Novel Serpinopathy in Rat Kidney and Pancreas Induced by Overexpression of Megsin

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The intracellular polymerization of abnormal serine protease inhibitors (serpins) results in liver or neuronal cell abnormalities recently identified as “serpinopathies.” It was demonstrated in transgenic rats that overexpression of megsin, a recently discovered serpin located in the kidney, produces renal and pancreatic lesions characteristic of serpinopathies. Megsin expression is elevated in a variety of organs, including kidney and pancreas. Periodic acid-Schiff-positive, diastase-resistant intracellular inclusions develop only in the kidney and the pancreas. They correspond to electron-dense deposits, shown to contain megsin by immunohistochemistry and immuno-electron microscopy. In the kidney, inclusions are located mainly in the endoplasmic reticulum of glomerular epithelial, distal, and collecting duct cells, and are associated with massive proteinuria and an impaired renal function. In the pancreas, similar inclusions are found in the exocrine and Langerhans islet cells, where islet β cells are reduced as a result of apoptosis. They are associated with diabetes with low insulin levels. The animals have an impaired growth and die within 10 wk. Rats that overexpress a mutant megsin, characterized by a deficient conformational transition activity, do not develop the serpinopathy, suggesting that some conformational flexibility of the serpin is required for the development of serpinopathy. This model of serpinopathy is the first to involve the kidney and the pancreas.


SeRINE PROTEASE INHIBITORS (SERPINS) FORM A SUPERFAMILY OF PROTEINS THAT REGULATE SEVERAL PHYSIOPATHOLOGIC PROCESSES, INCLUDING INFLAMMATION AND COAGULATION, AND PLAY CRITICAL ROLES IN CELLULAR FUNCTIONS, SUCH AS APOPTOSIS AND ANGIOGENESIS. THEY SHARE A COMMON STRUCTURE, COMPOSED OF THREE β SHEETS (A THROUGH C) AND AN EXPOSED MOBILE REACTIVE LOOP THAT PRESENTS A PEPTIDE SEQUENCE AS A PSEUDOSUBSTRATE FOR THE TARGET PROTEASES. THE REACTIVE LOOP/β SHEET A INTERACTION OF SERPINS IS CRUCIAL FOR THEIR ANTIPROTEASE EFFECTIVENESS. MODIFICATIONS OF ITS CONFORMATIONAL TRANSITIONS CAN CAUSE DISEASES, RECENTLY CATEGORIZED AS SERPINOPATHIES (1,2).

α1-Antitrypsin (α1AT) deficiency, a representative serpinopathy, predisposes humans to chronic liver disease. The mutated α1AT (α1ATZ) molecule is retained in the endoplasmic reticulum (ER) and, through a gain of function mechanism, triggers a series of eventually hepatotoxic events (3–6). The mutation opens the β sheet A and favors the insertion of the reactive loop of a second α1AT to form first a dimer and subsequently polymers that tangle in the ER as α1ATZ inclusion bodies in the liver (7–9). A similar process of polymerization is involved in another human serpinopathy, familial encephalopathy with neuroserpin inclusion bodies in the neurons (10).

We previously cloned and characterized a novel member of the serpin superfamily, megsin, from cultured human mesangial cells (11). To define better its characteristics, we developed a rat model with genetic overexpression of megsin. Unexpectedly, we discovered that this experimental model had all of the characteristics of a new serpinopathy. Elevated megsin expression was documented in several organs, but typical intracellular periodic-acid–Schiff (PAS)-positive, diastase-resistant, electron-dense megsin inclusions, characteristic of a serpinopathy, developed only in the kidney and the pancreas together with biologic renal and pancreatic dysfunction.

Materials and Methods
Megasin Transgenic Rat

The entire coding sequence of human megsin cDNA was ligated in the sense orientation with 3′ region of cytomegalovirus enhancer and
chicken β-actin promoter in pBS-CAG-2 (12). The megsin transgene was microinjected into one pronucleus of fertilized Wistar rat eggs, followed by transfer into the oviducts of pseudopregnant rats (13). For production of mutant megsin transgenic rat, the site-directed mutagenesis at the position of P14 (substitution of Thr for Arg at 334 residue) in the reactive loop of human megsin gene was performed using a standard two-step megaprimer PCR with mutated oligonucleotides. The mutant megsin (megsinT334R) transgene in the pBS-CAG-2 was microinjected into Wistar rat eggs. Animals were treated in accordance with the guidelines of the Committee on Ethical Animal Care and Use of Tokai University.

Northern Blot Analysis
Total RNA was isolated from snap-frozen tissues using ISOGEN (Wako Pure Chemicals, Osaka, Japan). RNA (20 μg) was electrophoresed on a 1% agarose-formaldehyde gel, followed by capillary transfer and hybridization using vector-specific fragment Bgl II and BamHI digested fragment of poly-A signal in pBS-CAC-2– or β-actin–specific fragment as a probe.

Western Blot Analysis
Tissue samples were homogenized in 100 μl of 0.35 M Tris-HCl (pH 6.8) that contained 10% SDS, 36% glycerol, 5% β-mercaptoethanol, and 0.012% bromophenol blue. Meigsin protein was detected by rabbit anti-human megsin peptide IgG (P2: 10 μg/ml) (14) and alkaline phosphatase-conjugated goat anti-rabbit IgG (Cappel, Durham, NC). Purified recombinant human megsin expressed in Chinese hamster ovary (CHO) cells (14) and anti-actin antibody (Sigma, St. Louis, MO) was used.

Biochemical and Immunologic Analyses of Serum and Urine Samples
Serum and urine were analyzed by a Biochemical autoanalyzer DRI-CHEM 3500V (Fujifilm, Tokyo, Japan) and a Hitachi autoanalyzer 7170 (Hitachi High-Technologies Corp., Tokyo, Japan), respectively.

Histopathology
Tissues were fixed in 10% neutral-buffered formaldehyde, embedded in paraffin, and sectioned at 4-μm thickness, followed by PAS or hematoxylin eosin staining. In some experiments, the section was treated with 0.1% diastase (Wako) at 37°C for 1 h.

Electron Microscopy
Tissues were fixed with 4% paraformaldehyde, 0.1 M sodium phosphate (pH 7.4) that contained 2.5% glutaraldehyde, and 1% osmium
tissues were counterstained with hematoxylin.

In Situ Hybridization Analyses

Transgene expression was assessed in glomeruli of megsin transgenic animals by in situ hybridization with the antisense oligonucleotide probe (5'-AGC GTG GAC TGA GGG AGT TGC TTT TCT ACA ATA TTA CT-3'), which corresponded to 1388 to 1425 of human megsin cDNA (GenBank accession no. NM 003784). A total of 100 pmol of the oligonucleotide probe was labeled with a digoxigenin oligonucleotide tailing kit (Boehringer Mannheim, Mannheim, Germany), and mRNA was detected as described previously with minor modifications (17).

Statistical Analyses

Data were expressed as means ± SD. ANOVA was used to evaluate the statistical significance of various differences. When the analysis detected a significant difference, the Scheffe $t$ test was used to compare results obtained from the experimental animals. In the TUNEL assay, the statistical difference was evaluated by the Scheffe $t$ test. The difference in the prevalence of renal and pancreatic dysfunctions between wild-type and mutated megsin transgenic rats was analyzed by the Fisher exact probability test. Values are considered significant at $P < 0.05$.

Results

Generation of Megsin Transgenic Rats

Of 61 putative founders, seven had megsin transgene detected by PCR analysis of the genomic DNA. Two independent lines (lines K and L) were established. By Northern blot analysis in line K (Figure 1A), megsin was ubiquitously expressed, but expression levels differed among organs: Expressions were relatively high in the heart, kidney, and pancreas; moderate in the lung; and low in the brain and liver (Figure 1B). Again, protein expression was higher in line K than in line L rats (data not shown).

Growth, Survival, and Biologic Characteristics of Megsin Transgenic Rats

Homozygotes failed to gain weight (Figure 2A) and died within 10 wk (Figure 2B). Heterozygotes, by contrast, had normal growth curves and survival rates, at least during the first 10 wk. Biologic data obtained in experimental animals aged 6 to 9 wk are given in Table 1. Homozygotes but not heterozygotes had clear evidence of renal and pancreatic dysfunction, as shown by a nephrotic syndrome (proteinuria, hypoproteinemia, and elevated cholesterol levels) with deterioration of renal function and hyperglycemia with insulin deficiency. Phenotypes were independent of sex.

Renal Pathology

Numerous large PAS-positive globules were observed within the cytoplasm of glomerular epithelial cells, distal tubular cells, and collecting ducts in 8-wk-old homozygotes of line K (Figure 3A). Similar but smaller PAS-positive granules were present in some proximal tubular cells. We did not detect mesangial proliferation, capillary loop thickening in the glomeruli, tubulointerstitial fibrosis, or infiltration of inflammatory cells. In heterozygotes, a significantly smaller number of PAS-positive inclusions with essentially the same distribution pattern were observed.

On electron microscopy, huge electron-dense inclusion bodies were noticed in podocytes (Figure 3B), distal tubular cells, and collecting ducts of homozygotes. They were located in the dilated cisternae of rough ER. Glomerular basement membrane...
and foot processes of podocytes remained intact. Quantitative analysis of foot process effacement also revealed that the arithmetic mean width of the foot process in wild-type rats (0.31 ± 0.68 μm) was not different from that in homozygotes (0.32 ± 0.01 μm). In homozygotes, a significantly lower number of smaller electron-dense inclusions were found with the same distribution pattern. The PAS-positive inclusions were characterized further by immunohistochemistry. The large inclusions in glomerular and tubular epithelial cells reacted with an anti-human megsin polyclonal antibody (Figure 3C). Their size and distribution were similar to those of the PAS-positive granules. The intracellular localization of megsin was evaluated further by immunoelectron microscopy with a rabbit anti-human megsin IgG peptide and a protein A-gold solution. Gold particles were detected in the huge electron-dense homogeneous inclusion bodies within the dilated rough-surfaced ER of podocytes and epithelial cells of proximal and distal tubules (Figure 3D). The intracellular PAS-positive inclusions proved to be diastase resistant (Figure 3E).

A TUNEL assay of the kidney of wild-type and homozygous rats detected no apoptotic cells in glomeruli of any rat strain (Figure 4). Apoptosis was also negligible in the tubular cells of wild-type animals, but, by contrast, TUNEL-positive, presumably apoptotic tubular cells were observed in homozygous transgenic rats (Figure 4, right). The number of TUNEL-positive tubular cells per high-power field averaged 2.3 ± 0.6 in homozygous transgenic rats and 0.5 ± 0.3 in wild-type rats (P < 0.05).

No correlation was found (P > 0.1) between blood glucose levels and blood urea nitrogen as a parameter of the kidney damage. Furthermore, homozygotes that were treated with insulin to maintain normal blood glucose levels developed similar renal phenotypes (n = 2; data not shown).

**Pancreas Pathology**

In homozygotes, PAS-positive inclusions similar to those of the kidney were observed both in acinar cells and in Langerhans islet cells. In addition, the number of islet β cells was significantly reduced (Figure 5A). The inclusions were diastase resistant (Figure 5B). In homozygotes, smaller PAS-positive inclusions were present in lower number, in the acinar cells and in the Langerhans islet cells. The latter cells were relatively better preserved than in homozygotes.

On electron microscopy, huge electron-dense inclusion bodies were noticed in the acinar cells of homozygotes (Figure 5C). They were located in the dilated cisternae of rough ER and nuclear envelope. Apoptotic bodies including pyknotic nuclei and condensed cell organelae were present. In homozygotes, a significantly lower number of smaller electron-dense inclusions were observed in the pancreas.

Immunohistochemical studies demonstrated that the PAS-positive inclusions were composed of megsin (Figure 5D), whereas immunoelectron microscopy revealed megsin within dilated cisternae of rough-surfaced ER in the acinar cells (Figure 5E).

The TUNEL assay failed to detect apoptotic cells in the pancreas of wild-type animals. In homozygotes, by contrast, TUNEL-positive acinar cells were present (Figure 6). We also observed TUNEL-positive β cells in the islets, although their number was very low as a result of disappearance of the cells. Quantitative analysis showed that the number of TUNEL-positive acinar cells per high-powered field was 5.2 ± 0.8 in transgenic rats and 0.3 ± 0.2 in wild-type rats (P < 0.001).

**Other Organ Pathology**

In contrast to the kidney and the pancreas, PAS-positive inclusions were only rarely detected in the lung of homozygotes (Figure 7A). Liver structure was intact, but glycogen

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**Table 1. Biochemical analyses of blood and urine from the experimental animals**

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type Rat (n = 14)</th>
<th>Megsin Transgenic Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heterozygotes (n = 11)</td>
<td>Homozygotes (n = 12)</td>
</tr>
<tr>
<td>Age (wk)</td>
<td>7.4 ± 1.2</td>
<td>7.1 ± 0.9</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total protein (g/dl)</td>
<td>6.06 ± 0.68</td>
<td>5.73 ± 0.60</td>
</tr>
<tr>
<td>blood urea nitrogen (mg/dl)</td>
<td>15.6 ± 1.8</td>
<td>17.9 ± 4.5</td>
</tr>
<tr>
<td>creatinine/body wt (mg/dl per kg)</td>
<td>1.0 ± 0.4</td>
<td>1.0 ± 0.4</td>
</tr>
<tr>
<td>glucose (mg/dl)</td>
<td>139 ± 31</td>
<td>163 ± 24</td>
</tr>
<tr>
<td>insulin (ng/ml)</td>
<td>3.6 ± 2.8</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>total cholesterol (mg/dl)</td>
<td>77.8 ± 12.2</td>
<td>75.7 ± 8.1</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total protein (mg/d)</td>
<td>3.8 ± 3.6</td>
<td>4.4 ± 2.9</td>
</tr>
<tr>
<td>total protein/body wt (mg/d per kg)</td>
<td>15.9 ± 12.6</td>
<td>30.3 ± 19.7</td>
</tr>
<tr>
<td>creatinine clearance (ml/min)</td>
<td>0.41 ± 0.45</td>
<td>1.65 ± 1.21</td>
</tr>
</tbody>
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*P < 0.05 versus wild-type or heterozygotes rats.
*P < 0.001 versus wild-type or heterozygotes rats.
*Below the detection limit.
storage in hepatocytes was markedly decreased (Figure 7B). No pathologic changes were noticed in the other tissues on light microscopy (data not shown). On electron microscopy, neither electron dense deposits nor dilated ER lumen were found in the liver and heart.

**Overexpression of a Mutated Megsin in Transgenic Rats**

MegsinT334R has a mutation at the position of P14 (corresponding to the proximal hinge region of the reactive loop), with a resultant abolishment of its serine protease inhibitory activity (our unpublished observation). The rigidity of the mutated hinge region might account for the loss of inhibitory activity and could also hinder polymerization of the transgene products. To test this hypothesis, we produced megsinT334R transgenic rats. Only heterozygotes were available. Whereas expression levels of the transgene in the kidney and the pancreas were similar in wild-type and mutant megsin heterozygote transgenic rats (data not shown), PAS-positive inclusions were not detectable by microscopic analysis in either the kidney or the pancreas from two different lines of mutant megsin heterozygotes at the age of 20 wk ($n = 40$ for each line; Figure 8). By contrast, they were observed in all wild-type megsin heterozygotes examined at the same age. MegsinT334R heterozygotes had a normal growth and exhibited no blood and urine abnormalities suggestive of renal or pancreas dysfunction, whereas, by contrast, 13.3% (11 of 83 for line K) of the wild-type megsin heterozygotes presented such abnormalities at the age of 20 wk ($P < 0.05$).

**In Situ Hybridization Analyses in the Transgenic Animals that Overexpress Megsin**

Expression patterns of the transgene were assessed in the glomeruli by *in situ* hybridization. Results were compared in

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*Figure 3. Pathologic change of the kidney. (A) Periodic acid-Schiff (PAS) staining of a wild-type and megsin transgenic rats (heterozygote and homozygote) at the age of 8 wk. Numerous, large, PAS-positive globules were observed within the cytoplasm of glomerular epithelial cells, distal tubular cells, and collecting ducts. (B) Electron microscopic analysis of the glomerulus of a megsin transgenic rat (homozygote). Note the presence of electron-dense inclusion bodies (arrow) in rough endoplasmic reticulum (ER). Foot processes of podocytes remained intact. (C) Immunohistochemical detection of human megsin in glomeruli of a wild-type (left) and megsin transgenic rat (right) at the age of 8 wk. (D) Immunoelectron microscopic analysis of human megsin in podocytes of a megsin transgenic rat at the age of 8 wk. (E) PAS staining pretreated with (right) or without (left) diastase of a megsin transgenic rat (homozygote) at the age of 8 wk. The intracellular PAS-positive inclusions proved diastase-resistant. Magnification, $\times 200$ in A, homozygote and a wild-type rat, and $\times 600$ in a heterozygote rat; $\times 2500$ in B; $\times 200$ in C; $\times 10,000$ in D; $\times 200$ in E.*
wild-type and mutated-megsin transgenic rats as well as in wild-type megsin transgenic mice.

Despite very different phenotypes among the various transgenic strains and species, expression pattern was ubiquitous in glomerular cells and essentially the same in the rats that overexpressed mutated-megsin, megsinT334R, and wild-type megsin and in the mice that overexpressed wild-type megsin (Figure 9).

**Discussion**

We provide the first example of a serpinopathy involving both the kidney and the pancreas. This experimental serpinopathy develops in transgenic rats that overexpress megsin, a novel, recently identified serpin. It is characterized by the cellular accumulation of megsin in both organs, forming PAS-positive, diastase-resistant inclusions on light microscopy and electron-dense deposits within a dilated ER on electron microscopy. The presence of megsin is demonstrated further by immunohistochemistry and immunomicroscopy. In homozygotes, growth is impaired and premature death ensues. Development of both morphologic and functional disorders in two independent transgenic lines (lines K and L) excludes effects of the site of integration of the transgene and confirms that the manifestations are transgene specific. The disease is more severe in line K than in line L, probably as a result of dose-dependent effects of megsin: Northern blot analysis shows more abundant mRNA levels in line K than in line L transgenic rats. A similar dose dependence has been observed in other serpinopathies (4,7,18,19).

In the kidney, megsin accumulates within glomerular epithelial cells, distal tubules, and collecting ducts, but only tubular cells show apoptotic changes. Renal functional abnormalities including renal failure and a nephrotic syndrome with hypoproteinemia and hyperlipidemia ensue. Whether similar disorders can be encountered in humans remains to be determined, because up to now, no renal serpinopathy has yet been described.

In the pancreas, megsin accumulates within exocrine and endocrine cells. In the exocrine cells, apoptosis ensues without detectable functional consequences. In the β cells, apoptosis also ensues, but by contrast, β cells disappear with attendant diabetes with low insulin levels, hyperglycemia, and glucosuria. The observed renal lesions clearly differ from those of diabetic nephropathy. Furthermore, severity of the renal phenotype is not correlated with blood glucose levels, and insulin treatment fails to improve renal dysfunction. Altogether these observations incriminate megsin overexpression per se in the genesis of renal lesions, although a potential contribution of high blood glucose cannot be formally ruled out.

Megsin is also overexpressed in other organs, without globule formation and cellular disappearance. Extremely small PAS-positive inclusions were exceptionally identified in the lung. There were no apparent abnormalities in heart and lung function. Liver intracellular glycogen was strikingly reduced, and in several homozygotes, serum levels of transaminases increased markedly but the rise failed to reach statistical significance for the whole group. The relationship between these liver abnormalities remains to be elucidated.

Why is megsin overexpression toxic only for the kidney and the pancreas? One explanation might relate to higher intracellular concentrations of megsin in these organs. All homozygotes have a high megsin expression as well as disease manifestations that prove lethal within 10 wk. By contrast, heterozygotes have lower megsin expression levels and only 13.3% of them develop an impaired growth and biologic abnormalities at the age of 20 wk. Clearly, lower megsin expression delays and reduces renal and pancreatic involvement. A relationship between cellular damage and the degree of serpin expression has been documented in other serpinopathies and incriminated in the preferential involvement of either liver or neuronal cells (1). Still, on Northern blot analysis, performed only in heterozygotes, megsin expression in the heart equals that observed in kidney and pancreas, a finding confirmed by Western blot analysis in both homo- and heterozygotes. Nevertheless, PAS-positive inclusions are absent in the heart. Therefore, in addition to actual megsin levels, individual cellu-

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**Figure 4.** Transferase-mediated dUTP nick-end labeling (TUNEL) staining of the kidneys. Representative pictures of TUNEL staining of the kidneys derived from a wild-type (left) and a megsin transgenic homozygous rat (right) at the age of 8 wk. TUNEL-positive, apoptotic, tubular epithelial cells but not glomerular epithelial cells were observed in the transgenic rats. Magnification, ×200.
lar susceptibility must influence the eventual expression of the disease. Low susceptibility is unlikely related to high cellular turnover rate because turnover of heart muscle cells is certainly not higher than that of renal tubular cells.

All reported serpinopathies are characterized by a low or normal production of an abnormal serpin whose inadequate cellular handling leads to toxic accumulation. Megsin serpinopathy, by contrast, is caused by an increased synthesis of a normal protein, flooding the protein handling system, with accumulation and subsequent polymerization within the ER. The megsin transgene used to produce megsin transgenic rats contains a full sequence of the coding region of megsin, including a natural and an adequate signaling sequence to allow exit from the ER. In preliminary studies, we have shown that transfection of the full length of megsin cDNA into cultured mesangial cells induced the excretion of the transgene product in

Figure 5. Pathologic analysis of the pancreas. (A) PAS staining of a wild-type (left) and a megsin transgenic rat (right) at the age of 8 wk. PAS-positive inclusions are observed both in exocrine epithelial cells and in Langerhans islet cells of the homozygous rats. (B) PAS staining after treatment with (right) or without (left) diastase of tissues from a megsin transgenic rat (homozygote) at the age of 8 wk. The PAS-positive inclusions both in exocrine epithelial cells and in Langerhans islet cells proved diastase-resistant. (C) Electron microscopic analysis of a megsin transgenic rat. Electron-dense huge inclusion bodies were noticed in rough ER of the acinar cells. (D) Immunohistochemical detection of human megsin in acinar cells of a wild-type (left) and a megsin transgenic rat (right) at the age of 8 wk. (E) Immunoelectron microscopic analysis of human megsin in acinar cells of a megsin transgenic rat at the age of 8 wk. Magnification, ×400 in A; ×200 in B and D; ×2500 in C; ×10,000 in E.
the culture medium (data not shown), suggesting that the transgene was processed adequately in these circumstances. Thus, serpinopathies develop when the cell is unable to cope with a serpin not only when it is abnormal but also when it is overproduced. Preliminary data from our laboratory disclose that intact megsin aggregates \textit{in vitro} in a dose-dependent manner: Purified normal megsin (1 mg/ml) incubated in 20 mM acetate buffer (pH 4.0) at 37°C for 12 h polymerizes as detected by nondenaturing acid PAGE (pH 4.0) according to the methods reported elsewhere (20).

It is interesting that overproduction of a mutant megsin, lacking flexibility at the loop hinge region as well as protease inhibitory activity, does not result in a serpinopathy. Preservation of megsin function thus seems required for the development of disease manifestations in our transgenic model. The T334R mutation in the megsin gene might decrease its polymerization perhaps through an alteration of the loop mobility. Recent studies investigating mechanisms of polymerization of serpins suggest that a mutation in the hinge region indeed affects polymerization of some serpins (21,22). The hypothesis that polymerization and the subsequent accumulation of megsin is prevented by a conformational transition change in the hinge region of mutated megsin requires further investigations.

Just as for the kidney, no serpinopathy has yet been reported to affect the pancreas. Disappearance of β cells might be mediated by a mechanism analogous to that highlighted by Ron (23)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image1.png}
\caption{TUNEL staining of the pancreas. Representative pictures of TUNEL staining of the pancreas derived from a megsin transgenic (homozygote) rat at the age of 8 wk. TUNEL-positive, apoptotic, acinar cells (right). TUNEL-positive apoptotic β cells within rarefied Langerhans islets (left). Magnification, ×400 in right; ×200 in left.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image2.png}
\caption{PAS staining of the lung (A) and the liver (B) in a wild-type (left) and a homozygote megsin transgenic rat (right) at the age of 8 wk. PAS-positive inclusions were rare in both the lung and the liver of the megsin transgenic rat, whereas cellular structures remained intact. Glycogen storage in hepatocytes was markedly decreased. Magnification, ×200 in A; ×400 in B.}
\end{figure}
in diabetic Akita mouse, a counterpart of maturity-onset dia-
abetes of the young in human type 2 diabetes. In this condition,
the accumulation of polymers of the abnormal isoinsulin chain
results in eventual \( \beta \) cell apoptosis. The initiation of \( \beta \) cell
disappearance by an excessive production of a normal serpin
molecule extends the significance of this mechanism.

We reported previously that transgenic mice that overex-
press megsin develop a different phenotype that includes mes-
angial expansion and hypercellularity (12). The phenotypic dif-
ference between megsin transgenic mice and rats cannot be
attributed to different expression patterns but might reflect
different pathogenic mechanisms. Indeed, expression patterns
of the transgene revealed by \textit{in situ} hybridization were similar
in all tested strains and species. In mice, moderate overexpres-
sion of megsin might enhance the inhibition of its target pro-
teases with accumulation of whole proteins that are normally
degraded. Histologic abnormalities develop late, at the age of
40 wk. In rats, by contrast, the markedly higher (by one order
of magnitude) level of megsin results in early serpin polymer-
ization and cellular toxicity within \(<10\) wk. The difference
between mice and rats is reminiscent of the mechanisms impli-
cated in pulmonary emphysema and liver damage observed in
patients with \( \alpha_1 \) AT deficiency. Whereas a loss of function of
the serpin reduces the inactivation of various elastases with atten-
dant lung emphysema, a gain of function as a result of the
aggregation of serpin as a consequence of aberrant intermolec-
ular linkage results in liver dysfunction. In the transgenic mice,
the late development of pathologic changes in the mesangium
is time dependent as it requires accumulation of extracellular
matrix and proliferation of mesangial cells as a result of over-
functioning megsin. Thus, mesangial expansion and hypercel-
lularity result from an imbalance between proteases and a

\[\text{Figure 8. PAS staining of the kidney (left) and the pancreas (right) in a mutated megsinT334R transgenic rat (line D) at the age of}
20 wk. PAS-positive inclusions were not detectable. Magnification, \( \times 200 \).
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\[\text{Figure 9. \textit{In situ} hybridization analyses of transgenic animals overexpressing megsin. Expression pattern of the transgene, megsin or}
megsinT334R, in glomeruli of the transgenic animals (8 wk old) were compared. (A) A wild-type rat. (B) A heterozygote megsin
transgenic rat (line K). (C) A heterozygote megsinT334R transgenic rat (line D). (D) A wild-type mouse. (E) A heterozygote megsin
transgenic mouse (12). Transgene expression patterns in glomeruli were essentially the same in the transgenic animals overexpressing
either wild-type or mutated megsin. Magnification, \( \times 400 \).
\]
protease inhibitor, megsin, in analogy with lung emphysema observed in patients with α1,AT deficiency. In contrast, the serpinopathy observed in transgenic rats damages rapidly the ER as a result of polymerization of megsin, with attendant cellular dysfunction. It seems that the structural characteristics of megsin may be more important than megsin’s protease inhibitory properties. We speculate that the megsin transgenic rats fail to show mesangial expansion or hypercellularity because their short life expectancy prevents the consequences of an overfunction of megsin as a protease inhibitor.

The clinical relevance of our model requires further studies in the future. Of interest, although some patients with α1,AT deficiency develop membranoproliferative or mesangial proliferative glomerulonephritis, no reports have yet described characteristic inclusions in the glomeruli (24).

Accumulating evidence emphasizes an essential role of podocyte injury in proteinuria (25,26). This model is characterized by heavy proteinuria with eventual hypoproteinemia and hyperlipidemia. On electron microscopy, foot processes of the podocytes of megsin transgenic rats remain relatively intact despite the accumulation of megsin aggregates. This finding is not surprising because some patients with proteinuria, such as obstructive proteinuria (27), and certain animal models with heavy proteinuria (28,29) lack foot process effacement. Previous quantitative studies have also demonstrated a poor correlation between proteinuria and foot process effacement (30). Although undetected by TUNEL assays, podocyte apoptosis cannot be completely ruled out in the development of the proteinuria observed in transgenic rats, because apoptotic podocytes are readily washed off by the ultrafiltrate. Alternatively, the excessive accumulation of the transgene product may induce different responses in different cells, e.g., apoptosis in pancreatic cells but not in podocytes. In pancreatic cells, megsin may flood the capacity of the ER to deal with client proteins and induce stress responses, which are known to lead to apoptosis via activation of CHOP and caspase 12 in pancreatic cells (31,32). Whether this death pathway is inhibited in podocytes is an interesting topic to be pursued in the future.

Recent studies emphasize participation of podocytes in progression of kidney failure (25,33). Although accumulation of megsin in podocytes may have induced not only proteinuria but also a decline in renal function, we have found no histologic evidence of glomerulosclerosis in these animals. We cannot exclude a possible contribution of hemodynamic factors such as a decrease in effective circulating volume as a result of hypoproteinemia. The cause of the renal dysfunction in homozygotes remains to be elucidated.

Acknowledgments

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