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Increased Mast Cell Density in Renal Interstitium is Correlated with Relative Interstitial Volume, Serum Creatinine and Urea Especially in Diabetic Nephropathy but also in Primary Glomerulonephritis

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In primary glomerulonephritis the degree of interstitial fibrosis governs the renal function. Mast cells participate in renal interstitial fibrosis, but its role remains poorly understood. Some of human mast cells contain chymase and chymase may participate in local angiotensin formation.

Material consisted of 35 renal biopsies. The diagnoses included diabetic nephropathy, mesangial glomerulopathy, IgA glomerulopathy and membranous glomerulopathy. Chymase and tryptase-positive cells were stained by immunohistochemistry and counted. Relative interstitial volume (RIV) was measured by point counting method.

The density of tryptase-positive cells was 5.26 per 10 high power fields; the density of chymase-positive cells was 2.72. The counts were higher than in controls and highest in diabetic nephropathy. Creatinine serum level was related to density of chymase-positive cells (R=0.57), density of tryptase-positive cells (R=0.59) and RIV (R=0.77). On multiple regression analysis creatinine level was influenced by RIV but also by density of chymase-positive cells.

Our findings indicate that both types of mast cells are present in renal interstitium in diabetes and glomerulonephritis, and may influence the renal function. Chymase-positive cells may be more important in this regard.

Introduction

In glomerulonephritis, the disease involves the glomerulus first, yet the development of chronic renal failure depends on interstitial lesions. One of the cells participating in renal interstitial inflammation is mast cell. Tryptase and chymase are the two major proteases present in human mast cell granules [5]. Only a subset of mast cells is positive for chymase. In different body sites, mastocyte populations are differently represented [1, 2, 3, 24]. Mast cells were detected in different kind of renal disease, including IgA nephritis, membranous glomerulopathy, focal segmental glomerulosclerosis, lupus nephritis and diabetes but their role remains underestimated [9, 10, 14, 15, 19, 21].

Angiotensins (AT) are peptide hormones activated by converting enzyme (ACE). ATs influence systemic blood pressure and regulate renal blood flow, enhance proteinuria and influence renal disease progression. ATs action may be reverted by angiotensin convertase inhibitors (iACE) [20]. As chymase may constitute an alternative angiotensin converting mechanism [23], we decided to study the phenotype of mast cells in human renal disease.

Material and Methods

The material consisted of 35 representative renal biopsies. Cases of diabetic nephropathy and primary glomerulonephritis were included in the study. Ten specimens of healthy renal tissue from nephrectomy specimens for renal carcinoma served as controls. The material was fixed in buffered formalin, processed by routine method and embedded in paraffin. Four μm sections were cut from the paraffin blocks.

Primary anti-tryptase antibody (NCL-MCTRYP, Novocastra, UK) at 1:100 dilution and anti-chymase antibody (1A4, Abcam, UK) at 1:100 dilution were used. Antigen retrieval was carried out by microwaving in citrate buffer (pH 6.0) for 5 minutes at 700 W, then for 5 minutes at 600W for chymase stain and by trypsin digestion (30 min at 37°C) for tryptase stain. The ENVISION + System kit (DAKO, Denmark) detection system was used. 3-amino-9-

ethylcarbasole (DAKO, Denmark) was used as chromogen. The processing was done using DAKO Autostainter device (DAKO, Denmark).

The immunostained slides were examined under Zeiss Axioscop microscope (Zeiss GmbH, Germany) equipped with a 40x lens (PlanNeofluar, field of vision diameter 0.48mm). The entire section was scanned, and immunopositive cells as well as number of fields recorded. The results were expressed as number of positive cells per 10 high power fields. The person performing the assessment was neither aware of the diagnoses, nor of morphometry results.

The quantitative measurement of the relative interstitial volume was performed using the AnalySIS image analysis system. Silver methenamine stained, trichrome contrasted sections were used [4]. Image acquisition was done with Zeiss Axioscop microscope (Zeiss GmbH, Germany) and CCD ZVS-47DE camera (Optronics Inc, USA), connected to a standard PC. The software used consisted of the AnalySIS 3.2 image analysis system (Soft Imaging System GmbH, Germany) and custom made applications developed by one of the authors (K. O.). The measurements of the relative interstitial volume (RIV) were performed by point counting method, as described [18]. To estimate variability of RIV, variance of RIV between individual fields of vision was used (RIVVAR).

The values of serum creatinine and urea levels, daily proteinuria and arterial blood pressure at the time of biopsy were taken from the patients' records.

The statistical analysis was performed with Statistica 6.1 (StatSoft Inc, USA), using Kruskal-Wallis ANOVA, χ test, Spearman's correlation coefficient and stepwise multiple regression. The significance level was set to $p=0.05$.

Results

The material consisted of renal biopsies from 35 patients; 26 were male and 9 female. The diagnoses are shown in Table 1.

Mean age of the patients was 42.3 years (range 20-74, SD 15.2). In the diabetes group the age was 51.2, in IgA nephropathy group 27.7, in mesangial glomerulopathy 39.8 and membranous glomerulopathy 45.6.

Mean creatinine serum level was 109.9 μmol/l, range 47 to 380 μmol/l, SD 65.29. In diabetic nephropathy mean creatinine level was 177.1 μmol/l, in mesangial nephropathy 96.3 μmol/l, in IgA nephropathy 80.0 μmol/l, and in membranous glomerulopathy 80.9 μmol/l. Mean urea serum level was 7.7 mmol/l, range 3.4 to 31.0 mmol/l, SD 5.52. In diabetic nephropathy mean urea level was 13.0 mmol/l, in mesangial nephropathy 6.6 mmol/l, in IgA nephropathy 4.9 mmol/l, and in membranous nephropathy 5.5 mmol/l. Mean systolic blood pressure was 131.2 mmHg, range 110 to 160 mmHg, SD 12.51. In diabetic nephropathy mean systolic blood pressure was 143.3, in mesangial nephropathy 130.0, in IgA nephropathy 125.8, and in membranous nephropathy 132.0. Mean diastolic blood pressure was 81.7mmHg, range 60 to 110 SD 10.46; in diabetic nephropathy mean diastolic blood pressure was 86.7, in mesangial nephropathy 79.5, in IgA nephropathy 85.8, and in membranous nephropathy 80.0.

The density of mast cells was highest in diabetic nephropathy and lowest in membranous glomerulopathy; this was true for both tryptase- and chymase-positive cells. However, mast cell density even in membranous glomerulopathy was higher than in controls. The exact figures are shown in Table 2. The differences in mast cell density between different groups were statistically significant both in reference to chymase-positive, and tryptase-positive cells ($p<0.0012$ and $p<0.002$ respectively). In post-hoc analysis the differences between diabetic nephropathy and controls were significant ($p<0.015$ and $p<0.006$), as well as diabetic nephropathy and membranous glomerulopathy ($p<0.022$ and $p<0.025$). The differences between diabetic nephropathy and mesangial glomerulopathy were less obvious ($p<0.088$ and $p<0.085$). The ratio of chymase-positive to tryptase-positive cells was similar in all studied entities, in diabetic nephropathy 0.529, in mesangial glomerulopathy 0.493, in IgA nephropathy 0.431, in membranous glomerulopathy 0.442, against 0.403 for all cases. The differences were not statistically significant ($p<0.73$), but this ratio was significantly lower (0.145, $p<0.001$) in controls.

TABLE 1
Diseases under study

Diagnosis	No of cases (percentage)
Diabetic nephropathy	9 (25.7 %)
Mesangial glomerulopathy	10 (28.6 %)
IgA nephropathy	6 (17.1 %)
Membranous nephropathy	10 (28.6 %)

TABLE 2
Chymase and tryptase-positive cells' density

	Chymase-positive cells (mean, range, standard deviation)			Tryptase-positive cells (mean, range, standard deviation)		
Controls	0.09	(0 - 0.25	SD 0.108)	0.50	(0.10 – 0.90	SD 0.271)
Diabetic nephropathy	6.82	(0.06 – 15.85	SD 5.379)	12.11	(0.17 – 22.58	SD 7.993)
Mesangial glomerulopathy	1.11	(0 – 4.33	SD 1.343)	2.62	(0.35 – 9.2	SD 3.154)
IgAN	2.5	(0.54 – 4.45	SD 1.644)	5.67	(1.9 – 12.06	SD 3.614)
Membranous glomerulopathy	0.76	(0 – 2.67	SD 0.816)	1.50	(0.13 – 4.55	SD 1.332)
All cases	2.72	(0 – 15.85	SD 3.770)	5.26	(0.13 – 22.58	SD 6.224)

SD – standard deviation

The age of the patients was correlated to RIV (R=0.49, p<0.003) and RIVVAR (R=0.34, p<0.05) but correlation of age with density of chymase-positive, tryptase-positive cells, and chymase/tryptase ratio was not significant (respectively R=0.25, R=0.23, R=0.21). Creatinine serum level was related to density of chymase-positive cells (R=0.57, p<0.0004), density of tryptase-positive cells (R=0.59, p<0.0003), RIV (R=0.77, p<0.0001), RIVVAR (R=0.69, p<0.001). Correlation of creatinine to chymase/tryptase ratio was weaker and non significant (R=0.15). Urea serum level was related to density of chymase-positive cells (R=0.64, p<0.0001) density of tryptase-positive cells (R=0.57 p<0.0004), chymase/tryptase ratio (R=0.39 p<0.03), RIV (R=0.48, p<0.004) and RIVVAR (R=0.44, p<0.009). Blood pressure parameters were not significantly related to quantitative data, except for systolic blood pressure and chymase/tryptase ratio (R=0.38 p<0.05) and to some degree density of chymase-positive cells (R=0.34 p<0.07).

For testing the factors that influence kidney function parameters, multiple regression stepwise models were used. For creatinine, main factor was RIV (p<0.0001) as expected, but also density of chymase-positive cells (p<0.002). For urea level, only effect of RIV was significant (p<0.002) and chymase was just below the threshold of significance (p<0.063).

Discussion

Pathogenesis of interstitial lesions in glomerulonephritis is complex. The involved mechanisms include tubular epithelial cell activation, interstitial myofibroblast generation, extracellular matrix deposition and microvessel density reduction. These lesions depend on action of several inflammatory cells, mainly lymphocytes and macrophages but also mast cell. Mast cells are difficult to identify in the tissue sections by routine stain and may imitate other connective tissue or inflammatory cells. For detecting mast cells toluidine blue stain or immunohistochemistry may be used [9, 21, 22]. In renal disease, the mast cells were studied in the diabetic nephropathy [14], but also in other diseases, including glomerulonephritis [9, 10, 15]. Mast cells are found scattered in the interstitium, especially in fibrotic areas, the periglomerular areas and the medullary interstitium [9, 10]. The exact significance of mast cells in renal disease remains poorly understood. Ehara et al. found that mast cells in renal interstitium are spatially associated with fibroblasts and lymphocytes [9]. According to Hiromura et al. number of interstitial mast cells is correlated to creatinine level and leukocyte infiltration in several renal diseases. Hiromura noticed correlation between mast cells, renal function and interstitial fibrosis but not proteinuria [10]. Similar results were obtained by Roberts et al. [21]. In lupus nephritis mast cells are correlated to myofibroblasts and interstitial fibrosis, but do not influence the outcome [19]. Danilewicz et al. showed a significant correlation of mast cells with renal function, relative interstitial volume and myofibroblast density in membranoproliferative glomerulonephritis. Danilewicz found that in course of glomerulonephritis proteinuria decreases but mast cell number and interstitial fibrosis increase [6, 7]. Consequently, mast cells might be related to non-proteinuric factors of renal interstitial fibrosis. Yamada et al. examined renal interstitial mast cells in chronic allograft rejection, and have shown a correlation with interstitial fibrosis [25]. Chymase-positive mast cells in renal interstitium were detected by McPherson in autosomal dominant polycystic kidney disease [17].

Kondo et al. [15] showed a correlation of the degree of renal interstitial fibrosis and fibroblast proliferation with the number of infiltrating tryptase-positive mast cells. Similar effect on fibroblast culture was obtained by tryptase administration. Goto et al. have shown that mast cells

are abundant both in diabetic nephropathy and allograft rejection, and are correlated with the degree of interstitial fibrosis. Goto noticed also that tryptase-positive cells are more abundant that chymase-positive, but did not analyzed this finding further [11]. Tryptase and chymase may serve as mediators of inflammation and have been implicated in the development of tissue fibrosis in skin and lungs [1, 2].

Angiotensin is an important mediator in many renal diseases; thus the drugs that interfere with it are commonly used. They are known to reduce proteinuria and slow down disease progression in different renal disease [20]. The principal drugs are ACE inhibitors and AT-receptor blockers. The later seem more effective in human, but not in most animal models. This is explained by ACE-independent AT generation. One of the alternative convertases is chymase [12, 16]. Namely, rodents and primates differ in chymase-AT interactions [13]. There is evidence for such a mechanism in diabetic nephropathy [14, 16]. The mast cell chymase was also implicated in generation of TGF-beta, IL-1 and endothelins and degradation of the extracellular matrix [8].

Our results confirm those mast cells are present in renal interstitium not only in diabetes but also in the primary glomerular diseases. These cells are both tryptase and chymase-positive. The percentage of chymase-positive population is similar in all studied diseases, but higher than in a normal kidney. Density of mast cells is related to interstitial fibrosis and kidney function, but this is mainly true for the chymase-positive population.

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