Increased Density of Interstitial Mast Cells in Amyloid A Renal Amyloidosis

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Renal interstitial fibrosis is the final common pathway leading to end-stage renal disease in various nephropathies including renal amyloidosis. However, the role of mast cells (MCs) in the fibrotic process of renal amyloidosis is not fully understood. We compared the distribution of MCs in renal biopsies from 30 patients with AA type renal amyloidosis and 20 control cases. Immunoreactivity of renal MCs to anti-tryptase and anti-chymase was studied. Interstitial myofibroblasts were stained with anti-α-smooth muscle actin (α-SMA) antibody, and inflammatory cells were identified by anti-CD45, -CD20, and -CD68 mAbs. Positively stained cells were counted, and the relative interstitial and fractional areas of anti-α-SMA stained cells were measured. Anti-CD29 mAb was used to detect β1 integrin and anti-basic fibroblast growth factor (bFGF) mAb for the growth factor on MCs. MCs were rarely found in control samples. In contrast, samples showing amyloid deposition contained numerous tryptase-positive (MCT) (940.17 ± 5.4 *versus* 6.74 ± 1.1/mm^2) but fewer chymase-positive (MCTC) cells (20.7 ± 2.86 *versus* 1.7 ± 0.76/mm^2) in the renal interstitium. There was a significant relationship between interstitial MCT and creatinine clearance (r = −0.72), and between interstitial MCT and glomerular amyloid-index (GAI) (r = 0.723) and interstitial amyloid area (r = 0.824). Accumulation of MCs correlated significantly with the number of T lymphocytes (MCT: r = 0.694). There was also a significant relationship between mast cell (MC) number and the fractional area of α-SMA positive interstitium (r = 0.733) and interstitial fibrotic area (r = 0.6). Double immunostaining demonstrated intracytoplasmic presence of β1 integrin on 87% of MCT and correlated significantly with the interstitial amyloid area (r = 0.818, P = .001) and T-cell number (r = 0.639, P = .002). bFGF was also detected on 85.5% of MCTC correlating well with the interstitial α-SMA-area (r = 0.789). Our results indicate that MCs constitute an integral part of the overall inflammatory process and play a crucial role in interstitial fibrosis in renal amyloidosis.

KEY WORDS: Amyloid, Basic fibroblast growth factor, Fibrosis, Integrin, Kidney, Mast cell, Myofibroblasts.

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Human mast cells (MCs) are a multifunctional tissue-dwelling cells with fundamental roles in various conditions such as allergy, inflammation, asthma, keloid-formation, angiogenesis, rheumatoid arthritis, fibrosis, soft part tumors, and cutaneous melanoma. Immunohistochemical, biochemical, and functional studies have shown that there are two phenotypically distinct types of MCs within the tissues. On the basis of protease content, MCs may thus be categorized as those containing both tryptase and chymase (MCTC), or tryptase but not chymase (MCT) (1). The MCT phenotype seems to play a role in host defense, whereas MCTC phenotype appears to be "non-immune system-related" cell involved in fibrosis and wound healing (2).

Mast cell (MC) hyperplasia and/or accumulation is a common phenomenon in various diseases associated with either high level of serum amyloid A (SAA) and C-reactive protein (3, 4) or SAA-fragments (AA) deposition in tissue (5). It has been demonstrated that SAA-like proteins bind to ECM components and induce T-lymphocyte adhesion (6). Using *in vitro* studies, Herskoviz *et al.* (7) demonstrated that MCs adhered to the immobilized ECM-SAA complex through integrin recognition.

MCs have been documented in various renal lesions, including diabetes nephropathy (8), IgA nephritis (9), rapidly progressive glomerulonephritis (RPGN) (10), and acute cellular rejection of renal allograft (11). Pavao-Macalusco in 1960 (12) observed the presence of MCs in various renal diseases (including three cases of renal amyloidosis) using metachromatic staining. There is a general

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agreement that MCs increase in number in chronic diseases and in the progression of different renal lesions characterized by the presence of tubulointerstitial lesions, including interstitial fibrosis (8, 10, 11), which are considered as poor prognostic markers of various renal diseases including renal amyloidosis (13). Changes in the area of interstitial fibrosis correlate better than the amount of amyloid deposition with renal function (13). The pathologic changes induced by MCs are in part due to serine proteases synthesized and released by MCs, such as tryptase and chymase as well as other bioactive mediators, because these are mitogenic for fibroblasts and known to induce collagen synthesis (2, 14, 15). However, the role of MCs in renal tubulointerstitial lesions has not yet been fully analyzed.

The aim of the present study was to demonstrate (1) the presence and phenotypes of MCs in interstitial renal tissues with amyloidosis A, (2) β1 integrin-expression on MCs located around amyloid deposition, and (3) determine the relationship between MCs and interstitial fibrotic process.

MATERIALS AND METHODS

Patients and Tissue Samples

Between 1969 and 1999, a total of 13,000 patients underwent percutaneous renal biopsy at Fukuoka University Hospital and other affiliated hospitals. Amyloid A nephropathy was diagnosed in 57 cases. In this study, renal tissue samples of 30 adult (more than 16 years old) patients (14 males and 16 females; mean age, 57.4 $\pm$ 2.1 years) with amyloid A nephropathy were selected at random. Only samples containing at least 10 glomeruli on paraffin blocks were included in this study. All kidney samples showed amyloid deposition in various amounts and different locations by light, immunofluorescence, and electron microscopy. The presence of amyloid was also confirmed in all kidney samples by a positive apple-green birefringence under polarized light in Congo red-stained preparations. Control samples consisted of 20 randomly selected renal biopsy samples from adult patients (9 males and 11 females; mean age, 56.2 $\pm$ 3.7 years) with thin glomerular membrane disease but normal extraglomerular renal structure and free of immune deposition, glomerulosclerosis, and inflammation. None of the patients had peripheral blood abnormalities and all had normal renal function and normal arterial blood pressure (less than 160/95 mmHg) and no or only mild proteinuria (less than 1.0 g/day). Clinical and laboratory data were also documented at the time of renal biopsy. The study protocol was approved by the Human Ethics Review Committee of Fukuoka University and a signed consent form was obtained.

Tissue Preparation

Tissue sections were stained with hematoxylin and eosin, Masson trichrome, periodic acid-Schiff, and periodic acid-methenamine-silver stain. To identify MCs, sections were stained by toluidine blue. Amyloid was demonstrated by Congo red staining, including pretreatment with permanganate and polarizing light as well as with immunohistochemistry.

Electron Microscopy (EM)

A proportion of the biopsy sample was fixed in 1.4% phosphate buffered glutaraldehyde, postfixed in OsO4, and embedded in Epon resin. Ultrathin sections were cut and stained by uranyl acetate and lead citrate. All samples were examined by an electron microscope.

Immunohistochemistry

All paraffin-embedded samples were stained for immunoreactivity to amyloid-A (AA) protein using a polyclonal human AA protein (DAKO, Glostrup, Denmark) at 1:100 dilution and standard APAAP (alkaline-phosphatase-anti-alkaline-phosphatase) complex, based on the immunoperoxidase method previously described by Looi (16). Sections were finally stained with 0.01% new Fuchsin (Merck, Germany), 0.01% NaN, 10 mg naphthol AS-BI phosphate (Sigma, St. Louis, MO), 0.1 mL of n.N-dimethyl formamide (Wako, Osaka, Japan) in 40 mL of 0.2 M Tris-HCl buffer (pH 8.2).

The staining method used in the present study for MCs has been described previously (10). Anti-human tryptase and chymase monoclonal antibodies were purchased from Chemicon (Temecula, CA). Anti-CD68, anti-CD20, anti-CD45, and anti-α-smooth muscle actin (α-SMA) mAbs were purchased from DAKO. Deparaffinized serial sections (3-μm-thick) were washed in 0.05 M Tris-HCl buffer containing 0.145 M NaCl, pH 7.5 (TBS), followed by pretreatment with protease for sections stained later for chymase or CD68, or autoclave pretreatment (121° C for 10 min) for sections stained for α-SMA. Sections were initially treated with 1% skim milk (Difco, USA) and incubated for 1 hour at 20° C with the primary antibody (anti-chymase antibody, 50 mg/mL, anti-tryptase antibody, 30 mg/mL), each dissolved in 3% bovine serum albumin. For negative control, we used vehicle alone or non-immunized animal immunoglobulin. After incubation, APAAP method was adopted, and sections were stained with new Fuchsin described above. Tissue for positive control consisted of biopsy samples from the small intestine for chymase and tryptase and renal vasculature for α-SMA.

Double Immunostaining

Double immunofluorescence. After treating deparaffinized sections with 0.005% protease (DAKO, Osaka, Japan) for 10 min, they were incubated with the first mouse antibody against human chymase (Chemicon) for 60 min at 20° C. After we washed them with TBS, sections were incubated with TRITC (rhodamine)-conjugated second rabbit-antibody against mouse immunoglobulin (DAKO) for 60 min at 20° C. Then, sections were treated with 1% skim milk for 30 min to minimize the background level. Sections were again incubated with the first rabbit antibody against human tryptase (BioPur, Switzerland) for 60 min at 20° C. After we washed them with TBS, sections were incubated with FITC-conjugated swine-antibody against rabbit immunoglobulin (DAKO) for 60 min at 20° C. After incubation, nuclei were stained with 4′6-diamidino-2-phenylindole (Sigma). Sections were then mounted and examined under a fluorescence microscope (Axioplan, Carl Zeiss Jena, Germany) with a fluorescence imaging system (Isis, Meta Systems, Germany). This method was used for the following double staining: anti-tryptase and/or chymase was combined with (1) anti-AA component (DAKO), or (2) basic fibroblast growth factor (Santa Cruz Biotech, Santa Cruz, CA) or anti-CD29 (Serotec, UK) antibodies.

Double Immunohistochemistry

This method was used for detection on the same tissue section the following molecules: tryptase or chymase with α-SMA, and tryptase or chymase with amyloid A protein. The method was similar to that described above, with the exception of staining MC-proteases by 3,3′-diaminobenzidine while α-SMA and amyloid A were stained by new-Fuchsin.

Morphometric Analysis

Several indexes were used to describe the severity of pathologic process based on previously published reports (10). (1) Relative interstitial area: A standard point counting method was used to quantitate the relative interstitial area (Aint) of the renal cortex. In this method, sections stained by the PAM method were examined under a high magnification (400×) using a 121-point (100 squares) eyepiece micrometer of 1 mm^2. A total of 10 consecutive non-overlapping cortical fields (area, 0.625 mm^2) were analyzed in each section of the biopsy. Points overlying the tubular basement membrane and interstitial space were counted while those falling on either Bowman's capsule or peritubular capillaries were ignored. Points falling on glomerular structures or on larger vessels were excluded from the total count. The results were expressed using the following formula: Aint = (number of grid intersections on the cortical interstitium/total number of grid intersections) × 100. (2) Interstitial immunoperoxidase staining for α-SMA was quantitated by the same point counting method described above. The fractional area was also calculated using the above formula. (3) Fractional area stained by Masson-trichrome was quantitated by the same point counting method described above and the results were expressed as a fractional area using the above formula. Interstitial fibrotic area represented the blue-colored extraglomerular, extratubular and extravascular area. (4) Interstitial immunoperoxidase staining for anti-AA antibody was quantitated by the same point counting method described above. The fractional area was also calculated using the above formula. All amyloid AA-stained extraglomerular, extratubular, and extravascular areas not surrounded by basement membrane and in direct contact with the interstitial connective tissue were identified as interstitial amyloid deposition. (5) The numbers of interstitial lymphocytes (CD20, CD45), macrophages (CD68, Ki-1), CD29, or bFGF positive chymase and tryptase positive MCs present in the cortical interstitial area were counted under a high magnification (400×) in 10 adjacent non-overlapping cortical fields (total area, 0.625 mm^2 per biopsy specimen). Only cells with clearly identifiable nuclei were counted. Finally, the number of counted cells was expressed as cells per unit area (mm^2). (6) The amount of glomerular amyloid (glomerular amyloid index, GAI) was graded on a scale of 0 to 4, based on the method of Shiiki (17), with grade 0 representing no glomerular amyloid deposits and grade 4 representing large amounts of amyloid, respectively.

Statistical Analysis

Data were expressed as mean ± SEM. Difference between groups was examined for statistical significance using the Student's t test and one-way analysis of variance (ANOVA). Association of categorical variables was examined by the χ^2 test. Correlation between variables was assessed by linear regression analysis. A P value less than 5% denoted the presence of a statistically significant difference.

RESULTS

Table 1 presents selected clinical, laboratory, and histologic data of the control and study groups. In all renal samples, immunohistology showed the presence of rounded, elongated, or polygonal cells and each contained a central nucleus and abundant cytoplasm containing numerous tryptase- or chymase-positive granules or both, identifying

TABLE 1. Clinical, Laboratory, and Morphologic Parameters of Participating Patients

	Control	Amyloidosis AA
n	20	30
Age	51.5 ± 2.5	57.5 ± 2.1
Male/female	10/10	14/16
Proteinuria (g/day)	0.56 ± 0.04	4.0 ± 0.5[a]
Serum creatinine (mg/dL)	0.65 ± 0.04	1.93 ± 0.2[a]
MCT/mm^2	7.14 ± 1.3	40.2 ± 29.7[a]
MCTC/mm^2	1.9 ± 0.8	20.7 ± 2.8[a]
T cell/mm^2	15.7 ± 3.7	311.4 ± 51.9[a]
Ifib/mm^2	0.78 ± 0.2	10.5 ± 0.8[a]
α-SMA/mm^2	0.4 ± 0.1	16.6 ± 1.8[a]
b1 + MCT/mm^2	4.4 ± 0.9	34.9 ± 6.9[a]
bFGF + MCTC/mm^2	0.57 ± 0.9	17.7 ± 2.5[a]

Data are mean ± SD.
[a] $P < .001$, compared to the control.

these cells as MCs. A representative tissue sample containing MCT is shown in Figure 1A. Chymase was not observed in cells lacking tryptase. MCs were localized in the peritubular, perivascular, or periglomerular region in close proximity to AA deposits (Fig. 1B). Occasionally, the MCs were attached to the amyloid deposit (Fig. 2). MC degranulation was often observed at these sites. These MCs were usually not only degranulated, but also depleted of granules.

Furthermore, intraglomerular MCs were never found in our tissue samples, although a few intratubular MCs were identified. MCs were mostly located on the periphery rather than within areas of lymphocytic accumulation. Furthermore, MCs were observed in α-SMA positive interstitial area, independent of the presence of amyloid deposits.

The density of both phenotypes of MCs was significantly higher in renal amyloid group than in the control (MCT: 40.17 ± 5.4 *versus* 6.74 ± 1.1/mm^2, MCTC: 20.7 ± 2.86 *versus* 1.7 ± 0.76/mm^2). Furthermore, MCs constituted the third most abundant interstitial inflammatory cell population in renal amyloidosis.

Relationship Between MC Phenotype and Pathologic Findings

We also analyzed the relationship between MC phenotype and pathologic findings. There was a significant relationship between glomerular amyloidosis index (GAI) and number of tryptase-

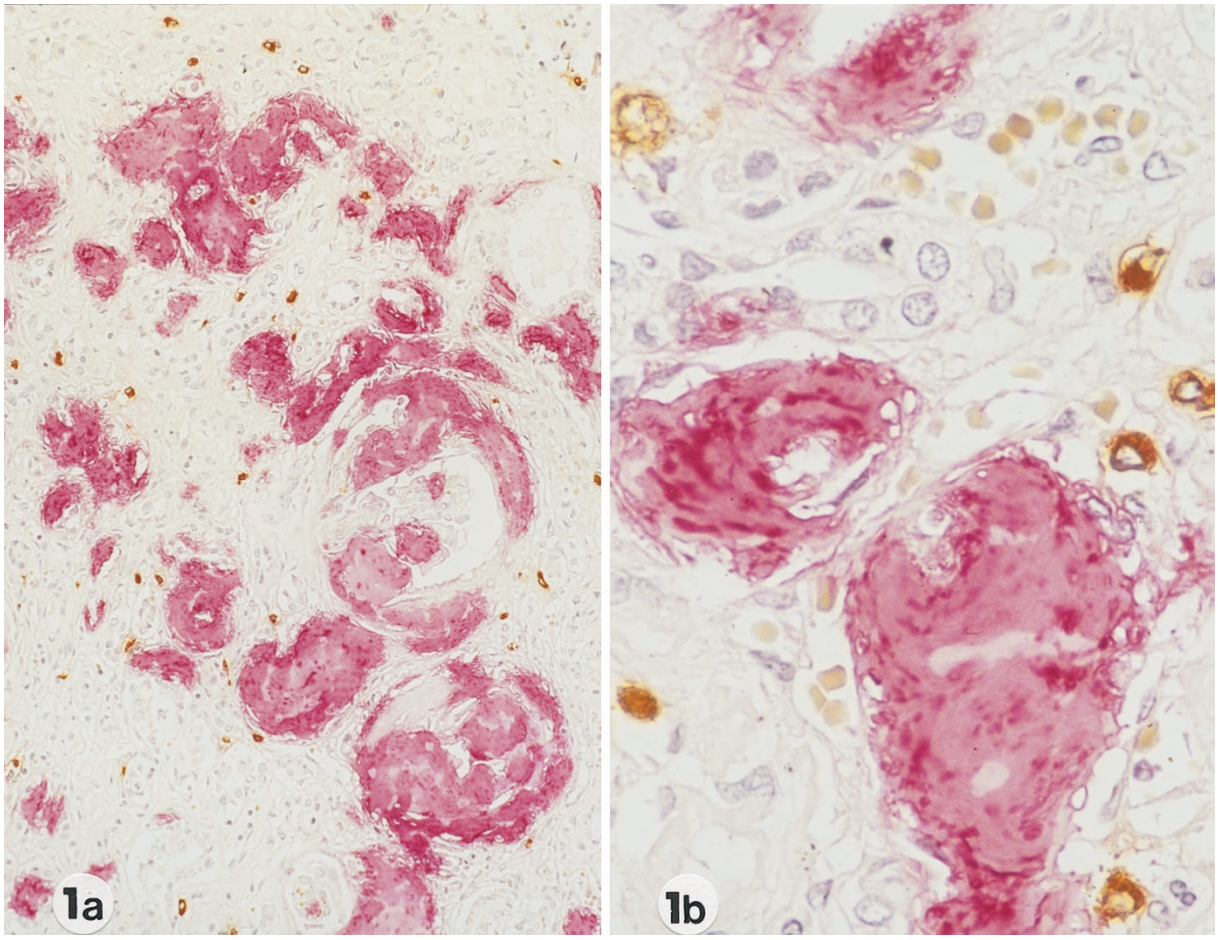


FIGURE 1. Photomicrographs showing interstitial distribution of mast cells stained with anti-tryptase (3,3′-diaminobenzidine) in amyloid kidney, and accumulation of these cells around amyloid deposits stained with anti-amyloid-A (new-Fuchsin stain; original magnification, **A**: 200×; **B**: 500×).

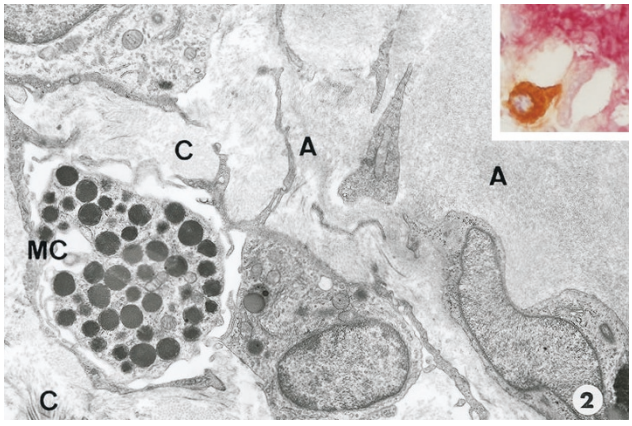


FIGURE 2. Electron micrograph showing a mast cell (***MC***) embedded in extracellular matrix of the renal interstitium containing collagen fibers (***C***) and amyloid fibrils (***A***). Note the typical conus-shaped arrangement of amyloid fibrils (**A-A**) (original magnification, 19,000×). Inset: Mast cells stained with anti-chymase (3,3′-diaminobenzidine) in close proximity to interstitial amyloid stained with amyloid-A (new Fuchsin stain; original magnification, 1000×, oil immersion).

positive mast cells ($r = 0.723$; $P < .001$). Furthermore, there was also a significant relationship between the number of interstitial T-lymphocytes and MCT ($r = 0.694$; $P < .001$) as well as MCTC ($r = 0.696$; $P < .001$). However, there was no relationship between the density of B-cells and MCT ($r = 0.28$, $P > .05$). There was a significant relationship between interstitial CD68+ macrophages and MCT ($r = 0.458$; $P < .001$) and between the relative volume of the interstitium and number of MCT ($r = 0.812$; $P < .001$). Furthermore, a significant relationship was present between the interstitial amyloid area and number of MCT ($r = 0.824$; $P < .001$) as well as T-cell number ($r = 0.495$; $P < .01$). We also found a close relationship between the fractional area of α-SMA (FAα-SMA) and number of MCs (MCT: $r = 0.733$ and MCTC: $r = 0.807$; $P < .001$). In addition, there was a significant relationship between fractional area of fibrosis (FAfib) and MCT ($r = 0.66$; $P < .002$) as well as MCTC ($r = 0.63$; $P < .002$) (Fig. 3).

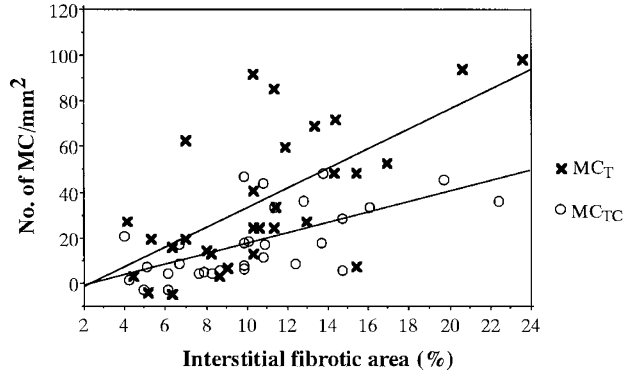


FIGURE 3. Correlation between the interstitial fibrotic area and number of chymase and tryptase-positive interstitial mast cells.

Double immunostaining showed immunostaining of β1 integrin by anti-CD29-mAb both on the surface and cytoplasm of MCs. On average, 87% (34.9 ± 6.9) of renal MCT showed β1 staining (Fig. 4). This staining was not dependent on the presence of amyloid deposition. Other cell constituents of the renal tissue, including tubular epithelial cells,

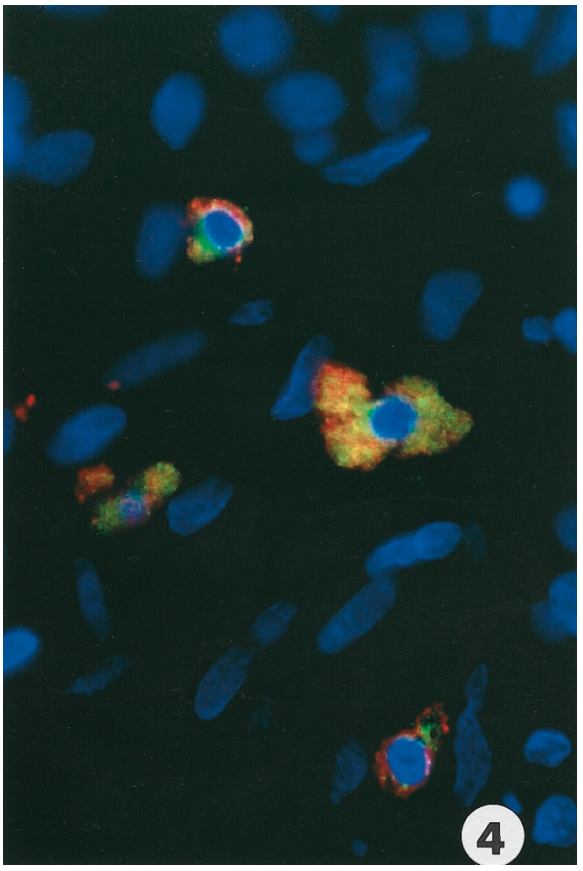


FIGURE 4. Photomicrograph showing β1 integrin stained with anti-CD29 (FITC-labeled) in the cytoplasm of mast cells stained with anti-tryptase (rhodamine-labeled) (double immunostaining; original magnification, 400×).

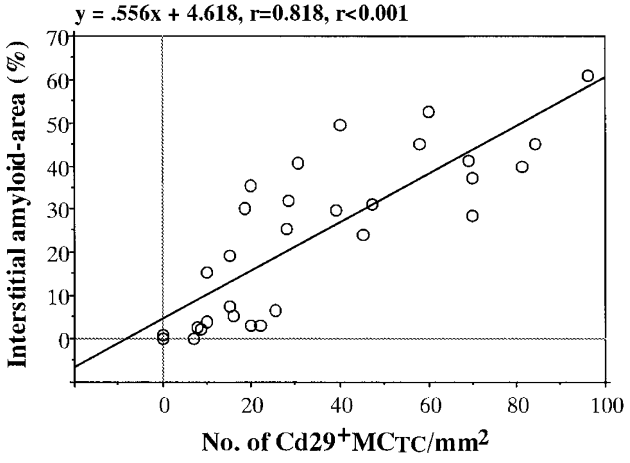
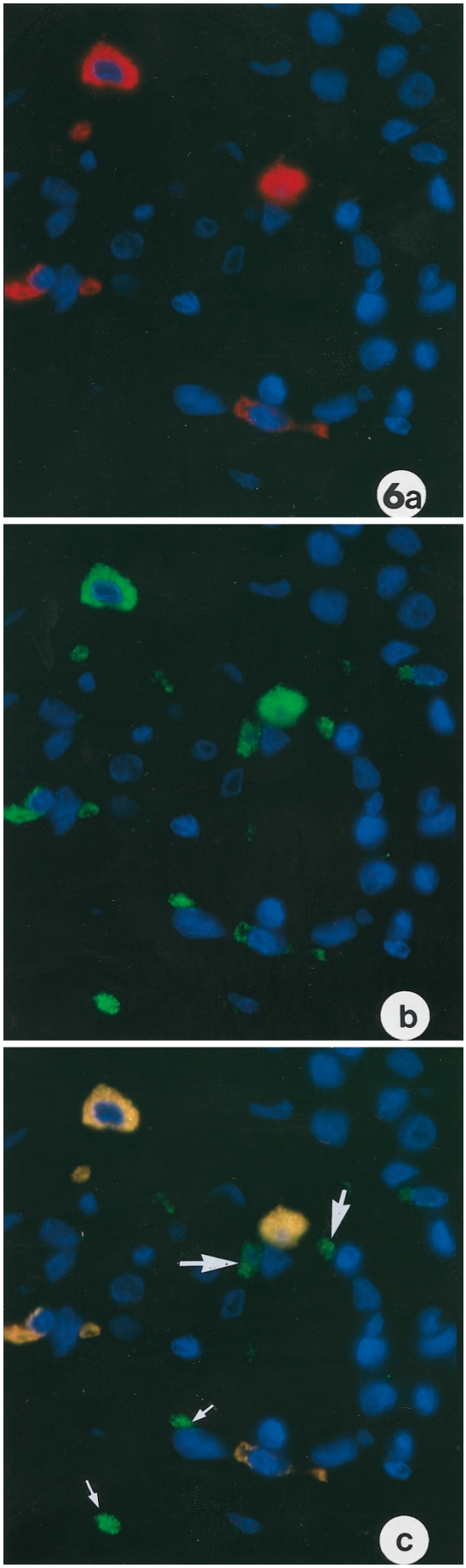


FIGURE 5. Correlation between the number of both chymase and CD29-positive interstitial mast cells (MCTC) and interstitial amyloid area.



vascular endothelial cells, and the majority of glomerular cells were also variable reactive for anti-CD29 mAb. A good correlation was demonstrated between the number of CD29+ MCTC and that of T-cells ($r = 0.639$; $P < .002$), FAα-SMA ($r = 0.694$; $P < .001$), as well as interstitial amyloid area ($r = 0.818$; $P < .001$) (Fig. 5).

Using the double immunostaining method, bFGF was identified in some interstitial MCs, and was located in the cytoplasm of 48% (19.3 ± 3.2) of interstitial tryptase-positive cells and 85.5% (17.7 ± 2.5) of MCTC cells, independent of the presence or absence of amyloid deposition (Fig. 6, A–C). There was a significant relationship between the number of bFGF+ MCTC and interstitial fibrotic area ($r = 0.721$; $P < .001$) as well as α-SMA+ interstitial area ($r = 0.789$; $P < .001$) (Fig. 7) and between bFGF+ MCTC and interstitial amyloid positive area ($r = 0.728$; $P < .001$).

Clinicopathological Correlates

At the time of biopsy, 18 patients had nephrotic syndrome and 14 patients had elevated serum creatinine concentration (more than 2.0 mg/dL). The number of MCs was not influenced by age or gender, but correlated significantly with creatinine clearance ($r = 0.72$; $P < .001$), and serum creatinine level ($r = 0.67$; $P < .001$). Furthermore, there was a significant correlation between the degree of 24-hour proteinuria and number of MCT ($r = 0.496$; $P < .001$). A significantly higher number of MCT were detected in patients with hypertension (more than 165/95 mmHg, $n = 6$, 65 ± 11.3 *versus* 24 normotensive patients: 34 ± 5.5 cells/mm^2; $P < .05$).

DISCUSSION

Human MCs play a pivotal role in the pathogenesis of acute allergic reactions, but they have also been observed to accumulate at sites of chronic inflammation and/or fibrosis in various disorders suggesting that MCs are also involved in fibrosis (18–21). Very little is known about MC accumulation in amyloidosis (22), particularly in renal amyloidosis. Pavone-Macaluso (12) counted numerous MCs in the renal parenchyma of three biopsies from patients with amyloidosis associated with pyelonephritis. In contrast, Bohle *et al.* (13) found only a few MCs in 11 biopsies from patients with AA

FIGURE 6. Renal interstitial mast cells stained with anti-chymase (rhodamine-labeled, **A**) containing intracytoplasmic bFGF protein (FITC-labeled, **B**). Photomicrograph **(C)** showing both colors are manifested in orange, whereas some cells (indicated by ***arrows***) are stained by only anti-bFGF and appear green in color (double immunostaining; original magnification, 400×).

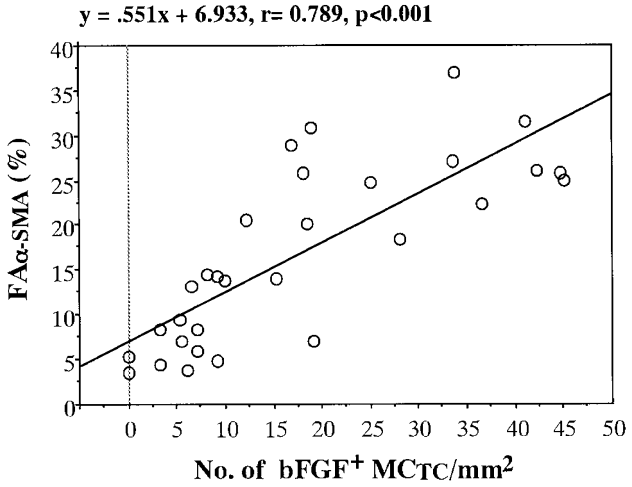


FIGURE 7. Relationship between number of both chymase and bFGF-positive interstitial mast cells (MCTC) and fractional α-SMA-positive interstitial area.

type renal amyloidosis, but did not discuss their importance or role in renal lesions. To our knowledge, our study is the first that provides a systematic analysis of the presence of MCs in AA-type renal amyloidosis.

We found a significantly high MC density in the renal interstitium of 30 patients with AA-type renal amyloidosis. Both subtypes of MCs were found in random distribution, mostly localized close to the interstitial and vascular amyloid deposits. Our analysis showed a significant correlation between the area of interstitial amyloid and the number of MCT ($r = 0.824$). MCs were also found in close proximity to glomeruli, which contained amyloid deposits and amyloid filled the Bowman's capsule, but these cells never appeared inside the glomerular capsule. MCs were found also in the nonamyloid interstitial area, although in fewer numbers.

The exact pathomechanism of MC involvement in renal amyloidosis is unclear. However, it is well known that in certain inflammatory diseases including AA amyloidosis, MC hyperplasia has also been observed with increased local and systemic levels of acute phase reactants such as SAA (3, 4, 23). In this regard, previous studies have shown that SAA and other SAA-like proteins adhere to ECM components (6). The binding site is located within the amino sequence of the AA molecule, the latter also contains cell adhesive epitopes within close proximity (24). Herskoviz *et al.* (7) demonstrated that human rSAA specifically binds to MCs, suggesting the possible presence of SAA receptor on MCs. In *in vitro* studies, they also documented MC and T-cell adhesion to the immobilized ECM-SAA complex through specific recognition sites on integrin (6, 7). Recently, β1-integrin (VLA-3, 4, 5)-expression has been demonstrated on human skin MCs, whose adhesion molecule mediates MC adhesion to fibronectin and laminin (25). Our present work using double immunostaining method documented for the first time that renal interstitial tryptase and/or chymase-positive MCs were also positive for β1 integrin. These cells were diffusely distributed in the interstitium, mostly close to amyloid deposits, but some also appeared in areas free of such deposits.

Despite the evidence of their presence, the pathologic role of MCs in renal tissues with amyloid deposition is still hypothetical. First, it seems that MCs form an integral part of the inflammatory process or reaction of the deposited amyloid in the renal tissue. In this study, we found a prominent T-cell inflammation that correlated significantly with the volume of interstitial amyloid deposits. In this regard, Bohle *et al.* (13) also demonstrated a marked increase in the number of T-cell in amyloidosis compared to normal. Our results also showed that the number MCs correlated with amyloid deposition, and also with T-cell numbers ($r = 0.69$), as was recently demonstrated in RPGN (6). On the other hand, MCs were located in close proximity to other inflammatory cells.

Previous studies have also shown that MC maturation and activation is regulated by various products (interleukin-3 and 4) of T-lymphocytes (26), whereas MC derivatives such as histamine, tryptase, chymase, TNF-α, and interleukins enhance inflammatory cell transmigration, maturation, and cytokine-release (2, 27). Previous reports suggested the involvement of apoptosis of either renal host cells (28) or T-cells (29) in the pathogenesis of both human and experimental renal amyloidosis. Further analysis is necessary to clarify the relationship between these two cell types in inflammatory processes as well in amyloidosis process.

Second, there is sufficient evidence to indicate that the presence of MCs enhances tissue fibrosis. A high MC density has been demonstrated at wound sites and fibrotic areas in various organs (18, 20, 21). Furthermore, previous studies have shown a significant relationship between the number of MCs and interstitial fibrosis in renal allografts (11), IgA nephritis (9), and RPGN (10). We also showed here a good correlation between the number of interstitial MCs and relative interstitial area as well as areas positive for α-SMA. The latter parameter represents the area of activated interstitial fibroblasts (myofibroblasts), which are important cellular components responsible for interstitial ECM production and accumulation (30).

In our cases, all MCs present in the interstitium were positive for intracytoplasmic bFGF, which is a strong mitogenic polypeptide (31). The close relationship between interstitial myofibroblasts and MCs is well-documented (10, 32). MC products exhibit proliferating activity for fibroblasts, inducing

phenotype modulation, which is manifested in α-SMA expression and regulation of collagen synthesis (14, 33, 34). Interstitial fibrosis in renal amyloidosis is well documented and is considered as a poor prognostic factor, more significantly than the amount of amyloid deposition (13, 35). In this regard, Bohle *et al.* (13) reported that long-term prognosis of renal amyloidosis is worse when interstitial fibrosis is present at the time of biopsy.

In summary, we have demonstrated in this study that in renal amyloidosis, MC accumulation is an integral part of interstitial damage and may be a factor responsible for the inflammatory and fibrotic processes. Our study is the first to demonstrate the presence of β1 integrins on renal interstitial MCs as a mediator of cell-cell and cell-ECM adhesion. The AA molecule seems to serve as a connector, binding to MCs (through the integrin), whereas the other site binds to the ECM, providing a cross talk between MCs and other inflammatory and/or resident renal cells. The presence of bFGF suggests that these cells may play a crucial role in the activation of interstitial fibroblasts, myofibroblast phenotype modulation, and finally fibrosis.

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Book Review

Klatt EC, Kumar V: Robbins Review of Pathology, 310 pp, Philadelphia, WB Saunders, 2000 ($29.95).

As the book review editor of *Modern Pathology*, I was faced with an interesting ethical dilemma: should I review this book that has almost an identical title as my own book reviewed in this Journal a few months ago? Both books were obviously written for the same medical student population. However, because most medical students buy several review books and usually base their decision more on the recommendation of their peers than professors, I wondered whether these two books are really competing with one another. Finally, because I have only nice things to say about the "competition," I decided to write this review, thinking that my positive reaction would be its best endorsement.

Without any equivocation, this is an excellent review book ideally suited to accompany Robbins' textbook. Technically, it is the successor of a similar companion compiled many years ago by Carolyn Compton, to which, however, it bears only rough resemblance. In essence, Drs. Klatt and Kumar have produced an entirely new book. With more than 1000 questions, it covers the entire field of pathology, or at least what every self-respecting second year medical student should know. Every question is accompanied by an answer, which is indexed to the relevant pages in the Robbins' textbook (short and long version).

It is important to note that among all currently available review books of pathology, this is the most clinically oriented. In this respect, it reflects closely the current orientation of the National Boards. The questions might not be verbatim replicas of those on the USMLE-1, but they give students a fairly good idea of what to expect on the national exam. Most questions are remarkably well constructed and are of high taxonomic grade. Such questions cannot be answered by rote memorization of predigested formulaic answers. The student will have to pose, think, and integrate bits of knowledge from several fields and use a deductive approach to solve these clinicopathologic problems. As such, the book probes not only students' knowledge of pathology but also their problem-solving skills. It is an excellent exercise in medical thinking and should prepare the students well for further clinical training.

A major new feature that sets this book apart from others is the excellent color photographs generously sprinkled throughout the book. Placed in proper clinical context, these photographs give students direction on how to study pathology, how to interpret visual information, and how to correlate morphology with symptoms or pathogenetic mechanisms. Professors also would be well advised to study the picture-related questions and learn from Drs. Klatt and Kumar how to use photographs of pathologic specimens to improve students' understanding of basic pathologic processes or how to construe questions pertaining to figures.

In my opinion, this new companion to Robbins' classical textbook breaks new ground and opens new vistas for both medical students and teachers. It should serve well in both learning and teaching pathology in a clinically oriented manner.

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