Pediatr Nephrol (2009) 24:1071–1075
DOI 10.1007/s00467-008-1044-2

BRIEF REPORT

Increased chymase-positive mast cells in children with crescentic glomerulonephritis

Hiroko Togawa · Koichi Nakanishi · Yuko Shima · Mina Obana · Mayumi Sako · Kandai Nozu · Ryojiro Tanaka · Kazumoto Iijima · Norishige Yoshikawa

Received: 11 August 2008 / Revised: 14 October 2008 / Accepted: 15 October 2008 / Published online: 3 December 2008

Abstract Mast cell-derived chymase is an angiotensin II-forming enzyme that appears to be involved in tubulointerstitial fibrosis in the kidneys. Previous studies have shown that the level of chymase increases in grafted kidneys after rejection and in adult patients with diabetic nephropathy. However, the significance of chymase in children with renal diseases has not been investigated. Using immunohistochemistry, we have investigated chymase expression in biopsy samples of renal tissue from 104 children with kidney diseases, including rapidly progressive crescentic glomerulonephritis (n=3), diabetic nephropathy (n=2), allografted kidney (n=3), membranoproliferative glomerulonephritis (n=6), immunoglobulin A nephropathy (n=33) and Henoch–Schönlein purpura nephritis (n=23). Increased numbers of chymase-positive mast cells were observed in the renal cortex of all three patients with crescentic glomerulonephritis (mean 26.0/mm^2; range 19.3–36.8/mm^2). Chymase-positive cells were also observed in the renal biopsy of an allografted kidney and in those from children with diabetic nephropathy. The mean number of chymase-positive cells in renal tissue samples characterized by each renal disease was significantly correlated with the mean intensity of the interstitial fibrosis in that same tissue sample (Spearman's rank correlation test p=0.0013; rank correlation coefficient 0.84). These findings suggest that mast cell-derived chymase plays an important role in juvenile crescentic glomerulonephritis.

Keywords Angiotensin II · Diabetic nephropathy · Allografted kidney · Immunohistochemistry · Immunoglobulin A nephropathy · Interstitial fibrosis · Rapidly progressive glomerulonephritis

H. Togawa · K. Nakanishi (✉) · Y. Shima · M. Obana · M. Sako · N. Yoshikawa
Department of Pediatrics, Wakayama Medical University,
811-1 Kimiidera,
Wakayama City, Wakayama 641-8509, Japan
e-mail: knakanis@wakayama-med.ac.jp

K. Nozu · K. Iijima
Department of Pediatrics,
Kobe University Graduate School of Medicine,
Kobe, Hyogo, Japan

R. Tanaka
Department of Nephrology,
Hyogo Prefectural Kobe Children's Hospital,
Kobe, Hyogo, Japan

Introduction

Mast cell (MC)-derived chymase is one of the serine proteases present in the secretory granules of MCs. It is an angiotensin II (ATII)-forming enzyme, similar to angiotensin converting enzyme (ACE). Chymase has no enzymatic activity under normal physiological conditions, but it is activated immediately after release into the extracellular matrix. Unlike ACE, the effects of chymase are limited to specific tissues. It has been suggested that chymase plays an important role in the progression of tubulointerstitial fibrosis via ATII. Recent studies have shown that chymase expression increases in rejected kidney grafts and in the kidneys of adults with diabetic nephropathy and immunoglobulin A nephropathy (IgAN) [1–4]. To date, however, the significance of chymase in children with renal diseases is still unclear.

Methods

Patients

Biopsy samples of renal tissue from 104 pediatric patients with renal diseases were examined (Table 1). The mean patient age± standard deviation (SD) was 10.6±4.9 years, and there was an equal distribution of sexes (52:52) in the patient cohort. Each renal specimen was subjected to routine histological examination, and the intensity of interstitial fibrosis was graded semiquantitatively based on the Banff classification of renal allograft pathology (from ci0 to ci3) [5]. A summary of three patients with crescentic glomerulonephritis showing rapidly progressive glomerulonephritis (RPGN) clinically is shown in Table 2. The estimated glomerular filtration rates (eGFRs) at renal biopsy by Schwartz's formula were 22.0, 8.8 and 7.6 ml/min per 1.73 m^2 body surface area (BSA). All of the patients with other renal diseases, except for those with allografted kidneys, showed normal eGFR (≥90 ml/min per 1.73 m^2 BSA). Of the patients with allografted kidneys, two underwent protocol biopsies (1 and 3 years after renal transplantation, respectively) and one underwent an event biopsy. Their eGFRs were 85.9 (1 year), 75.3 (3 years) and 11.6 (event) ml/min per 1.73 m^2 BSA, respectively. With IgAN, the extent of mesangial proliferation (focal or diffuse) was defined based on World Health Organization criteria [6]. Henoch–Schönlein purpura nephritis (HSPN) was graded using the International Study of Kidney Disease of Children's (ISKDC) grade [7]. Each patient's family gave informed consent for the renal biopsy and studies.

Immunohistochemical staining

Samples of biopsied kidney were snap-frozen and stored at −80°C until use, at which time they were then cut into 4-μm-thick sections using a cryostat and subjected to immunohistochemical staining. The sections were fixed in Carnoy's fixative before staining for chymase, which involved incubation with anti-human chymase monoclonal antibody (diluted 1:500; Chemicon International, Temecula, CA) for 1 h at room temperature. After washing, the

Table 1 Mast cell chymase expression in kidney tissues from children with various renal diseases

Diagnosis	Number of patients	Mean intensity of interstitial fibrosis[a, b] (range)	Number of patients showing chymase-positive cells (%)	Mean number of chymase-positive cells[b]/mm^2 (range)
Crescentic glomerulonephritis	3	3.0 (3–3)	3 (100.0%)	26.0 (19.3–36.8)
Diabetic nephropathy	2	0.5 (0–1)	1 (50.0%)	2.6 (0–5.1)
Allografted kidney	3	0.7 (0–1)	1 (33.3%)	2.2 (0–6.5)
Protocol biopsy	2	0.5 (1–1)	0 (0.0%)	0.0 (0–0)
Event biopsy	1	0.0	1 (100.0%)	6.5
Membranoproliferative glomerulonephritis	6	0.2 (0–1)	2 (33.3%)	0.5 (0–2.4)
Immunoglobulin A nephropathy	33	0.3 (0–2)	6 (18.2%)	0.6 (0–7.0)
Focal mesangial proliferation	22	0.1 (0–1)	4 (18.2%)	0.4 (0–2.4)
Diffuse mesangial proliferation	11	0.6 (0–2)	2 (18.2%)	0.9 (0–7.0)
Focal segmental glomerulosclerosis	6	1.2 (1–2)	1 (16.7%)	0.3 (0–1.9)
Henoch–Schönlein purpura nephritis	23	0.3 (0–2)	2 (8.7%)	0.3 (0–6.2)
Crescent < 50%	16	0.1 (0–1)	2 (12.4%)	0.4 (0–6.2)
Crescent ≥ 50%	7	0.7 (0–2)	0 (0.0%)	0.0 (0–0)
Minimal-change nephrotic syndrome	22	0.0 (0–0)	1 (4.6%)	0.1 (0–1.1)
Membranous glomerulonephritis	3	0.0 (0–0)	0 (0.0%)	0.0 (0–0)
Alport's syndrome	2	0.0 (0–0)	0 (0.0%)	0.0 (0–0)
Lupus nephritis, Class IV-G (A)	1	0.0	0 (0.0%)	0.0

[a] The intensity of interstitial fibrosis was graded semiquantitatively using the Banff classification of renal allograft pathology (from ci0 to ci3) [5]
[b] Spearman's rank correlation test showed that the p value and rank correlation coefficient were 0.0013 and 0.84 between them

Table 2 Crescentic glomerulonephritis showing rapidly progressive glomerulonephritis syndrome

Case number	Sex of patient	Age at biopsy (years)	Duration from detection to biopsy (days)	eGFR by Schwartz's formula at biopsy (ml/min per 1.73 m^2)	ANCA	Anti-GBM antibody	Circulating immune complex	Interstitial fibrosis[a]	Number of chymase-positive cells (/mm^2)
1	M	8	11	22.0	MPO+ PR3−	–	–	3+	36.8
2	F	6	20	8.8	MPO+ PR3−	N/D	–	3+	21.8
3	M	10	11	7.6	MPO− PR3−	–	–	3+	19.3

eGFR Estimated glomerular filtration rate; *ANCA* anti-neutrophil cytoplasmic antibody; *PR3* proteinase-3; *MPO* myeloperoxidase; *GBM* glomerular basement membrane; *N/D* not determined

[a] The intensity of interstitial fibrosis was graded semiquantitatively on a scale from 0 to 3+: none, 0; slight, 1+; moderate, 2+; and intense, 3+

sections were incubated with a secondary antibody conjugated to a peroxidase-labeled polymer using the Envision+ system (Dako Cytomation, Glostrup, Denmark). These samples were visualized with 3,3′-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. A double staining procedure using rabbit anti-human CD117 (c-kit) antibody (diluted to 1:400; Dako Cytomation) was performed to confirm that the chymase-positive cells were MCs. For negative control staining, we used the vehicle or the secondary antibody alone.

Counting of chymase-positive cells

All chymase-positive cells in the whole area of each section were counted, and the area of each section was measured using Image J ver. 1.37 software (National Institutes of Health, Bethesda, MD). This was performed blind, without knowledge of the clinical and pathological findings. The number of positively stained cells in each section was expressed as the absolute number of positive cells per square millimeter.

Statistical analyses

Spearman's rank correlation test was used to examine the association between the mean intensity of interstitial fibrosis and the mean number of chymase-positive cells of each renal disease.

Results

The number of patients examined, the mean intensity of interstitial fibrosis, the number and ratio of patients showing chymase-positive cells and the mean number of chymase-positive cells per square millimeter are shown in Table 1. Chymase expression was observed only in the interstitium.

Chymase expression was detected in the tissue samples of all three patients with crescentic glomerulonephritis. A representative micrograph showing chymase expression in renal tissue from a patient with crescentic glomerulonephritis is shown in Fig. 1. The mean number of chymase-positive cells in the renal cortex of these patients was 26.0/mm^2 (range 19.3–36.8). The renal specimens from all of the children with crescentic glomerulonephritis showed intense interstitial fibrosis.

The mean numbers of chymase-positive cells were 5.1 and 6.5/mm^2, respectively, in those patients with diabetic nephropathy and with allografted kidneys showing positive chymase expression (one patient each). The renal specimen from the patient with diabetic nephropathy with chymase-positive cells showed slight interstitial fibrosis, while that without chymase-positive cells showed no interstitial

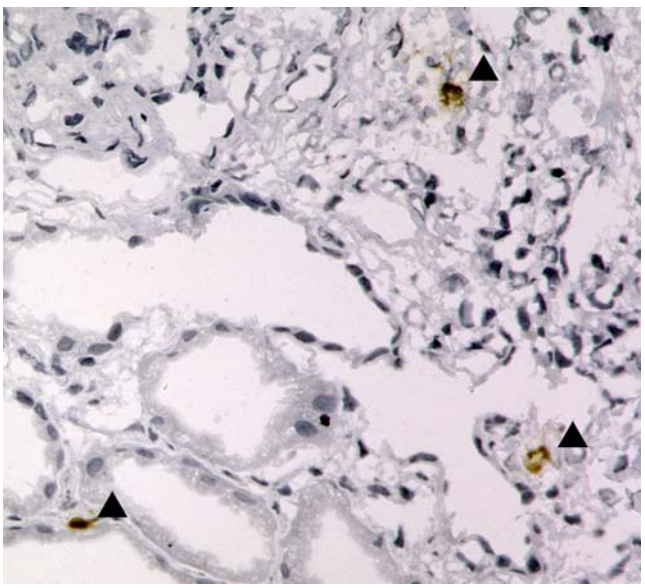

Fig. 1 A representative micrograph showing chymase expression in renal tissue from a patient with crescentic glomerulonephritis. *Arrowheads* Chymase-positive cells

fibrosis. The patient with an allografted kidney showing positive chymase expression underwent an event renal biopsy, which demonstrated acute tubule necrosis.

In tissue samples obtained from patients with membranoproliferative glomerulonephritis, the maximum number of chymase-positive cells was 2.4/mm^2. In those from patients with IgAN, there was no difference in the chymase-positive cell ratio between focal and diffuse mesangial proliferation. The maximum numbers of chymase-positive cells were 2.4 and 7.0/mm^2 in renal tissue samples showing focal and diffuse mesangial proliferation, respectively.

One patient of the six children with focal segmental glomerulosclerosis (16.7%) and one of the 22 with minimal-change nephrotic syndrome (4.6%) showed chymase positive cells.

Among the 23 HSPN patients, two showed chymase-positive cells. There was no association between ISKDC grade and chymase-positive cell ratio in HSPN.

The mean number of chymase-positive cells in tissue samples characterized by each renal disease was significantly correlated with the mean intensity of the interstitial fibrosis in that same tissue sample (Spearman's rank correlation test p=0.0013; rank correlation coefficient 0.84).

Discussion

The aim of this study was to clarify the involvement of MC-derived chymase in children with renal diseases. To our knowledge, this is the first report to describe chymase expression in children with renal diseases. We found a significantly increased number of chymase-positive MCs in all three patients with crescentic glomerulonephritis showing RPGN clinically. The accumulation of chymase-positive cells in the interstitium appeared to be correlated with the loss of renal function, and it was correlated with tubulointerstitial damage characterized by intense fibrosis. These findings suggest that chymase plays an important role in crescentic glomerulonephritis; as such, they are in agreement with the results of Tóth et al., who suggested the potential involvement of MCs in fibroproliferative change in the renal interstitium of adult patients with RPGN [8].

Mast cells contain large amounts of the serine proteases tryptase and chymase. Although both tryptase- and chymase-positive cells are present in the normal kidney, their overall number is very low. It has been reported that the number of chymase-positive cells in normal kidney is 0.58±0.38/mm^2 [1]. We focused on chymase in our study, even though the number of tryptase-positive cells is reportedly double that of cells positive for chymase [1] because of the ATII-generating property of that latter serine protease.

It has also been reported that the number of chymase-positive cells in rejected kidneys is positively correlated with the degree of fibrosis [1]. In our study, an event biopsy of one allografted kidney with acute tubule necrosis and no rejection showed an increased number of chymase-positive cells (6.5/mm^2). This finding suggests that chymase may play a role in acute tubule necrosis of allografted kidneys.

Huang et al. reported a markedly increased chymase expression associated with severe pathological changes in the kidneys of patients with diabetic nephropathy [3]. With the exception of mild tubulointerstitial fibrosis, no such findings were evident in our patients with diabetic nephropathy. The reason for this difference is unclear, but disease severity may have been partly responsible.

Studies in adult patients with IgAN suggest that MC-derived chymase plays a role in the progression of IgAN [4, 9]. However, in our population of children with IgAN, the findings suggest that chymase does not play a major role, perhaps because of a difference in disease progression level between children and adults. Interestingly, glomerular hypercellularity in the mesangial area is more prominent in children than in adults, while, in contrast, glomerular matrix expansion, crescent formation and interstitial damage are more severe in adults than in children [10]. These histological differences may be related to chymase expression patterns.

It is unknown whether chymase plays a disease-specific role in crescentic glomerulonephritis. In most of the children with renal diseases in our series, renal biopsy specimens did not show fibrosis. This may be the main reason why the numbers of chymase-positive cells were not so high in most of the diseased tissue samples we studied.

Although an increase of chymase is usually interpreted as a sign of pathological involvement, recent data have suggested that it can restore kidney homeostasis [11]. It has been reported that chymase regulates the activities of pro-matrix metalloprotease (MMP)-2 and -9 [12].

It has been demonstrated that at least 40% of angiotensin I can be converted to ATII by a pathway other than ACE in the normal kidney [13]. Although the effect of chymase inhibitors in renal diseases has not yet been investigated, they may have potency as renoprotective agents.

In conclusion, the findings of our study suggest that MC-derived chymase plays a pivotal role in disease progression in children with crescentic glomerulonephritis showing RPGN. However, the number of patients we investigated was very small, and additional analysis will be required to establish the role of chymase in children with kidney diseases.

Acknowledgments This study was presented at the 51st Annual Meeting of Japanese Society of Nephrology, Fukuoka, Japan, 2008.

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