Mast cell chymase expression and mast cell phenotypes in human rejected kidneys

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Mast cell chymase expression and mast cell phenotypes in inflammatory cells, intimal thickening of intrarenal arteries, tubular atrophy, and interstitial fibrosis [1, 2]. Although inflammatory cells such as macrophages and T lymphocytes increase in rejected renal allografts, it has been reported that mast cells (MCs) also increase in the renal parenchyma of rejected renal allografts [3–5].

Mast cells are thought to play a role in the pathogenesis of a variety of chronic inflammatory diseases. According to their neutral protease content, human MCs have been divided into two phenotypes: those that contain only tryptase, termed MCt, and those that contain tryptase and chymase, termed MCtc [6]. These MC phenotypes exhibit variable distribution, a spectrum of protease content and differing responsiveness to activating stimuli [7–9], suggesting that MC subtypes have different functions in health and disease, although these functions remain to be elucidated. Evidence for a new MC phenotype, designated MCc, which contains chymase but not tryptase, has been shown in several human tissues [10–12]. More recently, in vitro studies have shown that interleukin-4 (IL-4) promotes morphological maturation of human cultured MCs in accord with the increase in chymase expression [13, 14]. These data suggest that cytokines within the microenvironment may influence the chymase expression of human MCs in vivo.

In human kidney transplantation, chronic rejection is a major cause of morbidity and mortality among transplant recipients. Chronic renal rejection is histologically characterized by interstitial infiltration of mononuclear inflammatory cells, intimal thickening of intrarenal arteries, tubular atrophy, and interstitial fibrosis [1, 2]. Although inflammatory cells such as macrophages and T lymphocytes increase in rejected renal allografts, it has been reported that mast cells (MCs) also increase in the renal parenchyma of rejected renal allografts [3–5].

Mast cells are thought to play a role in the pathogenesis of chronic rejection is poorly understood. Recently, distinct phenotypes of MCs have been described in humans by the demonstration of one protease, chymase. Hence, we questioned whether chymase in MCs could play a role in the pathogenesis of renal rejection in humans.

Methods. We investigated MC chymase expression and MC phenotypes, using immunohistochemical single- and double-staining techniques, in nephrectomy (N = 13) and biopsy (N = 8) specimens of human rejected kidneys. Tissue chymase levels were determined by enzymatic assay for chymase activity. We also examined the association between MC chymase expression and the degree of interstitial fibrosis in these renal allografts.

Results. Based on chymase positivity, rejected kidneys were divided into two groups, a chymase-negative [Chy(−)] group and a chymase-positive [Chy(+) ] group. Quantitative analysis showed that the number of chymase-positive MCs and tissue chymase levels were significantly higher in the Chy(+) group than in the Chy(−) group. Furthermore, the interstitial fibrotic area in the Chy(+) group was significantly larger than that in the Chy(−) group. Immunodouble staining analysis also demonstrated that a new MC phenotype, positive for chymase but negative for tryptase, was present in the human rejected kidney.

Conclusions. These results show that increased expression of chymase in MCs is related to the severity of interstitial fibrosis in human rejected kidneys.

Key words: tryptase, chymase, mast cells, kidney rejection, transplantation, angiotensin II, interleukin-4, renal allograft, interstitial fibrosis.

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Table 1. Relevant clinical data

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Abbreviations are: mo, months; yr, years; M, male; F, female.

Biopsy specimens

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<tr>
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<tr>
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<td>—</td>
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</tbody>
</table>

Abbreviations: mo, months; yr, years; M, male; F, female.

A nephrectomy specimen N-10 and biopsy specimen B-5 were obtained from the same patient.

METHODS

This study was based on an analysis of 13 renal allografts that had been surgically removed because of chronic rejection and 5 renal biopsy specimens obtained from patients with chronic rejection. The relevant clinical data are summarized in Table 1. In the nephrectomy cases, 11 patients had received a cadaveric kidney, and 2 received a kidney from a living-related donor. In the biopsy cases, all patients had received a cadaveric kidney. The nephrectomy specimen of case N-10 and the biopsy specimen of case B-5 were obtained from the same patient (Table 1). All patients were on triple immunosuppressive therapy (cyclosporine, prednisone, and azathioprine or mizoribine).

From each nephrectomy specimen from renal allografts, five tissue blocks were obtained; two blocks were snap frozen and used for immunodouble staining and enzymatic assay for chymase activity (discussed later in this article), and the remaining three blocks were fixed in methanol-Carnoy’s fixative (60% methanol, 30% chloroform, and 10% glacial acetic acid). This method of fixation has the advantage that immunohistochemical studies on chymase expression can be performed on paraffin-embedded sections [6]. All biopsy specimens from renal allografts were also fixed in methanol-Carnoy’s fixative.

Normal renal tissues obtained from four autopsied patients, including snap-frozen samples and tissue blocks fixed in methanol-Carnoy’s fixative, were examined as controls.

Tissue blocks, obtained from each transplanted or normal kidney and fixed in methanol-Carnoy’s fixative, were routinely processed and embedded in paraffin. Thirty serial sections were cut from each block at 3 μm thickness. Every first and second sections were stained with hematoxylin and eosin and Azan Mallory stain, respectively. To identify MCs histologically, every third and fourth sections were stained with astra-blue/safranin stain and toluidine blue stain, respectively [15]. The other sections were used for immunohistochemical staining. The snap-frozen samples were serially sectioned at 5 μm thickness and fixed in acetone. Every first section was stained with hematoxylin and eosin, and the other sections were used for immunohistochemical staining.

Immunohistochemistry

Antibodies. The primary antibodies used for the identification of MCs were anti-MC tryptase (AA1; Dako, A/S, Glostrup, Denmark) [16], anti-MC chymase (MAB-1254; Chemicon, Temecula, CA, USA) [17] and anti–c-kit (Dako) [18]. Anti-macrophage antibodies (HAM56 and EBM11; Dako) were used for the identification of macrophages. For endothelial cell identification, two antibodies (F8/86, Dako; and EN-4, Monosan, Am Uden, The Netherlands) were used. To identify T lymphocytes, two anti-T lymphocyte antibodies, UCHL-1 (Dako) and Leu4 (Becton and Dickinson, San Jose, CA, USA), were used. For the identification of IL-4, anti–IL-4 antibody (Genzyme, Cambridge, MA, USA) was used.

Single staining. Sections were incubated with the primary antibodies either overnight at 4°C or for one hour at room temperature. The antibodies against c-kit, macrophage (EBM11), endothelial cell (EN-4), T lymphocyte (Leu4), and IL-4 did not work well with methanol Carnoy’s fixed sections and were used only on frozen sections. The labeled streptavidin-biotin complex system with 3-amino-9-ethylcarbazole color development was used. Sections were faintly counterstained with hematoxylin.

The specificity and results obtained with anti-MC tryptase and chymase antibodies were checked by omitting the primary antibodies and using a nonimmune mouse IgG antibody (Dako) as negative control.

Immunodouble staining. To identify cell types that express chymase, tryptase, or IL-4, we performed immunodouble staining. Moreover, for the identification of c-kit
expression in chymase-positive cells, some sections were double stained with c-kit and chymase.

In immunodouble staining for EN-4/chymase, EBM11/chymase, c-kit/chymase, IL-4/chymase, or tryptase/chymase, one primary unlabeled antibody (EN-4, EBM11, c-kit, IL-4, or tryptase) and one biotinylated antibody (chymase) were used according to modifications of procedures previously reported [19]. The following incubations were performed in a sequential order: primary unlabeled antibody, peroxidase-conjugated goat anti-mouse immunoglobulin (Dako), normal mouse serum (Dako), biotinylated chymase antibody (Chemicon), and alkaline phosphatase-conjugated streptavidin (Dako). Finally, peroxidase was visualized with 3-amino-9-ethylcarbazole (red: EN-4, EBM11, c-kit, IL-4 and tryptase) and alkaline phosphatase with fast blue BB development (blue: chymase).

In immunodouble staining for chymase/Leu 4, one primary unlabeled antibody (chymase) and one fluorescein isothiocyanate (FITC)-conjugated antibody (Leu 4) were used [19]. The procedure was as follows: chymase antibody, alkaline phosphatase-conjugated goat anti-mouse immunoglobulin (Dako), normal mouse serum (Dako), FITC-conjugated Leu4 antibody (Becton and Dickinson), FITC-conjugated goat anti-rabbit immunoglobulin (Dako), and peroxidase-conjugated goat anti-rabbit immunoglobulin (Dako). Alkaline phosphatase was visualized with fast blue BB (blue: chymase) and the peroxidase with 3-amino-9-ethylcarbazole development (red: Leu4).

Antibody/enzyme conjugates were diluted in Tris-HCl (50 mmol/L, pH 7.8)-buffered saline (TBS) + 1% bovine serum albumin (BSA). TBS washings were performed between all steps (three times 2 minutes), and all incubations were performed at room temperature.

Morphometric analysis

The number of tryptase-positive MCs, chymase-positive MCs, or IL-4-positive cells within the surface area of the cortex of each transplanted or normal kidney was quantitated, using a computerized morphometry system, MacSCOPE Ver 2.2 (Mitani Corporation, Fukui, Japan) and expressed as the absolute number of tryptase-positive MCs, chymase-positive MCs, or IL-4-positive cells per 1 mm² surface area.

In each nephrectomy specimen of transplanted kidneys, the surface area occupied by macrophages was quantitated by use of the computerized morphometry system and was expressed as a percentage of the total surface area of cortex, the so-called macrophage area. The surface area occupied by T lymphocytes—the so-called T lymphocyte area—was quantitated in a similar fashion and was likewise expressed as a percentage of the surface area of the cortex.

The degree of interstitial fibrosis in the transplanted kidneys was also assessed, using sections stained with Azan-Mallory stain. In this analysis, the surface area showing interstitial fibrosis within the cortex of the allograft was quantitated, using the computerized morphometry system, and was expressed as a percentage of the surface area involved.

The observer was blind to data regarding the patients’ characteristics. Intraobserver variability was determined on the basis of triplicate measurements. The mean ± SE difference among measurements was 3.4 ± 0.93%.

Enzymatic assay for chymase activity

Chymase activity in normal kidney tissues or nephrectomy specimens of rejected kidneys was measured by incubating tissue extracts for 10 minutes at 37°C with 770 μmol/L of angiotensin I as the substrate, in 150 mmol/L borax-borate buffer, pH 8.5, containing 8 mmol/L dipyridyl, 5 mmol/L ethylenediaminetetra-acetic acid (EDTA) and 770 μmol/L diisopropyl phosphorofluoridate. Angiotensin II converted from angiotensin I was determined using reversed-phase column [20]. The protein concentration was measured by protein assay reagent (Pierce Chemical Company, Rockford, IL, USA), using BSA as the standard.

Statistical analysis

The results are expressed as mean ± SE. Comparisons between two groups were performed using the unpaired Student t test and between ≥3 groups using analysis of variance (ANOVA) followed by Fisher’s PLSD test. Associations between variables were evaluated using Spearman’s correlation coefficient. P values of less than 0.05 were considered significant.

RESULTS

Quantitative analysis of tryptase-positive MCs and chymase-positive MCs

Normal kidneys. Normal kidneys, as controls, contained only occasional MCs, which were preferentially located in areas adjacent to interstitial vessels. Quantitatively, the mean number of tryptase-positive MCs in normal kidneys was 1.08 ± 0.35, and that of chymase-positive MCs was 0.58 ± 0.30 (Table 2).

Rejected kidneys. All rejected kidneys, including both nephrectomy and biopsy specimens, contained abundant MCs scattered throughout the thickened interstitium. Each of these rejected kidneys, moreover, showed massive infiltration of macrophages and T lymphocytes in the interstitium.

(a) Nephrectomy specimens. The mean number of tryptase-positive MCs was significantly higher (P < 0.05) in 13 nephrectomy specimens of rejected kidneys (12.04 ± 2.32) than in normal kidneys. Of the 13 nephrectomy specimens, 12 showed a marked increase in the
number of tryptase-positive MCs. However, the remaining one (case N-12) revealed normal range in the number of tryptase-positive cells, although this case showed an increased number of MCs histologically.

The number of chymase-positive MCs varied widely in the nephrectomy specimens. No significant correlations were found between the number of chymase-positive MCs and the interval from transplantation to hemodialysis, that from transplantation to nephrectomy, or that from hemodialysis to nephrectomy.

Based on chymase positivity, the 13 nephrectomy specimens were divided into two groups: (1) the chymase-negative group [Chy(−) group] and (2) the chymase-positive group [Chy(+) group]. The first group (N = 5) had an increase in the number of tryptase-positive MCs, but chymase expression in MCs was low, as in normal kidneys. The number of tryptase-positive MCs in this group increased more than eightfold compared with that of normal kidneys, but the difference between these two groups was not statistically significant (Table 2). In the second group (N = 8), the number of chymase-positive MCs in each allograft was more than fourfold compared with that of normal kidneys (Fig. 1A). In this group, the number of chymase-positive MCs was significantly higher (P < 0.01) than in the control and Chy(−) groups (Table 2).

With regard to the macrophage area and the T lymphocyte area, there were no statistically significant differences between the Chy(+) group and the Chy(−) group.

(b) Biopsy specimens. The mean number of tryptase-positive MCs in the five biopsy specimens (13.48 ± 3.04) was significantly higher (P < 0.01) than in normal kidneys. Based on chymase positivity, biopsy specimens were also divided into a Chy(−) group (N = 1) and a Chy(+) group (N = 4; Table 2). In the Chy(+) group of biopsy specimens, the number of chymase-positive MCs was significantly higher (P < 0.05) than in normal kidneys (Fig. 1B and Table 2). There were no significant correlations between the number of chymase-positive MCs and the interval from transplantation to biopsy.

Enzymatic assay for chymase activity

Chymase activity in normal or rejected kidney tissues, as determined by the capacity to convert angiotensin I to angiotensin II, showed similar trends to those in morphometric examinations (Fig. 2). Chymase activity in the Chy(+) group was measured with six nephrectomy specimens, because two nephrectomy specimens had intrarenal hemorrhage inhibiting chymase activity due to serpines, which are chymase inhibitors in blood. Chymase activity in the Chy(+) group exhibited a more than 21-fold increase compared with those of normal kidneys (P < 0.01) and a more than 6.5-fold increase compared with those of the Chy(−) group (P < 0.01; Fig. 2).

Mast cell phenotypes

Nephrectomy specimens. In 12 of the 13 nephrectomy specimens, but not case N-12, both MCr and MCrc phenotypes were found in the interstitium (Fig. 3A). The percentages of the MCrc phenotype in the total MC counts were 7.40 ± 3.46% in the Chy(−) group and 75.69 ± 11.02% in the Chy(+) group.

In contrast, specimens of case N-12 contained abundant chymase-positive but tryptase-negative MCs (MCr phenotype; Fig. 3B), which were histologically identified as MCs using an astra blue/safranin and toluidine blue stain. The immunodouble staining for chymase and c-kit clearly demonstrated that these MCs of the MCr phenotype were positive for c-kit (Fig. 4A). Moreover, the immunodouble staining for endothelial cells (or macrophages, or T lymphocytes) and chymase revealed that chymase positivity was not detected in these endothelial cells, macrophages, and T lymphocytes (Fig. 4B–D).

Biopsy specimens. In all biopsy specimens, both MCr and MCrc phenotypes were observed in the interstitium. The percentages of MCrc phenotype in the total MC counts were 5.70% in the Chy(−) group and 63.85 ± 10.96% in the Chy(+) group.

Degree of interstitial fibrosis

Nephrectomy specimens. All nephrectomy specimens of the rejected kidneys showed interstitial fibrosis, but of various degrees, associated with atrophy and a decrease in tubules, renovascular stenosis caused by intimal thickening, and infiltration of inflammatory cells (Fig. 5). The degree of interstitial fibrosis was independent of the interval between transplantation and nephrectomy. No significant correlation was found between the degree of interstitial fibrosis and the number of tryptase-positive MCs. However, there was a positive and significant correlation between the degree of interstitial fibrosis and the number of chymase-positive MCs (R = 0.745, P < 0.01). Moreover, the rate of fibrosis in the Chy(+) group

| Table 2. Quantification of chymase- and tryptase-positive mast cells in rejected kidneys |
|-----------------------------------------------|-----------------------------------------------|
| Sample                                      | Morphometric analysis |
|                      | N | Tryptase | Chymase     |
| Normal kidneys       | 4 | 1.08 ± 0.35 | 0.58 ± 0.30 |
| Rejected kidneys     |   |          |             |
| Nephrectomy specimens|   |          |             |
| Chy(−) group         | 5 | 9.10 ± 2.44 | 0.40 ± 0.18 |
| Chy(+) group         | 8 | 13.88 ± 3.40 | 12.63 ± 2.68 |
| Biopsy specimens     |   |          |             |
| Chy(−) group         | 1 | 9.00     | 0.51        |
| Chy(+) group         | 4 | 14.60 ± 3.64 | 9.00 ± 2.27 |

\(^{a}P < 0.05\) vs. normal kidneys  
\(^{b}P < 0.01\) vs. normal kidneys  
\(^{c}P < 0.01\) vs. Chy(−) group of nephrectomy specimens
Fig. 1. Sections showing mast cell (MC) chymase expression in human rejected kidneys. (A) Staining for chymase (red), nephrectomy specimen of the Chy(+) group. (B) Staining for chymase (red), biopsy specimen of the Chy(+) group. Both nephrectomy and biopsy specimens showed increased numbers of chymase-positive MCs. Original magnification ×150 (A and B).

Fig. 3. Sections showing three mast cell (MC) phenotypes: MC containing tryptase (MCt), MC containing tryptase and chymase (MCtc), and chymase-positive and tryptase-negative MC (MCc). (A) Immunodouble staining (tryptase, red/chymase, blue). A cell (long arrow) shows double staining (purple), indicating that this is a MC of MCtc phenotype. A cell (short arrow) with positivity for only tryptase (red) represents a MC of MCt phenotype. (B) Immunodouble staining (tryptase, red/chymase, blue), case N-12. Most cells (arrowheads) show staining positivity for only chymase (blue), indicating that these cells represent MCs of the MCc phenotype. A cell (short arrow) with positivity for only tryptase (red) reveals a MC of the MCt phenotype. Original magnification ×630 (A and B).

Fig. 4. Sections showing c-kit expression and chymase positivity in the interstitium. (A) Immunodouble staining (c-kit, red/chymase, blue). Most cells (arrows) show double staining (purple); chymase-positive MCs are positive for c-kit. (B) Immunodouble staining (endothelial cell, red/chymase, blue). (C) Immunodouble staining (macrophage, red/chymase, blue). (D) Immunodouble staining (T lymphocyte, red/chymase, blue). Endothelial cells, macrophages, and T lymphocytes do not show double staining (purple), indicating no chymase positivity in these endothelial cells, macrophages, and T lymphocytes. Original magnification ×720 (A) and ×450 (B–D).

Fig. 5. Sections showing the degree of interstitial fibrosis. (A) Chy(−) group. Azan Mallory stain shows mild fibrosis of the interstitium. (B) Chy(+) group. Azan Mallory stain reveals marked fibrosis of the interstitium with the disappearance of tubules. Original magnification ×160 (A and B).

Fig. 7. Section showing interleukin-4 (IL-4) expression. Immunodouble staining (IL-4, red/chymase, blue). IL-4 positivity is detected in a chymase-positive MC (arrow, purple) and some T lymphocytes (red). Original magnification ×620.

was significantly higher than that in the Chy(−) group (Figs. 5 and 6).

Biopsy specimens. Similar results were obtained in the biopsy specimens. Morphometric analysis revealed marked fibrosis in the Chy(+) group [Chy(+) group \(N = 4\), 16.13 ± 2.7%; Chy(−) group \(N = 1\), 7.87%].

Expression of IL-4

In the nephrectomy specimens, the number of IL-4-positive cells in the Chy(+) group exhibited a more than 28-fold compared with that in the Chy(−) group \((P < 0.01; \text{Table 3})\). The immunodouble staining for IL-4 and chymase, moreover, revealed that IL-4 was expressed in some of the T lymphocytes and chymase-positive MCs (Fig. 7).

DISCUSSION

Previous studies have revealed increased numbers of MCs in renal allografts, using toluidine blue stain or simply an antitryptase antibody \([3–5]\). However, no reports have been able to provide information on the protease heterogeneity of MCs because formalin fixation is
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Fig. 2. Chymase activity in normal and rejected kidneys. Vertical whisker bars represent SEM. *P < 0.01.

Fig. 6. Quantitation of the fibrotic interstitium in the cortex of rejected kidneys. Vertical whisker bars represent SEM. *P < 0.01.

Table 3. Quantification of interleukin-4 (IL-4)–positive cells in nephrectomized kidneys

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<td>Chy(−) group</td>
<td>5</td>
<td>1.26 ± 1.17</td>
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<tr>
<td>Chy(+) group</td>
<td>8</td>
<td>36.37 ± 5.99*</td>
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*P < 0.01 vs. Chy(−) group

An unreliable criterion for distinguishing between the types of MCs, particularly chymase-positive MCs [6, 15]. Thus, there have been no data regarding chymase-positive MCs in renal allografts. The present study, which used immunodouble staining techniques and was based on nephrectomy and biopsy specimens obtained from patients with chronic rejection, is the first to demonstrate that chymase is expressed in MCs of human rejected kidneys.

The ability to distinguish the types of human MCs in the same section clearly is a crucial step toward better understanding the role of these MC phenotypes under diverse pathological conditions. It is widely accepted that in humans there are two phenotypes of MCs, designated MCt and MCtc [6]. However, recent reports have demonstrated that a third MC phenotype, tryptase-negative and chymase-positive type, designated MCc, is present in human tissues [10–12]. The present study supports this concept, and to the best of our knowledge, the presence of MCc phenotype in human rejected kidneys also is another first demonstration.

A number of previous experimental studies have suggested that a relationship may exist between human MC maturation and protease expression, and that there may be factors that up-regulate chymase expression along with promotion of human MC maturation [13, 14, 21]. In the present study, we found that the number of IL-4–positive cells in the Chy(+) group was significantly higher than that in the Chy(−) group. This observation suggests that enhanced expression of IL-4 may be linked to the increase of chymase expression in MCs in human transplanted kidneys. Recent in vitro studies have shown that protease phenotypes of human MCs in culture are regulated by cytokines [13, 21] and that the chymase expression in human MCs is strongly promoted by IL-4 [14]. These experimental data and our present findings support the hypothesis that cytokines produced within the microenvironment may play a role in chymase expression of human MCs in tissue.

Chymases in mammals, including humans, have been shown to be stored in secretory granules of MCs [22, 23]. Recently, Urata et al reported that the presence of chymase-like immunoreactivity was observed in multiple cell types, including endothelial cells, interstitial mesenchymal cells, and MCs in the human heart [24]. However, the present study using immunodouble staining analysis demonstrated that endothelial cells of vessels in human renal allografts did not show positive immunostaining for chymase. The reason for the discrepancy between the findings of Urata et al and our own is not clear. It might be attributed to the difference in experimental condi-
tions, including differences in organs and differences in antibodies.

The present study clearly shows that the occurrence of increase in the number of chymase-positive MCs in human rejected kidneys is closely related to the severity of interstitial fibrosis in both nephrectomy and biopsy specimens. The present study also demonstrates that chymase in human rejected kidney tissues is enzymatically active. This is of considerable interest, since one could argue that chymase expression in MCs may play a role in the progression of interstitial fibrosis in human renal allografts. Chymase is known to be one of highly efficient angiotensin II-forming enzymes [25, 26]. Our previous studies demonstrated that chymase strongly contributes to angiotensin II formation in human arteries and cardiomyopathic hamster hearts [26, 27]. The present study, using angiotensin I as a substrate, showed that it is possible to produce angiotensin II in the interstitium of human renal allografts. Recently, evidence has accumulated that angiotensin II is one of the biological determinants involved in the process of interstitial fibrosis in the kidney [28]. In experimental animal studies, angiotensin II has been shown to stimulate the synthesis of transforming growth factor-β (TGF-β), a major fibrogenic factor, in obstructed rat kidneys [29, 30]. Very recent studies have also shown that angiotensin II promotes accumulation of extracellular matrix by inhibiting matrix degradation, indicating that angiotensin II plays a role in enhancing fibrosis [31, 32]. These experimental results suggest that interstitial fibrosis of the kidney may be mediated, at least in part, by increased levels of angiotensin II in renal tissue. Furthermore, recent in vitro experiments have demonstrated that MC chymase, but not tryptase, cleaves type I procollagen to a fibril-forming collagen molecule, suggesting that MC chymase has a role in the regulation of collagen biosynthesis and in the pathogenesis of interstitial fibrosis [33]. These experimental data and our present findings suggest the possibility that increased chymase expression in MCs plays a role in the production of angiotensin II and in the augmentation of collagen biosynthesis in renal tissue, both of which could promote further progression of interstitial fibrotic processes in human renal allografts.

In conclusion, the present study demonstrates that enhanced chymase expression in MCs, associated with the presence of MCc phenotype, is indeed found in human rejected kidneys. Furthermore, our results show that increased expression of chymase in MCs is related to the severity of interstitial fibrosis in human renal allografts. These findings add new information to the nature of the cellular mechanisms that underlie renal rejection in humans.

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