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Contribution of mast cells to the tubulointerstitial lesions in IgA nephritis

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Contribution of mast cells to the tubulointerstitial lesions in IgA nephritis.

Background. Mast cells have never been extensively investigated in renal disease, particularly glomerulonephritis. Recent improvements in monoclonal antibody production to mast cell specific enzymes have made it possible to study mast cells in tissues more accurately and easily. Mast cells have been found to secrete basic fibroblast growth factor (bFGF) in human pulmonary fibrosis.

Methods. Mast cells in 67 cases of IgA nephritis were investigated. Toluidine blue (TB) stainings at pH 5.0 and pH 0.5 were employed histochemically, and anti-human mast cell tryptase and chymase monoclonal antibodies were used immunohistochemically. Anti-bFGF antibody was also used immunohistochemically.

Results. Mast cells were scattered in the interstitium including in fibrotic areas. TB pH 0.5-positive mast cells were more numerous than TB pH 5.0-positive mast cells. Immunostaining with anti-tryptase monoclonal antibody detected more mast cells than the TB stainings. Mast cells in the interstitium of IgA nephritis had both tryptase and chymase. Immunoelectron microscopy showed that tryptase was exclusively localized in the specific granules of mast cells. The average number of tryptase positive mast cell in the interstitium of IgA nephritis was lower than that of T lymphocyte but more than that of macrophages. The average number of mast cells increased with the progression of interstitial fibrosis and had a significant correlation with 24-hour creatinine clearance. Using double labeled immunohistochemistry, some tryptase-positive mast cells had bFGF in their cytoplasm. Electron microscopy showed that mast cells were associated with fibroblasts and/or lymphocytes in the interstitium.

Conclusion. Mast cells are one of the constitutive cells in the interstitium of IgA nephritis patients and affect renal function by contributing to the interstitial fibrosis.

Mast cells are bone marrow derived [1, 2]. The cells have high affinity IgE receptors and are known to cause type I hypersensitivity when cross linked to the receptors [3]. The granules in mast cells contain various primary inflammatory mediators, such as histamine, various enzymes, and chemotactic factors. Moreover, mast cells secrete secondary mediators, prostaglandin, leukotrienes, and platelet activating factor. Mast cells have been extensively studied with respect to lung and skin diseases. Recent studies, however, have revealed that mast cells are actively involved in several diseases including rheumatoid arthritis [4], diabetes mellitus [5], and AIDS [6]. In particular, mast cells have been shown to play a role in fibrotic processes like scleroderma [7], pulmonary fibrosis [8], and keloid [9]. Interaction between fibroblasts and mast cells is essential for fibrosis. Fibroblasts secrete mast cell growth factors, one of which is known as c-kit ligand [10]. Recently, mast cells were identified as the cells that secrete basic fibroblast growth factor (bFGF) in pulmonary fibrosis [11].

The importance of tubulointerstitial lesions in renal disease [12, 13], including IgA nephritis, has been stressed [14]. Macrophages and T lymphocytes have been shown to be involved in the pathogenesis of tubulointerstitial lesions [15, 16] possibly through the secretion of growth factors and cytokines [13]. Although mast cells are known to play a role in drug-induced tubulointerstitial nephritis [17], they have never been studied extensively with respect to glomerulonephritis. In the present study we investigated mast cells in IgA nephritis making use of an immunostaining method with anti-human mast cell monoclonal antibody and histochemical Toluidine blue (TB) stainings at pH 5.0 and 0.5. We also tried to check whether the mast cells in the renal interstitium had bFGF or not. The results showed that mast cells were one of the constitutive cell types in the interstitium of IgA nephritis patients and contributed to the interstitial fibrosis resulting in the deterioration of renal function. In addition, mast cells were found to have bFGF.

Key words: basic fibroblast growth factor, IgA nephritis, pulmonary mast cell, toluidine blue staining, tryptase, fibrosis.

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METHODS

Sixty-seven cases of primary IgA nephritis in the file of our department were studied. Patient ages ranged from 4 to 62 years, and the mean age was 28.6 years. The male to female ratio was 1.2:1. Needle biopsy was performed in 63

Table 1. Antibodies used in this study

Antibody	Animal	Specificity
Anti-tryptase (AA-1)	mouse	mast cell
Anti-chymase	mouse	mast cell
UCHL-1	mouse	T lymphocyte
MAC 387	mouse	activated macrophage
CD68 (KP-1)	mouse	tissue macrophage
Anti-human IgE	rabbit	
Anti-b-FGF	rabbit	

Antibodies were from DAKO except b-FGF (basic fibroblast growth factor) and anti-chymase, which were from CHEMIKON and Biomedical Technologies, respectively.

cases and open biopsy in 4 cases. Some patients had bronchial asthma, but none were known to be allergic to the drug. Serum IgA values were calculated in 55 cases. Serum creatinine and blood urea nitrogen (BUN) were calculated in 58 cases. Urinary protein excretion was calculated in 35 cases. Twenty-four hour creatinine clearance was calculated in 27 cases. Biopsy samples were fixed immediately in 30% formaldehyde and embedded in paraffin. Other samples were snap frozen for immunofluorescence or fixed in 2.5% glutaraldehyde for electron microscopical observation. All cases showed the predominant deposition of IgA in the mesangial area. Control sections were obtained from 6 patients (39 to 72 years old, mean age 57 years) with nephrectomy due to renal cell carcinoma, and essentially normal areas without carcinoma invasion were selected for the study. Paraffin embedded tissues were cut to 3 microns and stained with TB pH 5.0 for 30 minutes or TB pH 0.5 (0.5% TB in 0.5 N HCl) for five days [18]. TB pH 0.5 was counterstained with nuclear fast red.

The clinical data were compared with the average number of cells in glomeruli or in the interstitium. The degree of mesangial proliferation was divided into three groups (minor, mild, moderate) and compared with the mast cell number. Those with interstitial fibrosis was also divided into three groups (mild, moderate, severe) and compared. Biopsy and control specimens were observed with a light microscope at $\times 200$ magnification using an attached eye graticle. Cells were counted by the authors without the knowledge about clinical data. Values were expressed as the average number of cells per square millimeter of interstitium or average number of cells per glomeruli.

Immunohistochemistry

The antibodies used for immunohistochemistry are listed in Table 1. The avidin-biotin complex method (DAKO) or indirect peroxidase method for double labeled immunohistochemistry was employed. Briefly, sections were deparaffinized and washed in Tris buffered saline (TBS). Pretreatment, 1% trypsin (DIFCO, Detroit, MI, USA) digestion for 30 minutes, was done before the incubation with all of the antibodies except UCHL-1. Peroxidase activity was blocked by incubation in 1% hydrogen peroxide in methanol for 20 minutes. Then sections were incubated in primary antibodies at 37°C for one hour. After a wash with TBS, biotinylated anti-mouse and rabbit immunoglobulins were applied for 15 minutes. Then streptavidin solution was applied according to the instructions of vendor. Antibody binding sites were visualized with diaminobenzidine (DAB) and hydrogen peroxide. As a control for immunohistochemistry, sections were incubated in normal mouse or rabbit serum without the primary antibody. Sections were counterstained with hematoxylin unless otherwise stated.

Serial frozen sections were incubated with anti-tryptase and chymase antibody. After washing, fluorescent labeled goat anti-mouse antibody was applied.

For double labeled immunohistochemistry, sections were first digested with hyaluronidase (Wako Pure Chemical Industries, Osaka, Japan) and incubated first in a rabbit antibody to basic fibroblast growth factor (Biomedical Technologies). After incubation and wash, swine antibody to rabbit (DAKO) was applied at room temperature for 30 minutes. Visualized with DAB and washed, the sections were digested with 1% trypsin for 30 minutes, then incubated with an antibody to mast cell tryptase or macrophage (MAC387) followed by fluorescein-conjugated goat antibody to mouse (Organon Technica). Observation was done with Olympus BX-50 microscope. Tryptase positive cells or MAC387 positive macrophages were checked for the existence of bFGF in their cytoplasm.

Electron microscopy

For the electron microscopic observation, glutaraldehyde fixed specimens were rinsed in phosphate buffer and postfixed in 1.5% osmium tetroxide for one hour. After dehydration, these specimen were embedded in Quetol 812. Ultrathin sections were cut with a diamond knife by LKB ultramicrotome and stained with uranyl acetate and lead citrate. Observation was with a JEM 1200 electron microscope. Control cases were not observed electron microscopically.

Immunogold labeling for electron microscopy

Ultrathin sections of glutaraldehyde and osmium fixed biopsy specimens were cut as described above and mounted on nickel mesh. These sections were etched by 0.5% hydrogen peroxide for five minutes. After a wash with phosphate buffered saline (PBS), the sections were incubated in anti-tryptase antibody diluted to 1:50 in PBS overnight. After a wash with PBS, 10 nm immunogold labeled goat anti-mouse IgG (British Bio Cell International, Cardiff, UK) was diluted to 1: 50 with PBS and applied for 30 minutes. The specimens were then washed with distilled water and stained with uranyl acetate. Control sections were processed without primary antibody.

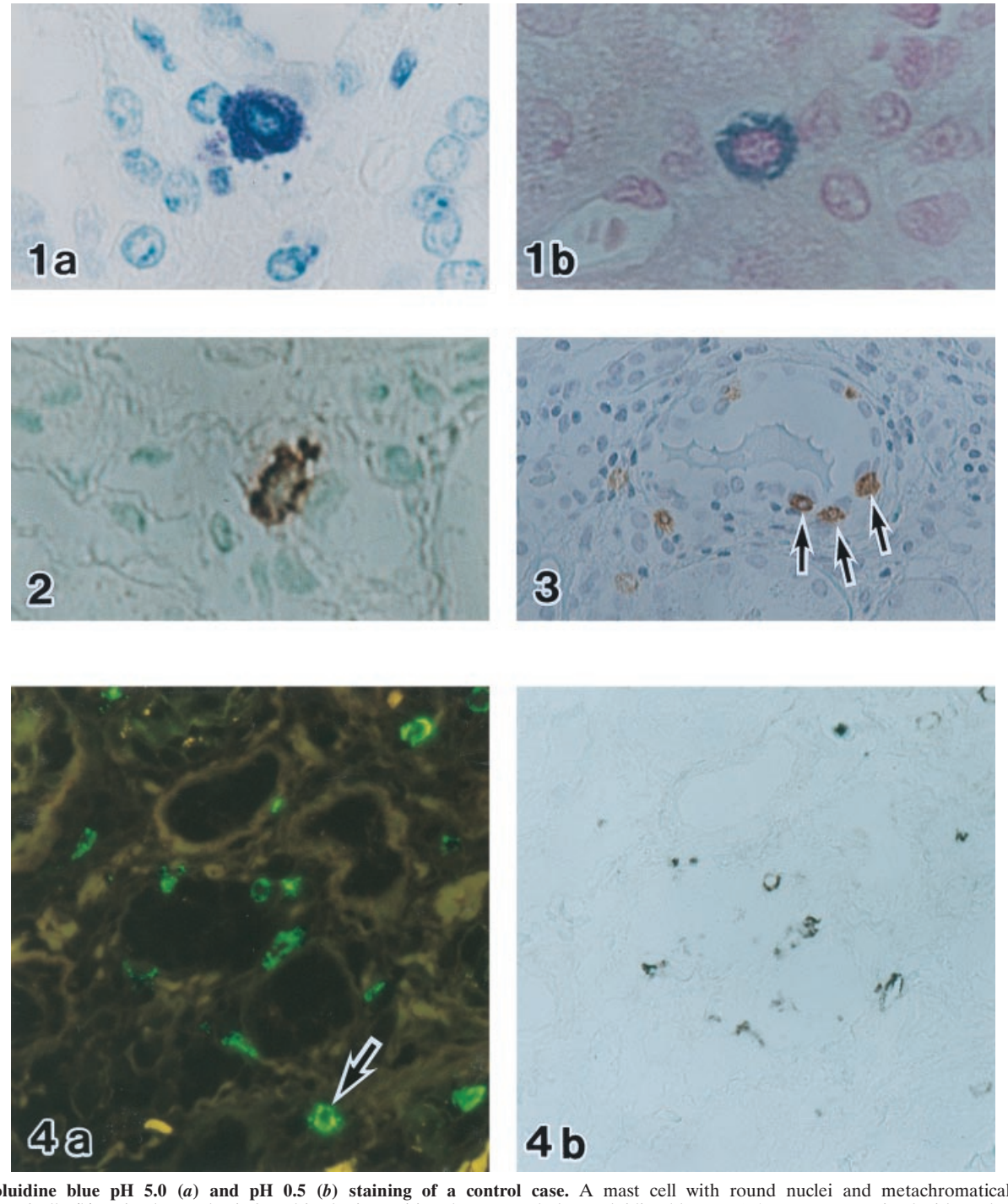

Fig. 1. Toluidine blue pH 5.0 (*a*) and pH 0.5 (*b*) staining of a control case. A mast cell with round nuclei and metachromatically (a) or orthochromatically (b) stained granules is found in the interstitium between tubules. Toluidine blue pH 0.5 was counterstained with nuclear fast red (×900).

Fig. 2. Immunostaining with anti-tryptase monoclonal antibody of a 25-year-old female with IgA nephritis. A mast cell that is polygonal in shape with cytoplasmic processes can be seen (counterstained with methylgreen; ×900).

Fig. 3. Anti-tryptase monoclonal staining of a 23-year-old female with IgA nephritis. Mast cells (arrows) have infiltrated the tubules (×350).

Fig. 4. Double immunostaining of a 51-year-old male patient with anti-tryptase antibody (*a*), and anti-basic fibroblast growth factor (bFGF) (*b*). Tryptase positive mast cells are scattered in fibrous interstitium and positive for bFGF in this field except one (arrow) (×250).

Statistics

Student's *t*-test or Aspin-Welch method were used for the analysis of the relationship between interstitial fibrosis and mesangial proliferation. Pearsons' correlation was used for the analysis of clinical data and the average number of cells. $P < 0.05$ was regarded as significant.

RESULTS

Histochemistry

In control sections, mast cells positively stained with TB were mononuclear with oval shaped cytoplasms. Granules of mast cells were metachromatically stained purple or purple red with TB pH 5.0 (Fig. 1a) and orthochromatically stained blue with TB pH 0.5 (Fig. 1b). TB-positive mast cells were scattered in the interstitium between tubules, around glomeruli or blood vessels. TB-positive mast cells were relatively abundant around large blood vessels or juxtacortical interstitium, however, no TB-positive mast cells were found in glomeruli. On average, significantly more mast cells were stained with TB pH 0.5 ($1.2 \pm 0.4/mm^2$, mean $\pm$ SE) than TB pH 5.0 ($0.7 \pm 0.2/mm^2$) (Table 2). Serial sections showed that a part of mast cells were positive for both TB stainings. In the IgA nephritis cases, the distribution pattern for mast cells was almost the same as in control except a few cells were found in the tubular lumen. On average, more mast cells were stained with TB pH 0.5 ($10.2 \pm 1.4/mm^2$) than TB pH 5.0 ($2.4 \pm 0.5/mm^2$). The average number of mast cell stained with TB in IgA nephritis was significantly more than that of control (Table 2). Similarly, in the controls, some mast cells stained positively for both TB pH levels (data not shown).

Immunohistochemistry

Cytoplasmic granules of mast cells stained positively with anti-tryptase monoclonal antibody. The distribution of mast cell positive for tryptase was the same as for TB stainings except that only one tryptase positive cell was found in a glomerulus in the controls (not shown). Tryptase positive cells were easier to identify than TB positive cells. Serial control sections showed that TB positive cells were positive for tryptase. TB negative and tryptase positive cells were sometimes found. In control, the average number of tryptase positive mast cells was $1.9 \pm 0.3/mm^2$, significantly more than that of TB pH 0.5 and TB pH 5.0 positive mast cells ($1.2 \pm 0.4/mm^2$ and $0.7 \pm 0.2/mm^2$, respectively; Table 2). Cytoplasmic processes were not visible in control specimens. In the IgA nephritis cases, some tryptase positive mast cells were a polygonal shape (Fig. 2). Cytoplasmic processes were sometimes visible. Only in one case of IgA nephritis were no tryptase positive cells present in the interstitium. On average, tryptase-positive mast cells ($23.0 \pm 2.9/mm^2$) were significantly more numerous than histochemically-stained mast cells in the IgA nephritis cases (TB pH 0.5 and TB pH 5.0, 10.2 ± 1.4 and $2.4 \pm 0.5/mm^2$, respectively; Table 2), although 9 out of 67 cases had more TB pH 0.5 positive than tryptase positive mast cells. The staining intensity for cytoplasmic granules varied from cell to cell and granule to granule. Although tryptase positive mast cells were haphazardly scattered in the interstitium as seen in the controls, mast cells in the IgA nephritis cases were frequently seen in fibrotic areas, or around atrophic tubules or dilatatic tubules with casts. Mast cells rarely infiltrated the tubules (Fig. 3). Basic fibroblast growth factor-positive cells were located separately each other in the interstitium of IgA nephritis cases. Double labeled immunohistochemistry with anti-bFGF antibody and anti-mast cell tryptase showed that a part of mast cells had bFGF in the cytoplasm (Fig. 4). The average number of bFGF positive cells ($4.5 \pm 1.0\ mm^2$) was significantly lower than that of tryptase positive cells (23.0 ± 2.9). Each case with IgA nephritis had different percentage of bFGF-positive mast cells that ranged from 0 to 65%. MAC387 positive macrophages were negative for bFGF. Basic fibroblast growth factor-positive cells were found in a few glomeruli of 3 cases with IgA nephritis. Macrophages and lymphocytes, but no tryptase positive mast cells, were found in the glomeruli of IgA nephritis patients. Few tryptase positive cells were found in the lumen of blood vessels in the interstitium. The average number of tryptase-positive mast cells ($23.0 \pm 2.9/mm^2$) was lower than the average number of T lymphocytes ($36.8 \pm 6.5/mm^2$) and the difference was significant. Tryptase-positive mast cells were more numerous than CD68 positive ($15.8 \pm 3.5/mm^2$) and MAC387 positive ($21.2 \pm 3.4/mm^2$) cells, though the difference was not significant. The area where inflammatory cells were accumulated was composed of T lymphocytes and contained few mast cells (Fig. 5). In control sections, some tryptase positive mast cells stained positive with CD68 antibody (not shown). In the control cases it was possible to distinguish CD68-positive mononuclear mast cells from CD68 positive macrophages by the latter's polygonal shape. However, this was difficult in the IgA cases because the mast cells were also polygonal. IgE was positive along the cytoplasmic membrane of the cells in the interstitium of 20 cases with IgA nephritis. IgE positive cells were found to be located separately from each other. Almost all of the IgE positive cells proved to be mast cells by serial sections. Three out of 20 cases were found to have diffusely scattered IgE positive cells, some of which were not able to identify the cell type. These cases were 14-, 24- and 25-year-old females having severe (2 cases) and moderate (1 case) interstitial fibrosis. Two of them had high serum IgE values (1935 and 2022 mg/dl) and one of them had a clinical history of a worsening renal function when a bronchial asthma attack occurred.

Table 2. Average number of mast cells in the interstitium

	Control	IgA nephritis
Toluidine blue pH 5	0.7 ± 0.2	2.4 ± 0.5
Toluidine blue pH 0.5	1.2 ± 0.4	10.2 ± 1.4
Tryptase positive	1.9 ± 0.3[a]	23.0 ± 2.9[a]

Data are the mean/mm^2 $\pm$ standard error.
[a] $P < 0.05$ vs. Toluidine blue stainings

Clinical parameters and mast cell

The average values of serum IgA, BUN and creatinine of IgA nephritis patients were 357 ± 16, 17.3 ± 1.1 and $1.1 \pm$

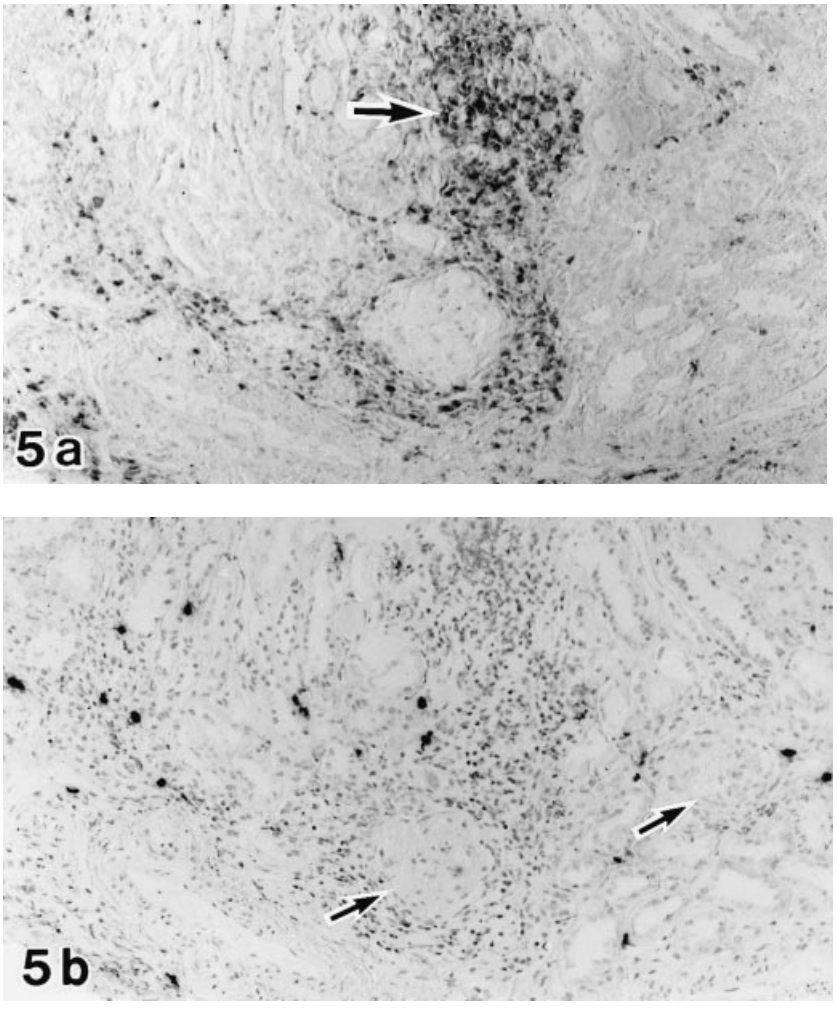

Fig. 5. The same area from a 25-year-old female with IgA nephritis is stained with UCHL-1 (*a*), and anti-tryptase monoclonal antibody (*b*). (a) UCHL-1 is positive where lymphocytes accumulated (arrow). (b) Tryptase positive cells are scattered in the interstitium. No positive cells are found in glomeruli (small arrows). In this field, the average number of UCHL-1 and tryptase positive cells in the interstitium is 344 and 42/mm^2, respectively (×150).

0.1 mg/dl, respectively. There was no or little correlation between the average number of each type of cell per glomeruli and clinical parameters (data not shown). Several clinical parameters correlated significantly with the number of cells in the interstitium (Table 3). All types of cells in the interstitium including tryptase positive cells correlated significantly with BUN and serum creatinine values. Urinary protein excretion was not correlated significantly with all types cells except tryptase positive cells. The average number of interstitial cells correlated significantly with 24-hour creatinine clearances, and the tryptase positive mast cells had an especially high correlation coefficient (r = 0.650; Fig. 6). Serum IgA values did not correlate with cell numbers for any cell type in the interstitium.

Table 3. Correlation between the clinical parameters and average number of cells in interstitium

	S_{IgA}	BUN	S_{Cr}	C_{Cr}	$U_{protein}$
MAC	0.038	0.625[a]	0.623[a]	0.618[a]	0.275
CD68	0.046	0.657[a]	0.702[a]	0.599[a]	0.280
UCHL-1	0.003	0.492[a]	0.590[a]	0.618[a]	0.344
Tryptase	0.010	0.407[a]	0.430[a]	0.650[a]	0.437[a]

Abbreviations are: S_{IgA}, serum IgA (mg/dl); BUN (mg/dl), blood urea nitrogen; S_{Cr}, serum creatinine (mg/dl); C_{Cr}, 24-hour creatinine clearance (ml/min); $U_{protein}$, urinary protein excretion per day (g/day).
[a] $P < 0.05$

Relationship between mesangial cell proliferation, fibrosis and mast cells

The degree of mesangial proliferation did not correlate with the number of mast cells. The average number of mast cells increased significantly with the progression of interstitial fibrosis (Table 4). The average number of the other interstitial cells also increased with the progression of interstitial fibrosis (Table 4). The average number of bFGF expressed cells in severe fibrotic group (10.3 ± 3.6) was significantly more than that in the mild and moderate fibrotic groups (3.2 ± 1.5, 4.0 ± 1.2, respectively; Table 4).

Electron microscopy

In IgA nephritis, the number and density of specific granules in mast cells were variable. Some mast cells were

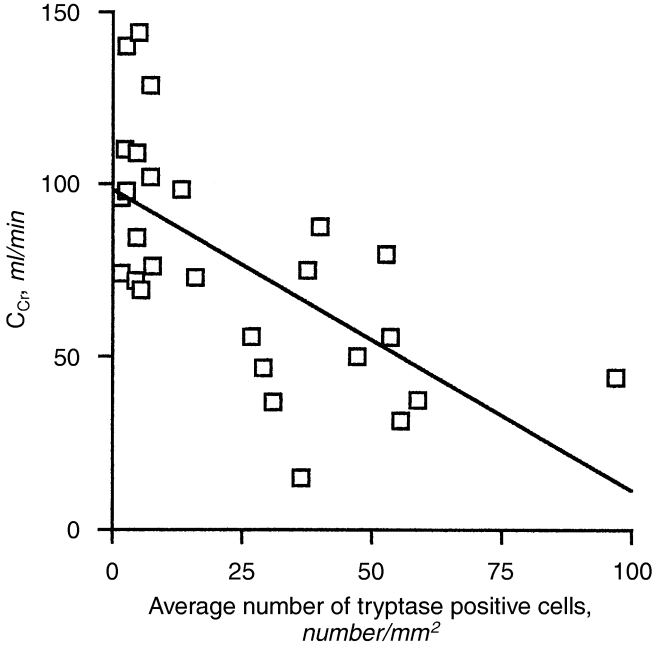

Fig. 6. Correlation between the number of cells and the 24-hour creatinine clearance. y = − 0.870x + 98.383; $r = 0.650$; $P < 0.05$.

Table 4. Relationship between the average number of cells in interstitium and the degree of interstitial fibrosis

	Tryptase	bFGF	UCHL-1	MAC 387	CD68
Mild ($N = 32$)	11.1 ± 2.5	3.2 ± 1.5	17.7 ± 2.8	11.2 ± 5.9	7.2 ± 1.0
Moderate ($N = 25$)	26.8 ± 4.5	4.0 ± 1.2	33.2 ± 7.1	18.0 ± 4.8	10.8 ± 3.4
Severe ($N = 10$)	45.5 ± 7.7	10.3 ± 3.6	102.3 ± 30.0	61.0 ± 10.9	55.6 ± 14.0

Data are mean ± SE. Definitions are: Mild, mild fibrosis of interstitium; Moderate, moderate fibrosis of interstitium; Severe, severe fibrosis of interstitium.

closely associated with fibroblast like cells and lymphocytes in the interstitium (Fig. 7). Other mast cells showed no association with other cell types. Using immunogold methods, specific granules of mast cells were specifically labeled with gold particles (Fig. 8). No other cells or cell organelles were labeled with the particles.

DISCUSSION

Using toluidine blue staining, we showed that in IgA nephritis and control TB pH 0.5-positive mast cells were significantly more numerous than TB pH 5.0-positive mast cells. The effect of formaldehyde should be considered when evaluating this result since formaldehyde fixation is known to reduce the number of mast cells stained with TB [18]. In rats, TB staining is useful for distinguishing subpopulations of mast cells. Connective tissue mast cells (CTMC) stain with TB pH 5.0 and mucosal mast cells (MMC) stain with TB pH 0.5 [19]. In humans, no clear distinction has been established with respect to TB stainings. In our study, a considerable number of mast cells were positive for both TB pH 5.0 and TB pH 0.5 stainings as shown in the serial sections. Therefore, these histochemical techniques are not useful tools to identify subpopulations of human mast cells. In humans, Irani et al distinguished two types of mast cells in Carnoy's fixed tissues based on the existence of two enzymes, tryptase and chymase, and designated them T and TC mast cells, respectively [20]. T mast cells have tryptase only and exist in the intestinal mucosa and alveoli of human lung. TC mast cells have both tryptase and chymase and exist mainly in the submucosa of the alimentary tract and skin. T and TC mast cells were thought to be analogous to the MMC and CTMC of rats, respectively. Unfortunately, we could not use the antibody to chymase to formalin fixed sections.

Serial frozen sections were checked for the existence of two enzymes in a mast cell. The results showed that almost all mast cells in the renal interstitium had both enzymes. This suggests that the mast cells in the kidney could be TC type. Curiously, however, chymase positive and tryptase negative mast cells existed in transplanted kidney (unpublished data).

In the past, only histochemical staining and electron microscopy were available to identify mast cells. Monoclonal antibodies, however, enable the detection of mast cells more accurately. Using the mast cell specific enzyme, tryptase, several monoclonal antibodies that recognize human mast cells have been developed [21, 22]. The monoclonal antibody we used, AA-1, was developed by Walls et al and stains mast cells specifically in formaldehyde fixed sections [21]. Our study showed that immunostaining with this antibody was a more sensitive technique for identifying mast cells than histochemical methods, even in formaldehyde fixed kidney specimens. Walls et al found no significant difference in sensitivity between formaldehyde fixation and Carnoy's fixation. Our immunoelectron microscopic analysis using immunogold methods to routinely process sections also revealed that anti-tryptase antibody specifically recognized specific granules of mast cells. This suggests that immunostaining with anti-tryptase monoclonal antibody detects almost all of the mast cells in the renal interstitium. One tryptase positive cell was detected in a glomerulus of a control case and a few tryptase positive cells were found in the capillary lumen of IgA nephritis cases. It is currently unknown what type of cell these were, though it may be a basophil since it contained only a small amount of tryptase (0.04 pg/cell [23] compared with 35 pg in dermal mast cells [24]). Another possibility is that this cell is a mast cell precursor, as is found in the rat [2]. Our study revealed that CD68 antibody recognized some mast cells in the interstitium of kidney, as in a previous report [25].

Inflammatory cells have been studied extensively in

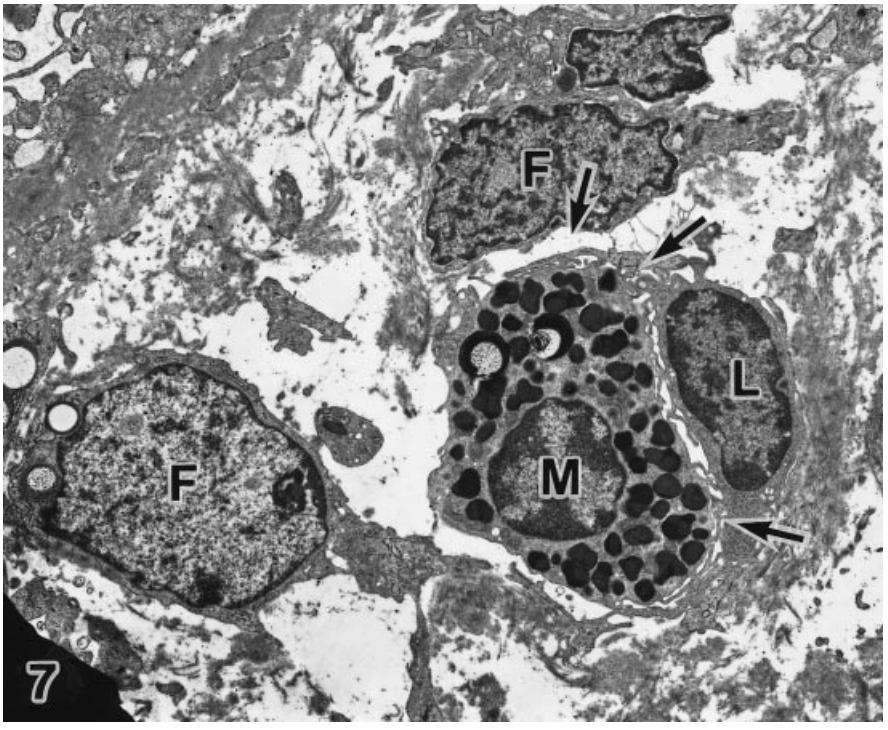

Fig. 7. Electron micrograph of a 44-year-old male with IgA nephritis. A fibroblast (F), lymphocyte (L), and mast cell (M) are associated and attached by processes (arrows) (×6000).

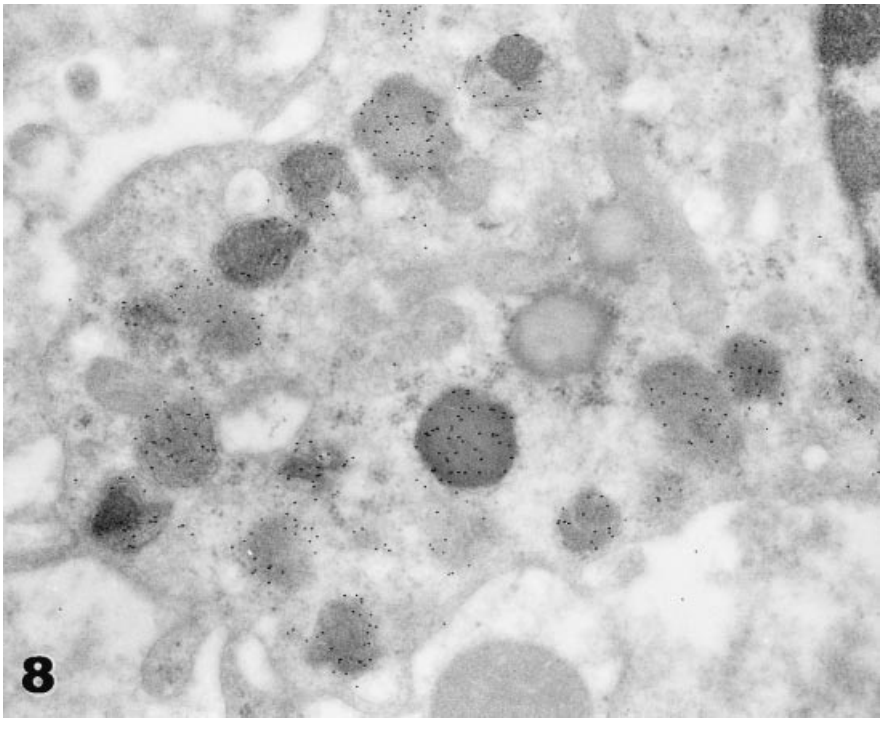

Fig. 8. Specific granules in a mast cell are selectively labeled with 10 nm gold particles (×34,000).

various types of glomerulonephritis, including IgA nephritis [14–16]. Macrophages and T lymphocytes are the predominant inflammatory cells in the renal interstitium. Generally, interstitial inflammatory cells are considered to be more important to renal function than cells in the glomeruli. There have been few studies about mast cells in kidney to our knowledge [26, 27]. Our results first demonstrate that mast cells are one of the constitutive cell types in the interstitium of IgA nephritis. Renal functional parameters (BUN, urinary protein excretion, 24-hr creatinine clearance) are significantly correlated with the number of mast cells. Basic fibroblast growth factor is expressed in

the cytoplasm of mast cells in the interstitium. Electron microscopical observation reveals that mast cells are often closely associated with fibroblast-like cells cooperating with lymphocytes and macrophages in the fibrous interstitium of IgA nephritis patients. Fibroblast and mast cells are known to influence each other [10, 11]. Mast cell granule synthesis has been reported to be dependent on fibroblasts [28]. Taken together, these data indicate that mast cells are actively involved in the fibrotic process with lymphocytes, macrophages, and fibroblasts in the interstitium in IgA nephritis.

Platelet-derived growth factor and transforming growth factor-beta have been shown to be expressed in kidney of patients with IgA nephritis [29]. Human dermal mast cell are already known to produce and secrete tumor necrosis factor alpha [30]. Interleukin (IL)-4, IL-5 and IL-6 are identified in the cytoplasm of mast cells in the nasal mucosa [31]. Recently, Qu et al found mast cells as a major source of bFGF in pulmonary fibrosis and skin hemangioma [11]. Also in our study, tryptase-positive mast cells were found to selectively express bFGF. Basic FGF correlated with the degree of interstitial fibrosis of IgA nephritis. In lung, bFGF is produced more in the early stage of disease [32]. This suggests that mast cells play different roles in the kidney; however, further study of growth factor expression and fibrosis is needed. As the bFGF was expressed in glomeruli, this growth factor might be produced from other, as yet unidentified cells.

The mechanism by which mast cells proliferate in the interstitium in IgA nephritis is not known. Mast cell proliferation has been observed in skin with scleroderma, where the mechanism of mast cell appearance is considered to involve IL-3 and/or IL-4 stimulation by helper T lymphocytes [33]. If the mast cells in the interstitium of the kidney is identical with the TC type mast cell in the gut, then T lymphocyte-dependent proliferation is unlikely because only the T type mast cells have been shown to be dependent on the T lymphocyte [6]. The average number of mast cells increased with the progression of interstitial fibrosis. It is not possible to decide from this study whether or not mast cell increased as a result of fibrosis.

IgE bearing cells were identified in some cases with IgA nephritis. Two cases had diffuse IgE positive infiltration in the interstitium with progressed fibrosis and a high serum IgE value. One case, moreover, had asthma that caused poor renal function after an asthmatic attack. These data suggest that type I hypersensitivity is involved in the formation of interstitial lesion in some cases of IgA nephritis. Drug-induced tubulointerstitial nephritis is sometimes caused by type I allergy [17]. However, in our cases with IgE positive cells, any changes such as plasma cell and eosinophil infiltration that could indicate a drug-induced interstitial nephritis were not observed. None of the patients in the present study had an allergy to the drug.

This paper is the first to our knowledge to describe the importance of mast cells in the interstitium in IgA nephritis. Mast cells should be investigated further in renal disease.

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REFERENCES

1. Kitamura Y, Shimada M, Hatanaka K, Miyano Y: Development of mast cell from grafted bone marrow cells in irradiated mice. *Nature* 268:442–443, 1977
2. Zucker-Franklin D, Grusky G, Hirayama N, Schnipper E: The presence of mast cell precursors in rat peripheral blood. *Blood* 58:544–551, 1981
3. Ishizaka T, Hirata F, Ishizaka K, Axelrod J: Stimulation of phospholipid methylation. Ca^{2+} influx, and histamine release by binding of IgE receptors on rat mast cells. *Proc Natnl Acid Sci USA* 77:1903–1906, 1980
4. Crisp AJ, Chapman CM, Kirkham SE, Shiller AL, Krane SM: Articular mastocytosis in rheumatoid arthritis. *Arthritis Rheum* 27: 845–851, 1984
5. Norrby K, Jakobsson A, Simonsen M, Sorbo J: Increased angiogenesis in diabetes. *Experentia* 46:856–860, 1990
6. Irani AA, Craig SS, DeBlois G, Elson CO, Schechter NM, Schwartz LB: Defficiency of the tryptase-positive, chymase-negative mast cell type in gastrointestinal mucosa of patients with defective T lymphocyte function. *J Immunol* 138:4381–4386, 1987
7. Rother MJ, Nowak M, Kerdel FA: The mast cell in health and disease. *J Am Acid Dermatol* 23:615–624, 1990
8. Schulman ES: The role of mast cells in inflammatory responses in the lung. *Crit Rev Immunol* 131:35–70, 1993
9. Craig SS, Deblois G, Schwartz LB: Mast cells in human keloid, small intestine, and lung by an immunoperoxidase technique using a murine monoclonal antibody against tryptase. *Am J Pathol* 124:428–435, 1986
10. Flanagan JG, Leder P: The kit ligand: A cell surface molecule altered in steel mutant fibroblasts. *Cell* 63:185–194, 1990
11. Qu Z, Liebler JM, Powers MR, Galey T, Ahmadi P, Huang X, Ansel JC, Butterfield JH, Planck SR, Rosenbaum JT: Mast cells are a major source of basic fibroblast growth factor in chronic inflammation and cutaneous hemangioma. *Am J Pathol* 147:564–573, 1995
12. Risdon RA, Sloper JC, Wardener HE: Relationship between renal function and histological changes found in renal biopsy specimen from patients with persistent glomerular nephritis. *Lancet* 1:363–366, 1968
13. Cameron JS: Tubular and interstitial factors in the progression of glomerulonephritis. *Pediatr Nephrol* 6:292–303, 1992
14. Abe S, Amagasaki Y, Iyori S, Konishi K, Kato E, Sakaguchi H: Significance of tubulointerstitial lesions in biopsy specimens of glomerulonephritis patients. *Am J Nephrol* 9:30–37, 1989
15. Alexopoulos E, Seron D, Hartley RB, Cameron JS: The role of interstitial infiltrates in IgA nephropathy: A study with monoclonal antibodies. *Nephrol Dial Transplant* 4:187–195, 1989
16. Arima S, Nakayama M, Naito M, Sato T, Takahashi K: Significance of mononuclear phagocytes in IgA nephropathy. *Kidney Int* 39:684–692, 1991
17. Heptinstall RH: Renal complications of therapeutic and diagnostic agents, analgesic abuse, and addiction to narcotics (chapt 23), in *Pathology of the Kidney* (vol 3, 3rd ed), edited by Heptinstall RH, Boston, Little, Brown and Company, 1983, pp 1195–1255
18. Wingren U, Enerback L: Mucosal mast cell of the rat intestine: A re-evaluation of fixation and and staining properties, with special

reference to protein blocking and solubility of the granular glycosaminoglycan. *Histochem J* 15:571–582, 1983
19. Strobel S, Miller HR, Ferguson A: Human intestinal mucosal mast cell: Evaluation of fixation and staining techniques. *J Clin Pathol* 34:851–858, 1981
20. Irani AA, Schechter NM, Craig SS, Deblois G, Schwartz LB: Two types of human mast cells that have distinct neutral protease compositions. *Proc Nat Acad Sci USA* 83:4464–4468, 1986
21. Walls AF, Jones DB, Williams JH, Church MK, Holgate ST: Immunohistochemical identification of mast cells in formaldehyde-fixed tissue using monoclonal antibodies specific for tryptase. *J Pathol* 162:119–126, 1990
22. Irani AA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB: Detection of MCt and MCtc types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* 37:1509–1515, 1989
23. Shalit M, Schwartz LB, Golzar N, vonAllman C, Valenzano M, Fleekop P, Atkins PC, Zweiman B: Release of histamine and tryptase in vivo after prolonged cutaneous challenge with allergen in humans. *J Immunol* 141:821–826, 1988
24. Schwartz LB, Irani AA, Roller K, Castells MC, Schechter NM: Quantitation of histamine, tryptase in dispersed human MCT and MCTC cells. *J Immunol* 138:2611–2615, 1987
25. Horny HP, Ruck P, Xiao JC, Kaisering E: Immunoreactivity of normal and neoplastic human tissue mast cells with macrophage-associated antibodies, with special reference to the recently developed monoclonal antibody PG-M1. *Hum Pathol* 24:355–358, 1993
26. Lemley KV, Kriz W: Anatomy of the renal interstitium. *Kidney Int* 39:370–381, 1991
27. Yoshimura A, Mori W: Renal tissue mast cells in liver disease. *Int Urol Nephrol* 23:511–516, 1991
28. Davidson S, Mansour A, Gailly R, Smorski M, Rofolovitch M, Ginsburg H: Mast cell differentiation depends on T cells and granule synthesis on fibroblasts. *Immunology* 48:439–452, 1983
29. Noronha IL, Weis H, Rambauser M, Ritz E, Waldherr R: Expression of IL-1β, PDGF, TGF-β1-β2, TGF-β3, and IFN-γ receptor (IFN-γ R) in IgA nephritis and in acute allograft rejection. (abstract) *J Am Soc Nephrol* 2:602, 1991
30. Walsh LJ, Trinchieri G, Waldorf HA, Whitaker D, Murphy GF: Human dermal mast cells contain and release tumor necrosis factor alpha, which induces endothelial leukocytes adhesion molecule 1. *Proc Natl Acad Sci USA* 88:4220–4224, 1991
31. Bradding P, Feather LH, Wilson S, Bardin PC, Heusser CH, Holtate ST, Howarth PH: Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects: The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. *J Immunol* 151:3853–3865, 1993
32. Henke C, Marineli W, Jessurn J, Harms D, Peterson M, Chiang L, Doran P: Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung inury. *Am J Pathol* 143:1189–1199, 1993
33. Claman HN: On slceroderma. Mast cells, endothelial cells, and fibroblasts. *JAMA* 262:1206–1209, 1989