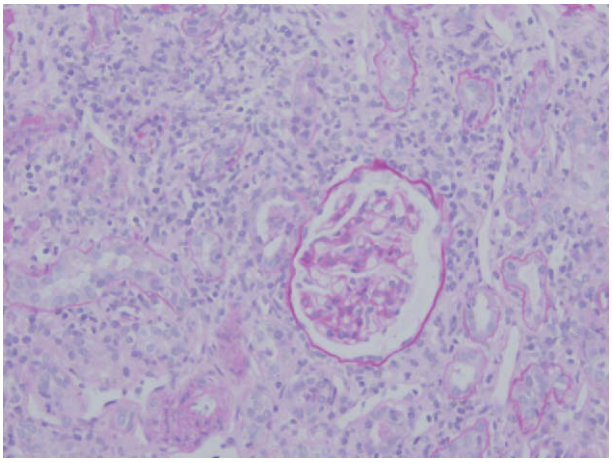

Figure 1. Light microscopy of a renal biopsy specimen shows acute tubulointerstitial reaction with edema and inflammatory cell infiltration. Glomerulus shows no abnormalities (periodic acid Schiff stain, 400×).

solution (phosphate-buffered saline containing 2% BSA, 2% fetal calf serum and 0.2% fish gelatin) for 30 minutes, followed by 1-hour incubation with antibody to chymase or tryptase, both diluted 1:50 with blocking solution. The sections were stained sequentially with the human peroxidase-conjugated Envision System (Dako Corp., Carpinteria, CA, USA), and developed with diaminobenzidine to produce a brown color. Nuclear counterstaining of the section was performed with Mayer's hematoxylin.

In addition, double immunostaining was performed to detect human mast cell subtypes. Anti-human chymase and anti-human tryptase antibodies labeled with Alexa Fluor 488 and Alexa Fluor 568, respectively, using Zenon Mouse IgG Labeling Kit (Molecular Probes, Eugene, OR, USA) were used in this study. The deparaffinized renal sections were incubated with anti-human chymase antibody and then incubated sequentially with anti-human tryptase antibody. After washing with PBS three times, the sections were mounted on slides with Perma Fluor™ aqueous mounting medium (Thermo Shandon, Pittsburgh, PA, USA).

Serial sections showed positive staining for chymase and tryptase with a similar pattern in the interstitial areas (Figure 2). The double staining of chymase and tryptase showed mostly MCtc in such areas (Figure 3). There was no positive staining in the glomeruli.

We diagnosed TINU syndrome from the clinical and histopathologic findings. Steroid pulse therapy with methylprednisolone 500 mg for 3 days was started on June 13, 2002. After pulse therapy, oral administration of prednisolone was initiated at an initial dosage of 40 mg/day. One week after steroid therapy, serum creatinine improved to 1.27 mg/dL. Prednisolone dosage was then gradually tapered and discontinued after 4 months. An anticoagulant (heparin) at 5,000 U/day was also administered with steroid therapy. One month after the therapy, urinalysis and renal function tests had almost normalized (Table). She was discharged from our hospital on July 10, 2002. At that time, the uveitis was also improved.

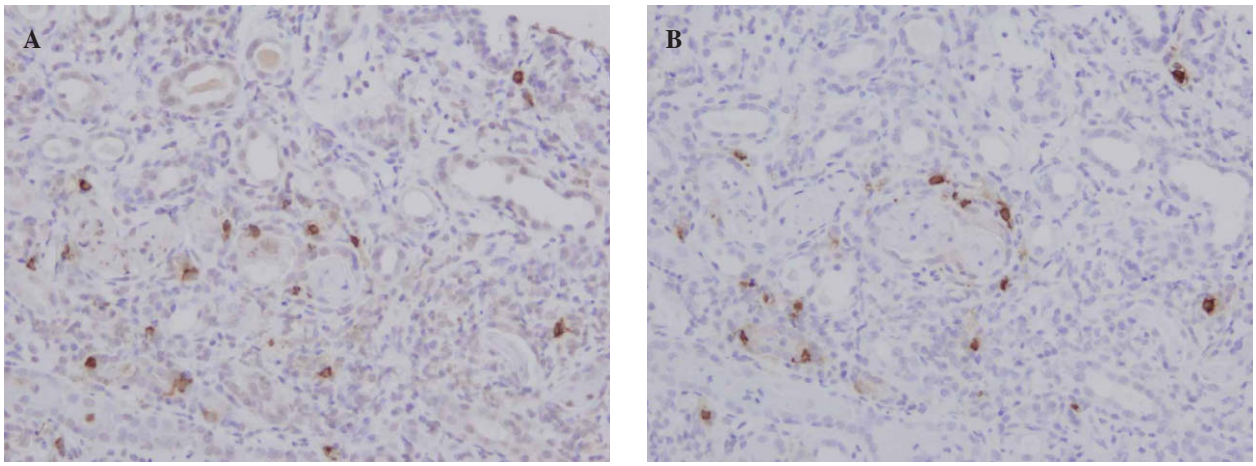

Figure 2. Immunostaining of: (A) tryptase; and (B) chymase in renal tissue. Serial sections show positive staining of chymase and tryptase in a similar pattern. Mast cells are seen only in the interstitial area (200×).

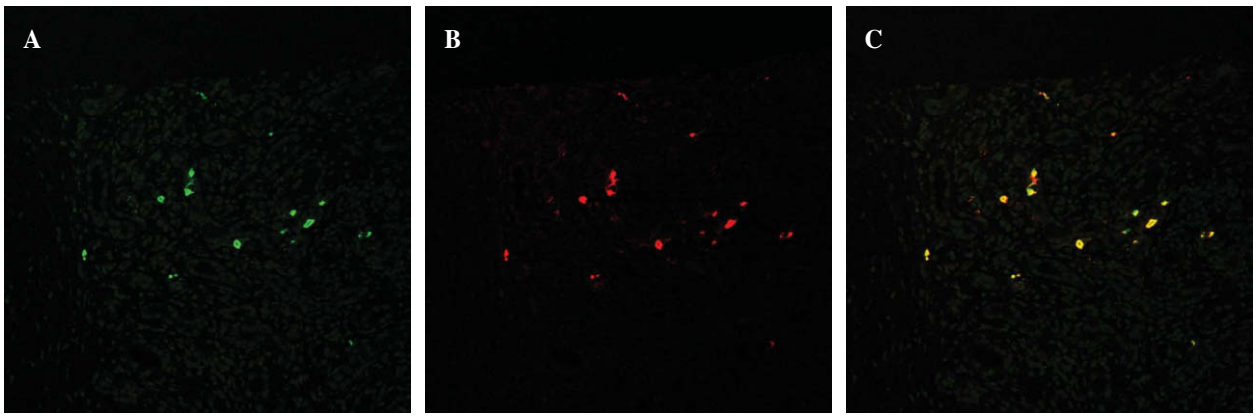

Figure 3. Immunofluorescence of: (A) chymase; and (B) tryptase in the interstitial area of renal tissue. (C) Double immunofluorescence of chymase and tryptase (MCtc) are dominant in the interstitial area. Negative staining in the glomerulus (100×).

Discussion

In this patient, there were no granulomatous changes in renal tissues although interstitial nephritis and uveitis were observed. All reported adult patients presented with body weight loss, elevated ESR and anemia, and 80% of patients did not have hypergammaglobulinemia, which is a consistent feature in adolescents. White blood cell count, C-reactive protein and ESR were elevated and urinary β_2-microglobulin was also elevated. Our patient had typical findings of TINU syndrome with interstitial lymphocyte infiltration and tubular atrophy without granulomas. Most reported cases showed completely normal glomeruli as in our patient. Large numbers of mast cells, which have a potential for serious disease progression, have been observed in patients with glomerulonephritis such as focal segmental glomerular sclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, and IgA nephropathy [8,9]. On the other hand, patients with minimal change nephrotic syndrome with normal renal function had small numbers of mast cells in renal tissues [8]. These reports suggest that mast cells in the tubulointerstitium may play an important role in tubulointerstitial fibrosis. Generally, tissue fibrosis is based on the balance between production and degradation of the extracellular matrix. Recently, Okazaki et al reported that the number of mast cells was correlated with hydroxyproline content in experimentally-induced fibrosis in rat lung and liver [10]. It was suggested that mast cells may contribute mainly to the degradation of the extracellular matrix. Therefore, it is possible that these cells may regulate extracellular matrix metabolism in the kidneys. Mast cell granule synthesis has been reported to be a mitogenic factor for cultured fibroblasts and enhanced collagen synthesis [11,12]. Ehara and Shigematsu found, by microscopic observation, that tryptase-positive mast cells were associated with fibroblast-like cells cooperating with lymphocytes and macrophages in the fibrous interstitium of patients with IgA nephropathy [5]. Moreover, mast cells have been shown to produce cytokines and growth factors that may contribute to fibrosis, such as tumor necrosis factor-α and transforming growth factor-β.

Immunocytochemical studies have shown the presence within the tissues of two mast cell phenotypes distinguishable by their neutral protease content; the MCt phenotype contains only tryptase and the MCtc phenotype contains both tryptase and chymase. MCt phenotypes appear to be "immune system-related" mast cells with a primary role in host defense. On the other hand, MCtc phenotypes appear to be "non-immune system-related" mast cells, which have functions of angiogenesis and tissue remodeling rather than immunologic protection. The mast cell phenotype in patients with IgA nephropathy or diabetic nephropathy is mainly MCtc [5]. Kurusu et al investigated whether mast cells in the tubulointerstitium actively contribute to the development of tubulointerstitial fibrosis in patients with IgA nephropathy [13]. These cells were observed in the tubulointerstitium, including the perivascular and periglomerular areas, but not in the glomeruli of patients with IgA nephropathy. There was a significant correlation between the number of mast cells per unit area in the whole tubulointerstitium and the degree of tubulointerstitial fibrosis or level of urinary protein excretion, but not with the level of urinary β_2-microglobulin. These results suggest that the number of mast cells in the tubulointerstitium may influence the prognosis in patients with IgA nephropathy [13]. Patients with rapidly progressive glomerulonephritis or renal amyloidosis had the MCt rather than the MCtc phenotype [14].

In TINU syndrome, the degree of renal insufficiency is closely correlated with the severity of tubulointerstitial fibrosis and the number of mast cells. Kondo et al suggested that mast cells, in addition to macrophages, might play an important role in the development of interstitial injury in TINU syndrome because the degree of renal interstitial lesions appears to be related to the number of these cells [4]. They also suggested that mast cells showed a stronger tendency to infiltrate the fibrotic areas than macrophages. To date, there have been no studies staining both tryptase and chymase in TINU syndrome patients. We examined the localization of mast cells using anti-human tryptase and anti-human chymase antibodies specific for mast cells in a patient with TINU syndrome. We also performed double immunostaining using tryptase and chymase to determine which phenotype is dominant. In this case, it appeared that most mast cells that infiltrated the fibrotic areas are MCtc. It is postulated that MCtc is one of the constitutive cells of interstitial fibrosis in patients with TINU syndrome.

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