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Chymase increases glomerular albumin permeability via protease-activated receptor-2

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Abstract

Increased infiltration of the kidney by mast cells is associated with proteinuria, and interstitial fibrosis in various renal diseases. Mast cells produce serine proteases including tryptase and chymase (MCC) that act via protease-activated receptors (PARs) to induce synthesis of fibrogenic cytokines by renal cells. In the present study, we investigated direct effect of MCC and role of PARs on glomerular albumin permeability (P_{alb}). Isolated rat glomeruli were incubated with MCC (0.1, 1, 10, and 100 ng/ml) for 5–30 min in presence or absence of PAR-1 and PAR-2 blocking antibodies. P_{alb} was determined from the change in glomerular volume in response to an albumin oncotic gradient. The effect of direct activation of PARs on P_{alb} was verified by incubating glomeruli with synthetic hexapeptide known to activate PAR-1 and PAR-2. MCC increased P_{alb} of isolated rat glomeruli in a dose- and time-dependent manner. Blocking PAR-2 prevented MCC-mediated increase in P_{alb}. RT-PCR analysis of glomerular RNA demonstrated the presence of constitutively expressed PAR-1, -2, and -3 and low levels of PAR-4. In addition, direct activation of PAR-2 by hexapeptide SLIGKV increased P_{alb} comparable to MCC, whereas PAR-1 activation by TFLLRN had no effect on P_{alb}. Our results document that MCC induces increase in P_{alb} and that this effect is mediated through PAR-2. MCC may also play a role in renal scarring. We propose that inhibiting MCC activity or blocking the activation of PAR-2 may provide new targets for therapy in renal diseases.

Key words: chymase, protease-activated receptors, glomerular albumin permeability

Abbreviations: MCC, mast cell chymase; PARs, protease-activated receptors; P_{alb}, glomerular albumin permeability.

Introduction

Mast cells are bone marrow-derived multifunctional cells that continue their maturation and differentiation in peripheral tissues [1]. A few mast cells are present in the normal human kidney [2], but their number increases significantly in a wide variety of renal diseases including primary or secondary glomerulonephritis [3–5], IgA

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nephropathy [6], diabetic nephropathy [7, 8], chronic interstitial fibrosis [9, 10], and allograft rejection [11]. Although an association between increased number of mast cells and progression of interstitial fibrosis in renal diseases has been established [9–11], there are conflicting reports regarding role of mast cells in mast cell deficient mice with anti-GBM antibody induced glomerulonephritis [12, 13]. The direct effect of mast cell proteases on glomerular structure and function has not been extensively studied.

Activation and degranulation of mast cells result in secretion of multiple inflammatory mediators, including histamine, cytokines, chemokines, leukotrienes, prostanoids, transforming growth factor-β (TGF-β1) and the neutral serine proteases, tryptase, and chymase [14, 15]. Mast cell-derived proteases are known to function through one or more G-protein-coupled membrane bound protease-activated receptors (PAR-1, -2, -3 and -4). While thrombin activates PAR-1, -3 and -4 [15], trypsin and mast cell-proteases activate PAR-2 [16–18]. Up-regulation of mast cell chymase (MCC) is considered to be responsible for renal fibrosis in diabetic nephropathy, allograft rejection and IgA nephropathy [7, 19, 20]. The physiological functions of PAR-2 and the proteases that activate this receptor in the glomerulus are largely unknown.

We have shown that increased glomerular albumin permeability (P_{alb}) is an early indicator of glomerular injury by several agents including profibrotic cytokines such as TGF-β1 [21], TNF-α [22], proteases [23], and free radicals [24]. We have shown that P_{alb} increases prior to the onset of proteinuria in animal models of several diseases including puromycin nephrosis [25] and hypertension [26]. The role of mast cell proteases and PARs in the regulation of glomerular filtration barrier function is not known.

We hypothesized that MCC acts through PAR-2 receptors in glomeruli to increase macromolecular permeability that leads to proteinuria and initiates glomerulopathy. Presently, we sought to demonstrate the effect of MCC on the protein permeability barrier of isolated glomeruli in our *in vitro* assay, and to determine the effect of direct activation of PAR-2 receptors on P_{alb}.

Methods

Chemicals and supplies

All commonly used supplies and analytical grade chemicals for buffers, bovine serum albumin (BSA), and chymase were obtained from Sigma Chemical Company, St. Louis, MO. PAR-1 and PAR-2 activating peptides (TFLLRN and SLIGKV) and inactive scrambled peptides (LFTNRL and ILSVKG) that lacked consensus sequences but retained same amino acid compositions were synthesized by Syn Pep Corp. (Dublin, CA) and HPLC-purified at the Biotechnology Facility of the Kansas University Medical Center, Kansas City, KS. Mouse anti-human PAR-1 (WEDE 15) was purchased from Fisher Scientific. Mouse anti-human PAR-2 (SAM11) was purchased from Santa Cruz, South San Francisco, CA. Mouse isotype IgG2a was obtained from Chemicon, Temecula, CA.

Experimental animals

Male Sprague–Dawley rats (180–250 g) maintained on Purina rat chow and water *ad libitum* were used in all experiments. In all cases kidneys were removed via abdominal incision after the animals were anesthetized using Halothane (Halocarbon Laboratories, River Edge, NJ). Animal care occurred in accordance with NIH guidelines and the Animal Care and Use Committee at the Medical College of Wisconsin approved of all protocols.

Isolation of glomeruli

After the kidney capsule was removed, fine fragments of the outer 1–2 mm of renal cortex were prepared, and passed through consecutive screens of 80, 120, and 200 mesh size [27]. Glomeruli were recovered from atop the 200-mesh screen. Isolation of glomeruli was carried out at room temperature in medium containing (mM/l): sodium chloride, 115; potassium chloride, 5; sodium acetate, 10; dibasic sodium phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 1.2; calcium chloride 1; glucose, 5.5; L-alanine, 6; sodium citrate, 1; and sodium lactate, 4. BSA 5 g/dL or 1 g/dL was included in the medium as an oncotic agent. The pH of the medium was adjusted to 7.4 and oncotic pressure was measured using a membrane colloid osmometer (Model 4100, Wescor Inc. Logan, UT). Medium containing 5 g/dL BSA was used for isolation of glomeruli.

Glomerular expression of PAR-1, PAR-2, PAR-3, and PAR-4

Glomerular total RNA was extracted immediately following the isolation of glomeruli from the kidneys. RNA was extracted using Micro-Midi Total RNA Purification System (Invitrogen, CA) following the manufacturer's instructions. Isolated RNA was analyzed for its quality and quantity by measuring absorbance of the samples at 260 and 280 nm using Pharmacia Biotech DNA/RNA calculator. All RNA

samples had an OD_{260}:OD_{280} between 1.8 and 2.0, indicating clean RNA preparations.

Synthesis of the glomerular cDNA

One μg of total RNA was reverse transcribed using Super-Script III First strand synthesis system for RT-PCR (Invitrogen, CA) according to the manufacturer's instructions and the cDNA was stored at −20°C.

cDNA amplification

Glomerular cDNA was amplified with hot start PCR using PARs-gene specific primers, 1 μl of cDNA, and Platinum PCR Super Mix (Invitrogen). The sequences (5′–3′) of PARs used are:

PAR-1 Forward, AGGGGATGAGGAGGAGAAAA
Reverse, CAGGGGAAGGCTGACTATGA,
PAR-2 Forward, GAAGCTGACCACCGTCTTTC
Reverse, GGGGAACCAGATGACAGAGA,
PAR-3 Forward, CCTGCCATCTACATCCTGGT
Reverse, CCAGTCGTTCCCATTGAGAT and
PAR-4 Forward, ACAACAGTGACACGCTGGAG
Reverse, CATGAGCAGAATGGTGGATG.

Expected product sizes for PAR-1, PAR-2, PAR-3, and PAR-4 are 193, 175, 186, and 183 bp, respectively. For PCR, initial denaturation step was carried out at 95°C for 3 min and rest of the 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 35 s. The final step of extension was carried out at 72°C for 7 min. PCR products were separated on 2.0% agarose gel containing 0.01% ethidium bromide. Products size was determined by comparing with the DNA ladder. Relative intensity of the bands was semi quantified using Alpha Imager software (San Leandro, CA).

Treatment of glomeruli

In first set of experiments glomeruli were incubated with varying concentrations of MCC (0.1, 1, 10, and 100 ng/ml) for 30 min at 37°C and P_{alb} determined using volumetric change after inducing an oncotic gradient across the capillary wall (see below). In the next set of experiments, glomeruli were incubated with 10 ng/ml of MCC (determined to produce maximal effect on P_{alb} in previous experiments) for 5, 10, 15, 20, 25, and 30 min at 37°C and P_{alb} was determined. In both sets of experiments glomeruli alone in isolation medium were used as control. At least 15 glomeruli from minimum of three rats were studied for each condition.

To determine the role played by PAR-1 and PAR-2 in this effect of MCC on P_{alb}, glomeruli were pre-incubated with 5 μg/ml concentration of mouse anti-human PAR-1 (WEDE 15), mouse anti-human PAR-2 (SAM11) antibodies or normal mouse IgG for 120 min. Glomeruli were then washed three rimes with fresh isolation medium to remove unbound antibodies and incubated further with10 ng/ml of MCC for an additional 15 min at 37°C. P_{alb} was measured as described above. The concentration of antibodies (WEDE 15 and SAM 11) used in these experiments is within the range reported earlier and have been shown to be sufficient to block activation of PAR-1 and PAR-2, respectively [28].

To document if, the direct activation of PAR-1 or PAR-2 will have similar to chymase effect on P_{alb}, glomeruli were incubated with PAR-1 and PAR-2 activating or control peptides for 30 min at 37°C and P_{alb} determined as described previously. The synthetic PAR agonist peptides mimic the tethered ligand of the receptors and therefore activate PARs in a highly specific manner without protease activity, whereas scrambled control or inactive peptides lack the consensus sequences but retain the same number of amino acids [29].

Measurement of glomerular volume response (ΔV)

The volume response of glomerular capillaries to an oncotic gradient was measured as previously described [27]. Following incubation with experimental agents, glomeruli were affixed to a glass coverslip coated with poly-L-lysine (1 mg/ml) and observed using videomicroscopy before and 1–2 min after the isolation/incubation medium containing 5 g/dL BSA was replaced by medium containing 1 g/dL BSA. This exchange of medium produces an oncotic gradient across the glomerular capillary wall and results in net influx of fluid and an increase in glomerular volume. Glomerular volume was calculated from the average of four diameters of the video image and the increase in volume (ΔV) of each glomerulus in response to an oncotic gradient was expressed as:

$$\Delta V = (V_{\text{final}} - V_{\text{initial}})/V_{\text{initial}} \times 100\%.$$

Reflection coefficient of albumin (σ_{alb})

There is a direct relationship between the increase in glomerular volume (ΔV) and the oncotic gradient ($\Delta \Pi$) applied across the capillary wall [26]. We used this principle to calculate σ_{alb}, using the ratio of ΔV of experimental to ΔV of control glomeruli in response to identical oncotic gradients:

$$\sigma_{alb} = \Delta V_{experimental}/\Delta V_{control}.$$

Convectional permeability to albumin (P_{alb})

Convectional albumin permeability (P_{alb}) was defined as $(1 - \sigma_{alb})$ to describe the movement of albumin consequent to water flow [26]. When σ_{alb} is zero, albumin moves at the same rate as water and P_{alb} is 1.0. Alternatively, when σ_{alb} is

1.0, albumin cannot cross the membrane with water and P_{alb} is zero.

Statistical analysis

P_{alb} values are expressed as mean ± SEM. *N* represents the number of glomeruli studied. Values of groups were compared using ANOVA or unpaired *t*-test. Significance was defined as $p < 0.05$.

Results

Chymase increases glomerular P_{alb} in a dose- and time-dependent manner

Several proteinuric kidney diseases are characterized by increased renal infiltration with mast cells [3–9]. Likewise glomerular level of chymase is increased in various renal diseases [7, 19, 20]. The effect of MCC on glomerular function has not been described. In order to determine if MCC directly affects glomerular protein permeability, isolated glomeruli were incubated with MCC in various concentrations. MCC significantly increased P_{alb} of isolated glomeruli at concentrations as low as 1 ng/ml (0.35 ± 0.08, $N = 15$, $p < 0.05$) and this effect increased further with 10 or 100 ng/ml (0.78 ± 0.072, $N = 15$, $p < 0.001$, $N = 20$ and 0.86 ± 0.015, $N = 15$, $p < 0.001$, vs. control 0.04 ± 0.058, $N = 20$, respectively), Fig. 1. The lower concentrations of MCC tested (0.1 ng/ml) did not increase P_{alb}, (0.1 ± 0.05, $N = 15$ vs. control 0.04 ± 0.058, $N = 20$), Fig. 1. The concentrations of MCC tested are within the range reported in kidney diseases associated with increased infiltration of mast cells [19, 20].

Mast cell chymase at 10 ng/ml concentration caused a significant increase in P_{alb} within 10 min (0.48 ± 0.14, $N = 15$, $p < 0.05$ vs. control 0.02 ± 0.065, $N = 25$), with a trend towards increasing P_{alb} with longer incubation times (15 min: 0.67 ± 0.056, $N = 15$; 20 min: 0.55 ± 0.028, $N = 15$; 25 min: 0.74 ± 0.075, $N = 15$; 30 min: 0.8 ± 0.083, $N = 20$, Fig. 2). To determine progressive effect in these short time periods, we performed "Analysis of trends" using ANOVA and found that MCC-mediated increase in P_{alb} after 30 min of incubation was significantly higher compared to increase in P_{alb} after 10 min of incubation.

PAR-2 mediates the increased P_{alb} caused by MCC

MCC-mediated cellular functions are mediated through activation of PARs [17, 18]. In order to determine if PARs are involved in MCC-induced increase in P_{alb}, we pre-incubated glomeruli with medium alone, PAR-1 or PAR-2 blocking antibodies, or normal mouse isotype IgG2a and

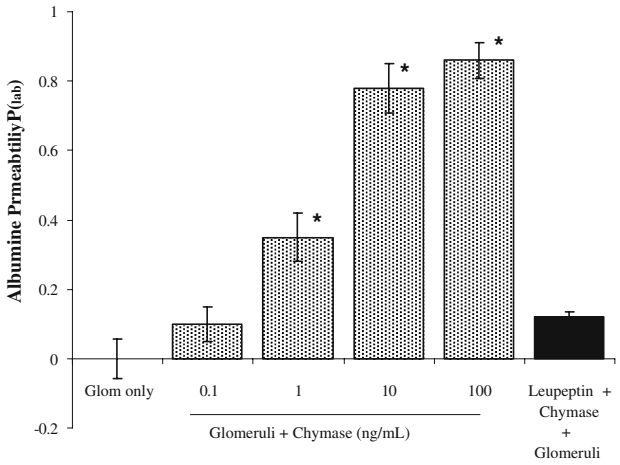

Fig. 1. MCC increases glomerular albumin permeability (P_{alb}) in a dose-dependent manner. Isolated rat glomeruli were incubated with various concentration of MCC (0.1, 1, 10, and 100 ng/ml) for 30 min at 37°C. MCC caused a significant increase in P_{alb} in a dose dependent manner. Pre-incubation with a non-specific protease inhibitor, Leupeptin (10 μg/ml) significantly prevented MCC (10 ng/ml) mediated increase in P_{alb}. Untreated glomeruli (glom only) were used as control Values for P_{alb} are expressed as mean ± SEM. *, $p < 0.001$ vs. glomeruli only control.

after washing unbound antibodies incubated glomeruli further with10 ng/ml of MCC and determined P_{alb}. Pre-incubation of glomeruli with PAR-2 antibodies prevented MCC to induce increase in P_{alb} (0.07 ± 0.14, $N = 15$, not different from control 0.006 ± 0.086, $N = 14$), whereas pre-incubation with medium alone, anti-PAR-1 antibody or isotype IgG2a did not prevent MCC to induce increase in P_{alb} (0.78 ± 0.12, $N = 15$; 0.70 ± 0.06, $N = 15$; 0.68 ± 0.14, $N = 15$ vs. control 0.006 ± 0.086, $N = 14$, respectively,

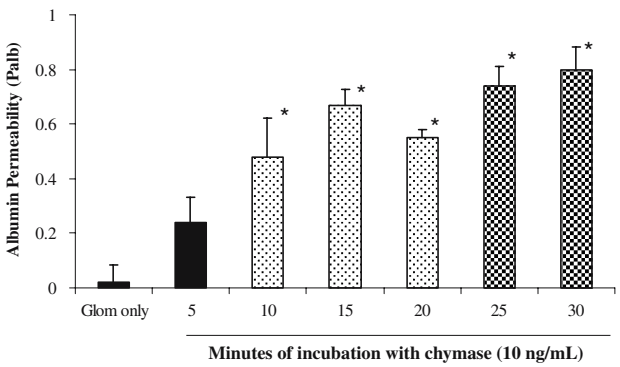

Fig. 2. MCC increases glomerular albumin permeability (P_{alb}) in a time-dependent manner. Isolated rat glomeruli were incubated with 10 ng/ml of MCC for 5, 10, and 30 min at 37°C. MCC caused a significant increase in P_{alb} within 15 min and this effect was further increased at 25 and 30 min of incubation. Untreated glomeruli (glom only) were used as control. Values for P_{alb} are expressed as mean ± SEM. *, $p < 0.001$ vs. glomeruli only control.

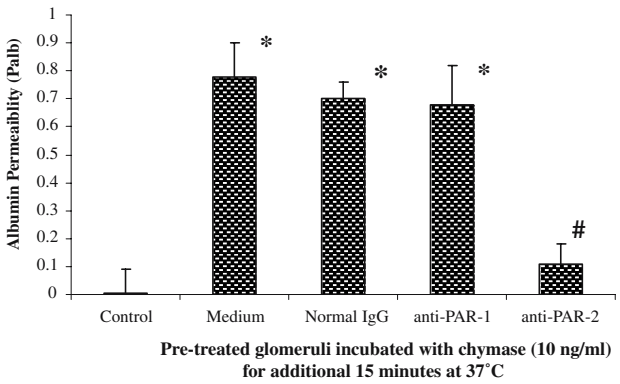

Fig. 3. Role of PAR isoforms in MCC-mediated increase in P_{alb}. Isolated rat glomeruli were pre-incubated with medium alone (control), normal mouse IgG (5 μg/ml), anti-PAR-1 antibody (5 μg/ml), or anti PAR-2 antibody (5 μg/ ml) for 60 min at 4°C. Glomeruli were washed thrice with isolation medium and further incubated with MCC (10 ng/ml) for additional 15 min at 37°C. MCC caused a significant increase in P_{alb} within 15 min in glomeruli pre-treated with medium alone, normal mouse IgG2a or anti-PAR-1 antibody, whereas it had no effect on glomeruli pre-treated with anti-PAR-2 antibody. Values for P_{alb} are expressed as mean ± SEM. *, $p < 0.001$ vs. control; #, $p < 0.001$ vs. medium, normal mouse IgG2a and anti-PAR-1 antibody.

Fig. 3). These results document that MCC acts by binding to PAR-2 binding sites.

Expression of PAR-1, PAR-2, PAR-3, and PAR-4 in rat glomeruli

After documenting that the MCC-induced increase in P_{alb} is abrogated by blocking PAR-2 binding sites, and that direct activation of PAR-2 by MCC can increase P_{alb}, it is important to document whether glomeruli express PARs and that only specific PARs participate in this process. We therefore, performed RT-PCR, to determine the expression of PAR-1, PAR-2, PAR-3, and PAR-4 in isolated glomeruli. PAR-1, -2, and -3 (Fig. 4, lanes 1–3, respectively) are strongly expressed compared to PAR-4 (Fig. 4, lane 4).

In order to further elucidate the role of PAR isoforms in P_{alb}, glomeruli were incubated with either PAR-1 or PAR-2 activating hexapeptides or with the inert peptides those lacked consensus sequences but retained same amino acid composition. These hexapeptides can directly bind and activate PARs and thus are useful reagents for investigating receptor functions without the use of proteases. We incubated glomeruli with active PAR-1 (TFLLRN) or PAR-2 (SLIGKV) or inactive scrambled synthetic peptides for PAR-1 (LFTNRL) or for PAR-2 (ILSVKG), and measured their effects on $P_{alb.}$ As shown in Fig. 5, PAR-2 agonist SLIGKV increased P_{alb} (0.68 ± 0.04, $N = 15$ vs. control 0.04 ± 0.058, $N = 20$, $p < 0.001$), whereas the PAR-1 agonist

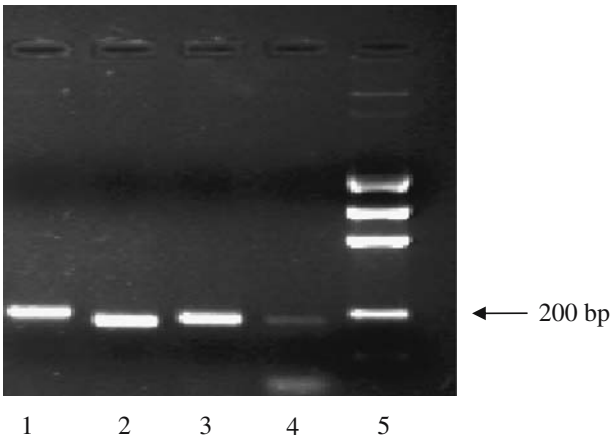

Fig. 4. Qualitative expression of PARs in rat glomeruli by RT-PCR. Normal rat glomeruli expressed relatively higher amount of mRNA for PAR-1, PAR-2, and PAR-3 (lanes 1–3, respectively), compared to PAR-4 (lane 4). Lane 5 is the standard ladder.

TFLLRN and the control peptides did not increase P_{alb} (PAR-1 active, 0.12 ± 0.07, $N = 15$; PAR-1 inactive, 0.02 ± 0.065, $N = 15$; PAR-2 inactive, 0.11 ± 0.07, $N = 15$, respectively).

Discussion

In the report, we have demonstrated a direct effect of chymase on the protein permeability barrier of the glomerulus that is independent of alterations in hemodynamic factors or effects

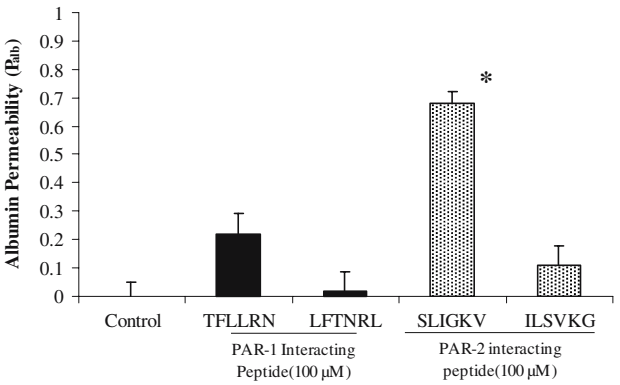

Fig. 5. Direct activation of PAR-2 increases glomerular albumin permeability (P_{alb}). Isolated rat glomeruli were incubated with 100 μM concentration of PAR-1 activating peptide TFLLRN or PAR-2 activating peptide SLIGKV for 30 min at 37°C and P_{alb} was measured. Peptides lacking consensus sequences LFTNRL (PAR-1) and ILSVKG (PAR-2) were used as controls. PAR-2 active peptide (SLIGKV) caused a significant increase in P_{alb}, whereas PAR-1 active, inactive and PAR-2 inactive, peptide had no effect on P_{alb}. Values for P_{alb} are expressed as mean ± SEM. *, $p < 0.001$ vs. control.

of recruited inflammatory cells. Chymase concentration of 10 ng/ml and a short incubation period of 15 min were sufficient to increase P_{alb} *in vitro*. MCC-induced increase in P_{alb} was blocked by antibodies against the ligand binding sites of PAR-2, indicating that PAR-2 receptors are involved in the regulation of glomerular permeability to albumin. These novel results are first to implicate PAR-2 as the mediator of the effect of MCC on glomerular filtration barrier function. In addition, direct activation of PAR-2 by specific PAR-2 activating hexapeptide also resulted in an increase in P_{alb} confirming their role in increased albumin permeability. We have also demonstrated the mRNA expression and relative abundance of PAR-1, -2, -3, and -4 in rat glomeruli. Though qualitative in nature, to our knowledge this is the first evidence which documents mRNA expression and relative abundance of all four PARs in rat glomeruli. Detailed quantitative analysis of presence of all four PAR types in glomeruli at genomic and protein levels is currently under investigation in our laboratory.

An association between increased infiltration of the kidney by mast cells and development of primary or secondary glomerulonephritis in a variety of human renal diseases has been established [3–11]. Activation and degranulation of mast cells result in secretion of a wide variety of inflammatory mediators including histamine, cytokines, chemokines, leukotrienes, prostanoids, TGF-β1, and neutral serine protease [30]. Based on their capacity to secrete neutral serine proteases, human mast cells have been characterized into three subtypes; (a) MC_T containing only tryptase, (b) MC_{TC} containing both tryptase and chymase, and (c) MC_C containing only chymase [30, 31]. The MC_T phenotype is believed to play a role in host defense, whereas the MC_{TC} phenotype seems to represent cells involved in non-immune system-related fibrosis [19, 31]. Though it has been speculated that mast cell tryptase may play a role in renal interstitial fibrosis its role in proteinuria has not been established [32]. In this study, we have focused our attention only on chymase and its effect on glomerular filtration barrier function.

Mast cell chymase is a distinct enzyme within the larger family of serine proteases, which is synthesized as an inactive zymogen, secreted as pro-enzyme and activated immediately upon release into the extracellular matrix [33]. In mammals this enzyme exists in two isoforms (α and β), which differ in species and functions [34, 35]. Humans synthesize only α-MCC [35], while dogs, rats, and mice synthesize both α and β-MCC forms [36]. MCC plays a significant role in ANG II generation by an "alternate" pathway. It has been suggested that at least 40% of ANG I to ANG II conversion may be MCC-mediated [34, 37, 38]. Tokuyama *et al*. documented a role for MCC in the generation of intrarenal ANG II in clipped kidney ischemic injury in dogs, whereas ACE-mediated ANG II generation was responsible for elevated ANG II contents in non-clipped kidneys [38]. MCC has also been shown to cleave big endothelin to produce 31-amino acid endothelin that might play a role in the progression of ANG II independent renal pathological changes [39]. MCC expression has been shown to be markedly up-regulated in glomeruli of diabetic and hypertensive kidney [7, 20], and in renal fibrosis in rejected grafts [19] and IgA nephritis [5].

Most proteases including serine proteases act through G-protein-coupled membrane bound protease-activated receptors (PARs). Currently four types of PARs are known [40–43]. Thrombin activates PAR-1, -3 and -4 [15], while trypsin and mast cell proteases activate PAR-2 and possibly PAR-4 [18, 16]. Mast cell proteases acting through PAR-2 activate renal resident cells resulting in the induction of TGF-β expression and matrix deposition in IgA nephropathy [44]. Similarly PAR-2 activating peptide or trypsin acting through PAR-2 has been shown to stimulate chloride secretion by Ca^{2+}-activated chloride channels in cultured M-1 mouse cortical collecting duct cells [45].

Proteases such as MCC, trypsin, or thrombin act as agonists for PARs and activate the receptor in a unique manner. Activation of PARs by proteases involves cleaving extracellular N-terminus of the trans-membrane pro-receptor, exposing a new N-terminus that functions as a tethered peptide ligand which then binds intra-molecularly to the cleaved receptor and activates it [46, 47]. This finding led to the development of synthetic peptides to study the activation of PARs. Synthetic peptides corresponding to the tethered ligands of PARs directly bind and activate the receptors and are useful reagents for investigating receptor functions. These short peptides serve to activate receptors without the use of proteases, which may have non-specific effects [28, 29]. Scrambled peptides of similar lengths are used as negative controls in these studies. Thus, a highly specific short chain peptide can substitute for each of the agonist proteinases that activate PARs.

The distinctive features of mast cell proteases and PARs have resulted in extensive studies on their function as important regulatory molecules. A recent review by Ritz *et al*. has emphasized the importance of MCC in nephropathy and why understanding the functional activity of this serine protease is important in acute and chronic renal diseases in general and specifically in diabetic nephropathy [7]. A better understanding of the role of mast cells and MCC in acute and chronic renal diseases is needed. Although several important questions remain unresolved, understanding the role of MCC and its receptor(s) in the regulation of the glomerular filtration barrier function is of great interest to us. The P_{alb} assay has permitted us to study the direct effect of on the filtration barrier. This sensitive *in vitro* assay is based on the use of freshly isolated rat glomeruli [27] that maintain an intact filtration barrier consisting of endothelial

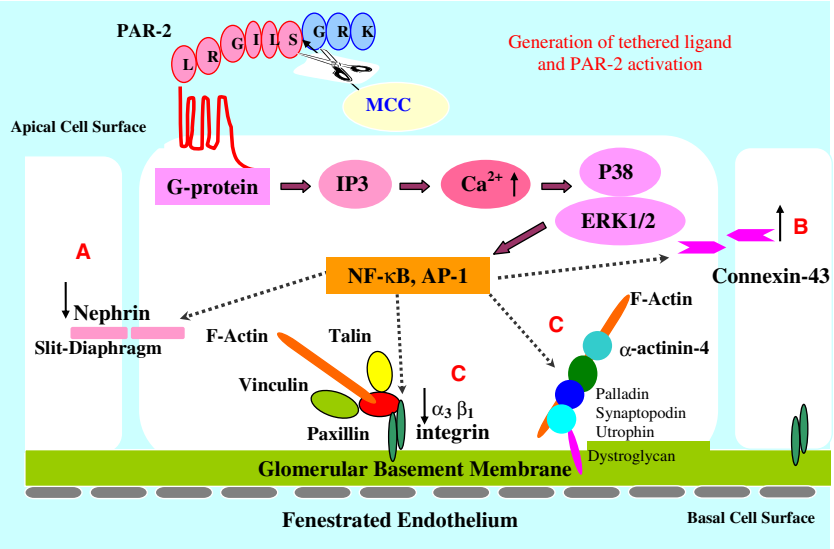

Fig. 6. Chymase mediated increase in podocyte macromolecular permeability via PAR-2: possible mechanisms involving intracellular calcium, ERK1/2, and NF-κB/AP-1 that result in increased permeability at (A) slit-diaphragm involving nephrin, (B) gap junctions involving connexin-43, and (C) cell body-basement membrane adhesions involving $\alpha_3\beta_1$-integrin and α-actinin-4 molecules.

cell, GBM, epithelial cells (GEC), and the negatively charged glycocalyx. The integrity of each of these structural components is required to study filtration barrier function [48]. We have used this assay extensively to study direct effect of various cytokines, reactive oxygen species and inflammatory and non-inflammatory mediators on glomerular macromolecular permeability [21–23, 49, 50].

Results of the current studies demonstrate expression of PARs in glomeruli and are first to show a direct effect of MCC on the glomerular protein permeability barrier. Incubation with MCC results in rapid and severe impairment of glomerular macromolecular permeability barrier (Fig. 1). A role for PAR-2 in the MCC-mediated increase in P_{alb} is indicated by the finding that blocking PAR-2 using anti PAR-2 antibodies prevented the MCC-induced increase in P_{alb} and that direct activation of PAR-2 resulted in increased P_{alb}. Though further studies are required to identify which glomerular cells participate in MCC-induced regulation of the glomerular filtration barrier function, we hypothesize that MCC-mediated increase in P_{alb} involves podocytes, major controller of the macromolecular permeability.

We speculate that MCC binds, cleaves, and activates PAR-2 receptors on podocytes. Activation of PAR-2 receptor increase intracellular calcium that in turn activates MAP Kinase (ERK1/2, P38) through phosphorylation. Activated MAP kinase most likely through transcription factors then effects cell–cell (nephrin, and connexin-43) and cell–matrix interaction molecules ($\alpha 3\beta 1$-integrin) at genomic and protein levels along with cytoskeleton proteins (F-actin and the actin associated protein, paxillin, and α-actinin-4) thereby modulating podocyte-controlled permeability of the glomerular barrier to macromolecular permeability (Fig. 6).

We postulate that proteinuria and possibly glomerulosclerosis are related to the presence of elevated MCC under pathological conditions. In light of our findings, MCC activity is a potential therapeutic target. Strategies may be aimed at altering the MCC activity, decreasing its production and/or blocking its binding to and activation of PARs, specifically PAR-2.

Acknowledgments

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