Increased mast cell number in human hypertensive nephropathy

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1Institute of Anatomy, Charité Universitätsmedizin Berlin, Free University and Humboldt University, Berlin; 2Department of Nephrology and Center for Cardiovascular Research, Charité Universitätsmedizin Berlin, Charité Campus Mitte, Humboldt University, Berlin; 3Otto Heubner Center, Charité Universitätsmedizin Berlin, Free University and Humboldt University, Berlin; and 4Department of Pathology, University of Erlangen, Germany

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Welker P, Krämer S, Groneberg DA, Neumayer HH, Bachmann S, Amann K, Peters H. Increased mast cell number in human hypertensive nephropathy. Am J Physiol Renal Physiol 295: F1103–F1109, 2008. First published August 6, 2008; doi:10.1152/ajprenal.00374.2007.—Mast cells have recently been related to nonallergic chronic organ damage and fibrosis. In the present study, we analyzed mast cell number, localization, and maturation in the kidney of a relatively unique group of middle-aged accident victims with primary essential hypertension and in normotensive controls (n = 8 per group, Caucasians, predominantly male). Hypertensive kidneys showed a significantly higher degree of arteriosclerosis. However, glomerular and tubulointerstitial matrix accumulation did not differ significantly to normotensive controls indicating a relatively early stage of hypertensive nephropathy. Using toluidine blue staining, renal mast cell number was found to be fivefold higher in hypertensive subjects compared with normotensive controls. Mast cells were primarily located in the peritubular interstitial spaces, some perivascular, but not in glomeruli. In a series of immunohistological staining studies, mast cell maturation grading showed that expression of early hematopoietic precursor cell marker CD34 did not differ between both groups. In contrast, mast cells were mostly positive for IgE receptor, tryptase, and chymase indicating a mature, differentiated cell phenotype in hypertensive nephropathy. Renal expression of stem cell factor was markedly upregulated in primary hypertension. Kidney macrophage and lymphocyte numbers were similar in both groups. In conclusion, human hypertensive kidney disease shows an early and conspicuous upregulation of stem cell factor along with an increased number of mature mast cells. The results suggest that renal mast cell accumulation may play a role in the pathogenesis of human hypertensive nephropathy.

CD34; chymase; tryptase; stem cell factor

HYPERTENSIVE NEPHROPATHY is a major cause of end-stage renal disease all over the world (14, 19). Of the many patients affected by high blood pressure, only a proportion develops clinically apparent renal injury and the underlying mechanisms responsible for a susceptibility to hypertensive nephropathy are still only incompletely understood. At the histological level, hypertensive kidney damage is characterized by expansion of extracellular matrix proteins, i.e., renal fibrosis, and an advancing number of infiltrating round cells especially in the interstitial space (12, 20, 26), although not primarily immune mediated. While contemporary research recently focused on the functions of renal tissue macrophages and T-lymphocytes for this pathological process, only a little be said as to the contribution of the mast cells. This distinct cell type is best known for its traditional role in type I allergies with immunoglobulin E (IgE)-mediated diseases such as bronchial asthma and anaphylactic reactions (16). However, mast cells have lately been more recognized as important modulators and mediators of innate immunity, chronic inflammation, tissue remodeling, and organ fibrosis (2, 17, 18).

Mast cells derive from CD34-positive multipotent bone marrow progenitor cells, circulate in small numbers in the blood, and enter the mucosal surfaces and connective tissue compartments of multiple organs (16). These cells characteristically express IgE and stem cell factor receptors (c-kit) on their surface. Their large intracytoplasmatic granules contain an array of primary and secondary mediators that influence resident and infiltrating cells in a paracrine manner. These mediators include mast cell-specific proteases, as tryptase and chymase, as well as a number of profibrotic acting cytokines (2, 16–18).

In rats following 5/6 nephrectomy, Jones et al. (9) recently provided evidence that mast cells play an active role in the pathogenesis of hypertensive nephropathy. To extend the relevance of this interesting experimental finding to the human situation, the present study took advantage from a recent unique study sample documenting that patients with primary hypertension have significantly fewer glomeruli per kidney than matched normotensive controls (11). The patients examined were middle-aged, white, and had a history of primary hypertension and/or left ventricular hypertrophy as well as matched normotensive controls. All had died in car accidents. This sample of renal tissues is unique in nature since there is no renal biopsy material available in early hypertensive nephropathy nor is there direct access to renal tissue in these patients. On the other hand, the price to pay is that these kidney samples were harvested postmortem, therefore allow only limited histological and immunohistological analyses and the number of subjects was rather small.

The present study was designed to characterize the renal distribution of mast cells and their maturation level in this exclusive set of sample of renal tissues with primary hypertensive nephropathy. The renal mast cell findings were compared with the degrees of arteriosclerosis, glomerulosclerosis, and tubulointerstitial fibrosis as well as to renal macrophage and lymphocyte cell number.

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METHODS

Study Population

From the previously described groups of 10 middle-aged Caucasian subjects (11) with primary hypertension and 10 matched controls, randomly sampled formalin-fixed renal tissue blocks (2 × 2 cm) were still available from 8 hypertensive patients and 8 normotensive controls. All materials were postmortem samples, thus allowing only a limited set of histological and immunohistological analyses compared with vitally fixed tissues. Inclusion criteria for the hypertensive cohort were a death having occurred before age 60 years, a medical history of primary hypertension, and/or left ventricular hypertrophy and hypertension-characteristic renal arteriolar lesions in histology. Written or oral informed consent was obtained from next of kin. The study was approved by the local authorities and ethics committee. The hypertensive group was 48 years old and weighed 87 kg, respectively. In both groups, all subjects except one were male.

Tissue Processing and Analysis

Four 4-μm sections of the paraffin-embedded kidney blocks were cut for histochemical and immunohistochemical analysis. All microscopic examinations were performed in a blinded fashion.

Histochemistry

Degree of hypertensive kidney damage. Arteriolar, glomerular, and tubulointerstitial changes were analyzed separately on periodic acid Schiff (PAS)-stained renal sections. Renal arteriolar lesions were judged on a four-point scale as absent (score 0), minor (score 1), moderate (score 2), or severe (score 3) as previously described (11). Tubulointerstitial and glomerular fibrosis was analyzed by a computer-based morphometric analysis system. This involved a Leica DM LB2 light microscope (Leica Microsystems, Wetzlar, Germany) connected to a PL-A662 video camera and the Axiovision 2.05 image analysis system (both Carl Zeiss Vision, München, Germany) and a 10 × 10 orthographic grid overlay as previously described (24). The relative degree of tubulointerstitial fibrotic lesions, i.e., matrix deposition, tubular atrophy, and dilation, was calculated in at least 15 randomly selected cortical areas per observed at ×200 magnification. The tubulointerstitial fibrosis score is expressed as percentage of the area affected in relation to the total area analyzed. Glomerular matrix expansion was evaluated by calculating the relative degree of the mesangial matrix-occupying area (glomerular matrix score, in percent) of 15 glomeruli of each subject (24).

Mast Cell Histochemical Staining

To identify mast cells in the kidneys, toluidine blue staining was performed at pH 0.5 for 24 h on 4-μm serial renal sections. Mast cell counting involved light microscopy and analyzing software (SPOT-Metamorph, Visiutron, Puchheim, Germany) (4).

Mast Cell Immunohistochemical Staining

Immunohistochemistry was performed as previously described (5) and mast cell maturation grading was carried out using a panel of mast cell markers. The following antibodies were used: CD34 (DakoCytomation, Hamburg, Germany), a mast cell-specific anti-tryptase antibody (DakoCytomation), an α-chain of the high-affinity IgE receptor antibody (FceRI, donated by J. Hakimi), a mast cell-specific protease chymase antibody (DakoCytomation). Previous studies demonstrated that expression of mast cell markers depends on their stage of maturation and differentiation (7). Variations in mast cell characteristic reflect the following: CD34 indicates early hematopoietic precursor cells. Early markers of mast cell differentiation are c-Kit and tryptase, thereafter FcεRIα, and a late marker is chymase. Renal lymphocytes were stained with a CD3 antibody and macrophages with a CD68 antibody (both DakoCytomation), respectively.

To quantify renal cell numbers, stained cells were counted using a grid covering 1/16 mm² at ×400 magnification in at least five microscopic fields. Counts were expressed as stained cells per millimeter squared, as described previously (4, 6). Measurements are based on at least four histological slides from each patient and control subjects examined in a blinded fashion.

To identify the molecular mechanisms underlying mast cell precursor immigration and differentiation, expression of the key mast cell chemoattractant stem cell factor (SCF) was assessed using an SCF antiserum (DakoCytomation). To control the staining specificity, tissue samples were incubated with no antibody or SCF antibody that had been preincubated with 2 μg/ml human recombinant SCF (ImmuNoTools, Friesoythe, Germany) for 1 h at 4°C. For quantification of SCF immunoreactivity, high-resolution digital images were obtained from each tissue. Using an image analysis system (MetaView Imaging System, Universal Imaging, West Chester, PA) and a preset fixed color threshold value, the number of positive (i.e., bright red) pixels was automatically calculated. The immunoreactivity of each tissue was expressed as optical density (ratio of the sum of optical pixel density/number of pixels).

The contribution for mast cells for local activation of the renin-angiotensin systems recently received much attention (15, 22). We were unable to detect renin, angiotensinogen, or angiotensin II in our samples, most likely due to the postmortem nature of the materials available.

Statistical Analysis

Data are expressed as means ± SD. Statistical analysis between the groups was performed by one-way ANOVA and a subsequent non-parametric Mann-Whitney U-test. A P value <0.05 was considered significant.

RESULTS

Histological Degree of Hypertensive Nephropathy

The kidneys of patients with primary hypertension showed a significantly higher arteriolar sclerosis score (1.8 ± 0.2 vs. 0.2 ± 0.1, P < 0.05), while glomerular [22 ± 4 vs. 18 ± 5%, P = not significant (NS)] and tubulointerstitial matrix (3 ± 1 vs. 2 ± 1%, P = NS) scores were not notably different from those of the normotensive control group (Fig. 1). These findings indicated that the samples investigated in this study are at a relatively early stage of hypertensive nephropathy.

Renal Mast Cell Number and Distribution

The renal mast cell numbers were quantified using histochemical toluidine blue staining. Compared with the normotensive controls, renal mast cell number was fivefold higher in the group of hypertensive patients (23 ± 6 vs. 114 ± 55 cells/cm², P < 0.01; Fig. 2A). The toluidine-stained mast cells were localized in the peritubular and perivascular interstitial spaces of the kidneys (Fig. 2, B and C). No mast cells were found in glomeruli. In the hypertensive samples, 86 ± 4% of the mast cells were localized peritubular and the normotensive renal sections 80 ± 5%, respectively.

Renal Mast Cell Maturation Grading

CD34 expression. Maturation grading was then carried out using a panel of mast cell markers. Representative
photographs are shown in Fig. 3. CD34 is a marker for early hematopoietic precursor cells and was detected in only a low renal number without significant differences between both groups (6.1 ± 3.4 vs. 5.2 ± 2.9 cells/cm², P = NS; Fig. 4). These findings indicate that the mast cell phenotype in early hypertensive nephropathy is differentiated and therefore mast cell markers for more advanced differentiation states were applied for further analysis.

Tryptase expression. Using an antibody against mast cell-specific tryptase, stained mast cells were found to be localized in the peritubular and perivascular interstitial spaces. Quantification and comparison of the number of tryptase-positive mast cells revealed a number of cells elevated approximately fourfold in the hypertension group (438 ± 275 vs. 111 ± 80 cells/cm², P < 0.01; Fig. 4).

α-Chain high-affinity IgE receptor (FceRI). Staining for FceRI demonstrated a typical distribution of positive mast cells in peritubular and perivascular interstitial spaces. Comparison of both groups indicated that a fivefold increase in the number of FceRI-positive mast cells was present in the hypertensive subjects (196 ± 51 vs. 38 ± 29 cells/cm², P < 0.01; Fig. 4).

Chymase expression. As a marker of advanced mast cell differentiation, the expression of the protease chymase was assessed. Chymase staining was present in peritubular and perivascular interstitial cells. Quantification of positively stained mast cells showed a sixfold elevation in mast cell numbers in hypertension (83 ± 50 vs. 14 ± 7 cells/cm², P < 0.01; Fig. 4).

SCF Expression

Expression of the mast cell chemokine SCF was examined in the specimen of the two groups and revealed a significant qualitative staining intensity. Expression of SCF was found abundantly in the hypertensive group, but only to a minor degree in the control group (705 ± 153 vs. 201 ± 50 relative staining intensity, P < 0.05; Fig. 5).

Renal Macrophage and Lymphocyte Number

Renal infiltration with macrophages and lymphocytes was determined to see whether the presence of mast cells in early hypertensive nephropathy is specific or rather a part of a general cell response of the immune system. Interestingly, and in contrast to renal mast cell number, renal counts for macrophages (257 ± 412 vs. 240 ± 134 cells/mm², P = NS) and lymphocytes (25 ± 10 vs. 23 ± 15 cells/mm², P = NS) were not significantly different between hypertensive and normotensive kidneys (Fig. 6).
DIscussion

More than 100 years ago, Paul Ehrlich was the first to discover a novel chunky cell type loaded with huge cytoplasmic granules in various tissues (3). Since these cells appeared so well-fed, he named them “Mastzellen” (mast cells). In the meantime, it has been found out that mast cells are an important limb of the immune system and act as key mediators of allergic reactions (16). Beyond this, more and more nonimmune activities of mast cells have been traced recently in acute and chronic organ dysfunctions (2, 17, 18).

The present investigation documents that a significantly elevated number of mast cells is present in early stages of human hypertensive nephropathy and thereby expands on the recent experimental study in hypertensive 5/6-nephrectomy (9). Using toiluidine blue histochemistry, mast cells were found predominately in the interstitial and perivascular spaces of the hypertensive kidneys, but not in glomeruli. Interestingly, the increase in mast cell number was seen while renal infiltration with other effector cells of the immune system, such as macrophages and lymphocytes, was not yet detectable.

Because hypertensive tissue injury is of nonimmune nature, the presence of mast cells in human hypertensive nephropathy is remarkable in itself. In the normal kidney, this cell type is constitutively expressed in small numbers only (3, 18). Reports on mast cell accumulation in renal disease are few, but this cell type has been shown mostly in immune kidney disorders, such as IgA nephropathy, membranous nephropathy, crescentic glomerulonephritis, and acute and chronic allograft rejection (2, 3, 18). In IgA nephropathy and chronic allograft rejection, renal mast number correlated positively with interstitial fibrosis and negatively with renal function. In nonimmune renal diseases, similar to this study, mast cell accumulation has been reported in diabetic nephropathy (2). Common to all these and to our own study is the finding that mast cells locate predominately in the renal interstitium and not in glomeruli.

Depending on their maturity stage, mast cells can release higher or lower amounts of specific biologically active peptides, proteases, and mediators (16, 18). Therefore, we analyzed the level of mast cell maturation in our set of hypertensive nephropathy samples. Using CD34 as a marker for early hematopoietic precursor cells, only low numbers of cells were found. Previous studies were unable to demonstrate double labeling of CD34 with other mast cell markers (toluidine blue, tryptase) in peripheral tissues (8, 10). Even in patients with systemic mastocytosis, double staining of CD34 and mast cell markers was only successful in bone marrow, but not in infiltrated organs. In the nonperfused kidney tissues of this study, CD34-positive cells may represent circulating hematopoietic cells, but as well cells that differentiate subsequently into renal tissue mast cells. In contrast to low renal CD43 positivity, higher numbers of cells were strongly positive for the IgE receptor, chymase, and tryptase, indicating that an increased number of mature mast cells is present in human hypertensive nephropathy.

The overlap of the different mast cell markers found in the present investigation requires some explanation. Mast cells in various organs are a heterogeneous population in terms of mediator or receptor expression. The phenotype of human renal mast cells has been characterized in few studies
so far. Beil et al. (1) described renal mast cells as cells exhibiting phenotypic properties similar to those of lung mast cells with low amounts of chymase in only few tryptase-positive cells. These data are consistent with the results of our study. In addition, the phenotype of mast cells is modified in pathological tissues (7, 21, 23). Previous studies demonstrated that the expression of mast cell characteristics depends on the stage of maturation and differentiation (25). Early markers of mast cell differentiation are c-Kit and tryptase, thereinafter FcεRIα, and a late marker is chymase. The variation in cell numbers positive for different mast cell markers in the present study suggests the migration and further differentiation of immature mast cells in tissues of patients with hypertensive nephropathy.

Upon stimulation mature mast cells can release a multitude of preformed and formed substances that are known not only to influence and modulate inflammation, but also organ function, tissue fibrosis, and blood pressure (2, 17, 18). These substances include histamine, chymase, tryptase, prostaglandin D2, leukotriene C4, thromboxane A2, renin, angiotensin II, endothelin, nitric oxide, cytokines like tumor necrosis factor-α, several interleukins and interferon-γ, chemokines including macrophage inflammatory protein-1 and monocyte chemoattractant protein-1, and growth factors such as platelet-derived growth factor, fibroblast growth factor, and transforming growth factor (TGF)-β1. The far-reaching power of mature mast cells is seen in cutaneous urticaria. In this clinical paradigm for mast cell function, a small number of these cells in the skin can trigger a dramatic change of large areas of the dermal surface.

In addition to the evidence that mature mast cells accumulate in hypertensive kidneys, this study shows that the expression of SCF is upregulated in hypertensive nephropathy. SCF is a key chemoattractant for mast cell precursor immigration and a key survival and differentiation factor for these cells (2, 4). This finding is consistent with the experimental data in hypertensive renal damage (9), indicating that similar mechanisms underlie mast cell precursor migration and maturation in both experimental and human hypertensive nephropathy. The findings are also in line with preliminary data of our group in a more advanced stage of hypertensive renal disease (10.9-fold increase in mast cell number by toluidine blue staining, n = 5; serum creatinine at biopsy 2.6 ± 0.8 mg/dl).

Due to the autopsy nature of this study, it remains difficult to draw clear conclusions as to the role of mature mast cells in human hypertensive nephropathy. Nevertheless, the experimental study by Jones et al. (9) provided strong indirect evidence for a profibrotic effect on mast cells in hypertensive renal disease. The group demonstrated that mast cells were predominantly localized to regions of tubular injury and peritubular fibrosis and expressed the key fibrosis mediator TGF-β1. Furthermore, the renoprotective action of angiotensin blockade went along with a significantly reduced renal mast cell number. Supportive of a profibrotic role of renal mast cells are in vitro studies showing that mast cell...
mediators could promote fibrosis (2, 9, 18). For instance, the mast cell-specific tryptase and chymase stimulate collagen synthesis and the processing of procollagen to fibril-forming collagens. Mast cells release a number of profibrotic growth factors such as TGF-β1, PDGF, and FGF and mast cell chymase can directly activate latent TGF-β1. In human IgA nephropathy and chronic allograft rejection, mast cells were predominately located in the renal interstitium and their number correlated positively with interstitial fibrosis and negatively with renal function (2, 13). In addition to renal fibrosis, a close correlation of mast cell accumulation and tissue fibrosis has been seen such as in several extrarenal diseases, such as scleroderma, keloid scars, pulmonary cardiac and hepatic cirrhosis, rheumatoid arthritis, and myelofibrosis (2).

![Fig. 5. Renal expression stem cell factor (SCF) in human hypertensive nephropathy compared with kidneys from normotensive controls using immunohistochemistry. Representative sections are shown for normotensive (A) and hypertensive kidneys (B). C: control without primary SCF antibody. D: SCF antibody was preincubated with human recombinant SCF to control for immunostaining specificity. Quantitative analysis of staining intensity is shown in E. *P < 0.05 vs. normotensive controls.](image)

![Fig. 6. Renal infiltration with macrophages (A) and lymphocytes (B) in human hypertensive nephropathy compared with normotensive controls. Macrophages were detected with a CD68 antibody and lymphocytes with an anti-CD3 antibody, respectively, and are expressed as positive cells per mm².](image)
MAST CELLS IN HUMAN HYPERTENSIVE NEPHROPATHY

In conclusion, an early increase in the number of mature mast cells is detectable in human hypertensive kidney disease and parallels upregulation of the key mast cell chemokine and survival factor SCF. The role of mast cell accumulation in the pathogenesis of both experimental and human hypertensive nephropathy warrants further investigation.

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