Matrix Metalloproteinases and Their Inhibitors in Aqueous Humor of Patients with Pseudoexfoliation Syndrome/Glaucoma and Primary Open-Angle Glaucoma

Ursula Schlötzer-Schrehardt,1 Jürgen Lommatzsch,¹ Michael Küchle,¹ Anastasios G. P. Konstas,² and Gottfried O. H. Naumann¹

PURPOSE. To determine the presence, activity, and quantitative differences of matrix metalloproteinases (MMPs) and their endogenous inhibitors (TIMPs) in aqueous humor and serum samples of patients with pseudoexfoliation (PEX) syndrome, PEX glaucoma (PEXG), primary open-angle glaucoma (POAG), and cataract.

METHODS. Aqueous humor and serum samples were collected from 100 patients with PEX syndrome, PEX glaucoma (PEXG), POAG, and cataract, respectively. Levels of MMP-1, -2, -3, -7, -9, and -12 and TIMP-1 and -2 were determined by zymography, Western blot analysis, and specific immunoassays. Activity assay kits were used to quantitate levels of endogenously activated MMP-2 and -9.

RESULTS. MMP-2, -3, -7, -9, and -12 and TIMP-1 and -2 were identified in human aqueous humor samples from all groups of patients with a six to sevenfold molar excess of TIMPs over MMPs. Whereas serum samples showed no significant differences, total MMP-2 and -3 and TIMP-1 and -2 were detected at significantly higher concentrations in aqueous samples from PEX eyes with and without glaucoma compared with cataractous eyes. MMP-2 and -3 and TIMP-1 were also detected in higher, but not significantly different, amounts in aqueous samples of POAG eyes. However, levels of endogenously activated MMP-2 were significantly decreased in both PEX and POAG samples. The ratio of MMP-2 to its principal inhibitor TIMP-2 was balanced in cataract samples, but was decreased in samples from patients with PEXG, resulting in an excess of TIMP-2 over MMP-2.

CONCLUSIONS. The findings suggest that complex changes in the local MMP-TIMP balance and reduced MMP activity in aqueous humor may promote the abnormal matrix accumulation characteristic of PEX syndrome and may be causally involved in the pathogenesis of both PEX glaucoma and POAG. (Invest Ophthalmol Vis Sci. 2003;44:1117–1125) DOI:10.1167/iovs.02-0365

Pseudoexfoliation (PEX) syndrome is a clinically significant systemic disorder of the extracellular matrix,¹ ² which represents not only the most common identifiable cause of open-angle glaucoma³ but also a risk factor for cardiovascular disease.⁴ ⁵ Increasing evidence suggests that PEX syndrome is a type of fibrosis associated with the excessive synthesis and deposition of an abnormal elastic fibrillar matrix in many intra- and extracellular tissues.⁶ ⁷ Active involvement of the trabecular meshwork in this abnormal matrix process leading to progressive accumulation of PEX material in the juxtanaca-
licular tissue is considered to be the primary cause of chronic pressure elevation in eyes with PEX syndrome.⁸ ⁹ However, the mechanisms responsible for this aberrant matrix process remain unknown. Excessive production and accumulation of abnormal matrix components may be due to increased de novo synthesis, decreased turnover of matrix components, or both. The principal ocular cells implicated in PEX material produc-
tion are those closely associated with the aqueous humor circulation (i.e., nonpigmented ciliary epithelium, iris pigment epithelium, iridal vascular cells, equatorial lens epithelial cells, and trabecular endothelial cells)¹ ² and are thus influenced by the substances contained therein. The composition of the aqueous humor may therefore play an important role in influ-
cencing the matrix metabolism of adjacent tissues.

Similarly, an excessive accumulation of extracellular material in the juxtanacatalicular tissue of the meshwork has been postulated to cause an increased outflow resistance in eyes with primary open-angle glaucoma (POAG),¹⁰ ¹¹ and an im-
paired trabecular meshwork matrix turnover, which is critical to the regulation and maintenance of aqueous humor outflow, has been implicated in the development of POAG.¹² ¹³

Extracellular matrix turnover is mediated by matrix metalloproteinases (MMPs), a large family of endopeptidases with variable substrate spectra,¹⁴ ¹⁵ the presence of which has been described in human aqueous humor.¹⁶ ¹⁹ However, no quantitative studies of aqueous levels of MMPs and TIMPs have appeared in the literature. The activity of these enzymes is regulated in part by specific endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Dysregulated expres-
sion of MMPs and TIMPs has been implicated in many disease processes accompanied by abnormal matrix production, such as fibrotic disorders,²¹ ²⁵ and a variety of other disease states. In the eye, abnormal expression of MMPs has been implicated, among many other disorders, in proliferative vitreoretinopa-thy,²⁴ ²⁶ secondary cataract formation,²⁷ and the pathogenesis of pterygia.²⁸ ²⁹ Therefore, MMPs and TIMPs are likely candidates to be involved in the abnormal extracellular matrix metabolism characteristic of PEX syndrome/glucoma (PEXG) and POAG.

The purpose of this study was to analyze members of the main MMP families—interstitial collagenases (MMP-1), gelat-
nases (MMP-2 and -9), stromelysins (MMP-3, -5, -7), and metalloelastase (MMP-12)—and their endogenous in-
hibitors (TIMP-1 and -2) in aqueous humor and serum of patients with PEX syndrome, PEXG or POAG and in control patients with cataract to determine the potential role of MMPs and TIMPs in the pathogenesis of PEX syndrome, PEX glau-
coma, and POAG. We performed Western blot analysis, sub-

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strate gel electrophoresis (zymography), activity assays, and enzyme-linked immunosorbent assays, with specific monoclonal antibodies used to determine the presence, activity, and quantitative differences of MMPs and TIMPs in the samples. Our data suggest that complex changes in the local MMP-TIMP balance and reduced MMP-2 activity in the aqueous humor may promote the abnormal matrix accumulation characteristic of PEX syndrome and may be causally involved in the pathogenesis of both PEXG and POAG.

METHODS

Samples
Aqueous humor was aspirated during surgery from 100 patients with PEX syndrome without glaucoma (72.6 ± 6.4 years), from 100 patients with PEX syndrome and secondary open-angle glaucoma (PEXG; 74.9 ± 6.2 years), from 100 patients with POAG (70.5 ± 6.8 years), and from 100 age-matched control patients with cataract (72.3 ± 5.2 years) during cataract surgery or trabeculectomy. Aqueous humor (80–100 μL) was withdrawn through an ab externo limbal paracentesis site with a 27-gauge needle on a tuberculin syringe. Meticalus care was taken to avoid touching intraocular tissues and to prevent contamination of aqueous samples with blood. The samples were immediately frozen in liquid nitrogen and stored at −80°C. Samples of serum were also collected from patients of each group and equally stored. Some of the patients with PEXG or POAG had undergone previous argon laser trabeculoplasty and received various antiglaucoma medications. Patients with other ocular or systemic disease, such as inflammatory diseases or diabetes mellitus, were excluded from the study.

Informed consent to aqueous humor and serum donation was obtained from the patients, and the research was in compliance with the tenets of the Declaration of Helsinki for experiments involving human tissue.

Protein Determination
Total aqueous protein concentrations were determined in aliquots (2 μL) of all aqueous humor samples, by using the Bradford assay reagent (Sigma-Aldrich; St. Louis, MO), with bovine serum albumin used as a standard.

SDS-PAGE Zymography
Proteolytic activities were detected in sodium dodecyl sulfate (SDS)-polyacrylamide gels (5%–15%) copolymerized with 1 mg/mL porcine skin type A gelatin (Sigma-Aldrich) as substrate for MMP-2 (gelatinase A) and MMP-9 (gelatinase B) or 1 mg/mL bovine milk casein (Sigma-Aldrich) as substrate for MMP-3 (stromelysin) and serine proteinases.30 Briefly, aliquots of aqueous humor (2 μg protein for gelatin zymography, 5 μg for casein zymography) and serum samples (20 μg protein for gelatin zymography, 50 μg for casein zymography) from all groups of patients were incubated in equal volumes of nonreducing sample buffer (10 mM Tris containing 1.5% [wt/vol] SDS, 10% [vol/vol] glycercol, and 2.5 μg/mL bromophenol blue) for 30 minutes at 37°C. After electrophoresis, the gels were washed twice in 2.5% (wt/vol) Triton X-100, and incubated in 50 mM Tris/HCl (pH 7.4) containing 150 mM NaCl, 10 mM CaCl2, 2 μM ZnSO4, and 0.005% polyoxyethylene-23-lauryl ether for 4 hours (gelatin) or 72 (casein) hours at 37°C. After staining with 0.1% (wt/vol) Coomassie blue (Coomassie Brilliant Blue R 250, Sigma-Aldrich) in 30% (vol/vol) methanol and 5% (vol/vol) acetic acid for 1 hour and destaining with 20% (vol/vol) ethanol and 5% (vol/vol) acetic acid, the proteolytic activities were detected as clear bands on a stained background. Molecular weight standards and trypsin (5 ng, bovine pancreas, Sigma-Aldrich) as a positive control were run in parallel. In some gelatinase experiments, one of the metalloproteinase inhibitors, EDTA (10 mM, Sigma-Aldrich) was included in the 2.5% Triton X-100 solution and in the substrate buffer. The zymograms from three similar experiments were subjected to densitometric analysis.

Enzyme Immunoassays of MMPs and TIMPs
To determine protein levels of the various MMPs and TIMPs, aliquots (100 μg protein) of aqueous humor, which was concentrated by acetone precipitation, and unconcentrated serum samples were electrophoresed on 12% SDS-PAGE gels under reducing conditions, after normalization for total protein concentration, and electrophoretically transferred onto nitrocellulose membranes with a semidy blotting unit. After the membranes were blocked with 1% normal goat serum in PBS/0.1% Tween-20 for 12 hours, they were incubated in mouse monoclonal anti-human MMP (MMP-1, -2, -3, -7, -9, and -12) and TIMP (TIMP-1 and -2) antibodies (Oncogene, Boston, MA) diluted 1:500 in PBS/0.05% Tween-20 for 1 hour at room temperature or overnight at 4°C. Immunodetection was performed with a goat anti-mouse IgG alkaline phosphatase conjugate (Oncogene) diluted 1:1000 in PBS/0.05% Tween-20 and nitroblue tetrazolium salt (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as a substrate.

Statistics
The data were statistically analyzed by the two-sample t-test and the Pearson correlation analysis. P ≤ 0.05 was considered statistically significant. To account for the multiple comparisons, the Bonferroni correction (BC) was performed for all tests of significance by multiplying the observed probability with the number of tests performed (n = 44).

RESULTS
Protein Concentration in Aqueous Humor Samples
The total protein concentration in the aqueous humor was significantly increased in PEX samples (0.570 ± 0.375 mg/mL, mean ± SD) compared with control samples with cataract (0.360 ± 0.141 mg/mL; P < 0.01). There was also an increase in total aqueous protein content of POAG samples (0.466 ± 0.305 mg/mL) compared with cataract samples, but the difference was not statistically significant.
Zymography of Aqueous Humor and Serum

Substrate gel electrophoresis (zymography) was performed to detect gelatinolytic and caseinolytic activities of MMPs in aqueous humor and serum samples from patients with cataract, POAG, PEX syndrome, or PEXG. Ten aqueous humor samples and five serum samples were analyzed in each group. By means of SDS-mediated dissociation of MMP-TIMP complexes, this method detects both activated forms and latent pro forms of gelatinase A (MMP-2), gelatinase B (MMP-9), and stromelysin (MMP-3), because SDS in the gel and sample buffer activates the pro enzymes without a change in molecular weight.

Zymography of gelatin-containing gels showed one major band of gelatinolytic activity at a molecular mass of 70 to 72 kDa, consistent with the molecular mass of the pro form of MMP-2 in aqueous humor samples from all patients (Fig. 1A); there was, however, a distinct increase in the amount of gelatinolytic activity in PEX samples compared with control samples as assessed by densitometric analysis (Fig. 1B). Minor bands were also apparent at molecular masses of 66 kDa consistent with an activated form of MMP-2 and 120 kDa, probably corresponding to MMP-TIMP complexes that were not completely dissociated by the SDS treatment. The 66-kDa band was present in the aqueous humor of patients with cataract, but was either absent or markedly weaker in the POAG and PEX groups. The 120-kDa band was more prominent in the PEX samples assessed by densitometric analysis.

Zymography of serum samples showed a major gelatinase band at 110 to 120 kDa and distinct bands at 200 to 210 kDa and 70 to 72 kDa, corresponding to the aqueous band (Fig. 1A). There were no significant differences in band patterns of serum samples between the groups. Trypsin, run as a positive control, showed a band at 24 kDa. The gelatinolytic activities observed in all samples were completely inhibited by treatment with 10 mM EDTA. The gelatinolytic activity of the 92-kDa MMP-9 was not detected in any of the aqueous humor and serum samples.

The casein-containing gels incubated for an extended period of 72 hours showed caseinolytic activities at molecular masses of 80 to 84 kDa and 66 to 68 kDa in the serum.
samples only, but no activities in the aqueous humor samples (Fig. 2).

**Western Blot Analysis of MMPs and TIMPs in Aqueous Humor and Serum**

To determine which MMPs and TIMPs are present in human aqueous humor and serum, Western blot analyses were performed and probed with monoclonal antibodies to various MMPs and TIMPs. The results were obtained with pooled and concentrated samples from 10 patients with PEX syndrome and 10 control patients with cataract. The monoclonal antibodies to MMPs used in this study recognize both latent and active forms of enzymes.

Western blot analysis identified immunoreactive bands at 72 and 66 kDa corresponding to latent and active MMP-2, at 54 and 22 kDa corresponding to latent and active MMP-12, at 28.5 kDa corresponding to TIMP-1, and at 22 or 24 kDa corresponding to TIMP-2 in aqueous humor and serum samples from patients with PEX and control patients (Fig. 3). Relative band intensities of MMP-2 and -12 and TIMP-1 and -2 appeared higher in PEX aqueous than in control aqueous. Weak bands at 28 and 59 kDa characteristic of MMP-3 were observed in some serum but not in the aqueous samples. Distinct protein bands characteristic of MMP-1, -7, and -9 were not detected in any of the aqueous or serum samples.

**Enzyme Immunoassays of MMPs and TIMPs in Aqueous Humor and Serum**

To measure quantitative differences in the concentrations of MMPs and TIMPs in aqueous humor and serum samples from individual patients with cataract, POAG, PEX syndrome, or PEXG, immunoassays and activity assays were performed. The results are summarized in Table 2, in which mean results and SD for each group are shown, together with significant differences between the groups. Figure 4 shows the distribution in individual patients of aqueous humor concentrations of MMP-2 and TIMP-1.

The concentration of MMP-1 was below the detection limit in most aqueous humor samples from all groups of patients. Serum levels of MMP-1 were generally low and showed no significant differences between the groups.

MMP-2 was detected in considerable amounts in aqueous humor samples from all groups of patients and ranged from 18.6 to 252.4 ng/mL. Despite this high interindividual variability, the total level of MMP-2 (pro enzymes and complexed forms) was significantly increased in aqueous humor of patients with PEX syndrome without and with glaucoma (P < 0.0001, significant after BC) compared with control patients with cataract. In patients with POAG, the difference (P < 0.01) was no longer significant after BC (Fig. 4A). The increase in MMP-2 in relation to total aqueous protein levels amounted to 8% in POAG, 50% in PEX syndrome, and 35% in PEXG samples compared with cataract samples and was only significant in the PEX groups. The free, not the complexed pro form of MMP-2, ranging from 1.5 to 63.7 ng/mL and representing 22% to 24% of the total amount in the aqueous humor, was markedly increased in patients with PEX syndrome or PEXG (P < 0.01, not significant after BC), but not in patients with POAG. The levels of intrinsically active MMP-2 decreased significantly from 1.5% of the total amount in the cataract group to 0.3% to 0.4% of the total amount in both the POAG and the PEX groups (P < 0.001, significant after BC). Serum levels of total, pro, and active MMP-2 showed no significant differences between the groups.

Total MMP-3 was detected in minimal amounts in the aqueous humor of all patients, ranging from 0.15 to 3.86 ng/mL. The differences in the POAG (P < 0.05) and both PEX groups (PEX syndrome: P < 0.01; PEXG: P < 0.0001) compared with the cataract group showed statistical significance after BC in the PEXG group only (Fig. 4B). When the MMP-3 increase was expressed relative to aqueous protein concentrations (POAG: 21%, PEX syndrome: 92%, PEXG: 127%) compared with cataract samples only, no significant differences were found.
### Table 2

Levels of Total and Intrinsically Active MMPs and TIMPs in Aqueous Humor and Serum of Patients with PEX Syndrome, PEXG, POAG, and Control Patients

<table>
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<th>PEXG</th>
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<tr>
<td>Total</td>
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<tr>
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<tr>
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<td>Active</td>
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<td><strong>TIMP-1</strong></td>
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<tr>
<td>Total</td>
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<td>11.6</td>
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<tr>
<td><strong>TIMP-2</strong></td>
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<td>5.9</td>
<td>11.9</td>
<td>11.9</td>
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<tr>
<td>Total</td>
<td>11.6</td>
<td>11.6</td>
<td>17.6</td>
<td>17.6</td>
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</table>

**SD.** NA not analyzed, ND not detected.

### Correlations

When the groups were evaluated separately, a significant correlation between the total aqueous protein concentration and total MMP-2 levels was established in patients with cataract, POAG, or PEXG and between MMP-3 and TIMP-1 levels in PEXG only ($P < 0.01$), whereas no correlation was established between aqueous protein and levels of TIMP-2 in any group. No correlation between aqueous protein levels and any of the factors could be established in the group of samples with PEX syndrome. MMP-2 and -3 levels correlated with each other only in the PEXG group ($P < 0.01$), but aqueous TIMP-1 and -2 levels did not correlate with each other, neither the amounts of total MMP-2 and pro-MMP-2 nor active MMP-2, respectively. Aqueous and serum levels correlated significantly only for TIMP-1 ($P < 0.01$) in the PEXG group, but not for TIMP-2, MMP-2, or MMP-3.
MMPs and TIMPs in Physiologic and Pathologic Conditions

MMPs represent a large family of endopeptidases that are capable of degrading all extracellular matrix molecules, thereby influencing cell biological activities. In addition, new proteolytic functions are increasingly described (e.g., release of sequestered growth factors from the ECM). MMPs have been grouped into four main subfamilies based on their specificity for different extracellular matrix components and include the collagenases (MMP-1, -8, and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10, and -11), the membrane-type MMPs, and others including matrilysin (MMP-7) and metalloelastase (MMP-12). The collagenases degrade fibrillar collagens types I, II, and III; the gelatinases degrade denatured collagens (gelatins) and native collagens types IV, V, and VII and also elastin and vitronectin; whereas stromelysins cleave type IV collagen, proteoglycans, fibronectin, laminin, and elastin.

These enzymes are synthesized and secreted as inactive proenzymes and are activated by proteolytic cleavage of a polypeptide from the molecule. MMPs are tightly regulated at the levels of transcription, secretion, and proteolytic activation.

**FIGURE 4.** Levels of total MMP-2 (A) and -3 (B) and TIMP-1 (C) and -2 (D) in aqueous humor of individual patients with cataract, POAG, PEX syndrome, and PEXG as measured by specific immunoassays. MMP-2 levels were assessed in 30, MMP-3 levels in 20, TIMP-1 levels in 30, and TIMP-2 levels in 15 individual samples of each group (*P < 0.01, not significant after BC; **P < 0.0001, significant after BC).
MMPs and TIMPs in Human Aqueous Humor

MMPs and TIMPs in Aqueous Humor

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<td><strong>MMP-2 total to MMP-2 active</strong></td>
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<tr>
<td>Aqueous</td>
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<td>111.1</td>
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<td>Serum</td>
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<td>333.3</td>
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<td><strong>MMP-2 to TIMP-2</strong></td>
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<tr>
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<td>20.0</td>
<td>1.4</td>
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<tr>
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<tr>
<td>Aqueous</td>
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<td>1.41</td>
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<td>333.3/166.6</td>
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of their precursors. In addition, MMP activities are further controlled by unspecific plasma- and specific tissue-derived inhibitors, such as α-macroglobulins and TIMPs, which prevent accidental activation by complex formation. Four TIMPs have been described to date that have the capacity to inhibit all active MMPs. TIMP-1 controls most MMPs, in particular MMP-1, whereas TIMP-2 is the major inhibitor of MMP-2.

MMPs have been implicated in normal matrix remodeling processes—for example, in embryonic development, morphogenesis, tissue homeostasis, and wound healing. Abnormal expression of MMPs and TIMPs or disturbances in the proteolytic balance between MMPs and TIMPs has been associated with a number of pathologic conditions including inflammatory diseases, cancer, cardiovascular disease, neurologic disease, and fibrotic conditions. In the eye, MMPs and TIMPs have been implicated in inflammatory and fibrotic conditions, including vitreoretinal disorders.

MMPs and TIMPs in Human Aqueous Humor

The presence of MMPs and their inhibitors has been previously reported in human aqueous humor, by using zymographic and immunoblot techniques. Whereas MMP-2 (gelatinase A) activity has been consistently detected as a major constituent of normal aqueous humor samples, MMP-9 (gelatinase B) activity has been variably identified in patients with cataract or uveitis. In addition, other investigators have identified MMP-1 and -3 and TIMP-1 with considerable interindividual variations. However, no quantitative studies of aqueous levels of MMPs and TIMPs have been performed.

The present study demonstrates the concentrations of a broad spectrum of MMPs and TIMPs in aqueous humor of patients with cataract, POAG, PEX syndrome, or PEXG, and establishes significant quantitative differences between the groups, despite considerable interindividual variations in levels of MMP and TIMP. The use of zymography, immunoblots, specific immunoassays, and activity assays allowed the determination of the presence, activity, and quantitative concentrations of MMPs and TIMPs in individual samples. We found that aqueous humor from all groups of patients contains relatively high quantities of pro-MMP-2, TIMP-1, and TIMP-2 with a six- to eightfold molar excess of TIMP-1 over -2, and only low amounts of MMP-3, -7, and -9, which were not detectable by zymography or Western blot analysis. Moderate amounts of latent and active MMP-12 were additionally detected by Western blot analysis only. The presence of MMP-7 and -12 and TIMP-2 has not been previously reported in human aqueous humor. MMP-1, however, was not identified in any of the samples, which may be due to the low sensitivity (1.7 ng/mL) of the assay used. Whereas MMP-9 was below the detection limit of the commercially available assay kits, we detected it in minimal amounts with a designed ultrasensitive immunoassay confirming the results obtained for cerebrospinal fluid of normal human subjects.

The high levels of TIMPs and the six- to sevenfold stoichiometric excess of TIMPs over MMPs in aqueous humor point to a predominant role of these specific inhibitors in the protection against accidental activation of MMPs in the absence of large amounts of unspecific serum-derived inhibitors. The ratio between MMP-2 and TIMP-2 was roughly balanced, however, suggesting generation of MMP-2/TIMP-2 complexes. Any disturbances of this delicate balance within the anterior compartment of the eye should influence cell biological activities. In contrast, the surplus of MMPs over TIMPs in serum samples indicates a role for unspecific inhibitors such as α-macroglobulins in addition to TIMPs in regulating circulatory MMP activities.

Aqueous levels of MMPs and TIMPs showed significant differences between patients with PEX syndrome, POAG, and PEX compared with patients with cataract, with the differences being more pronounced in the PEX samples than in the POAG samples. Aqueous samples from patients with PEX syndrome and PEXG showed a highly significant increase of total MMP-2, total MMP-3, and TIMP-1 and -2 compared with samples from patients with cataract. MMP and TIMP levels were approximately twofold those in the cataract samples. These differences were confirmed by immunoassays, Western blot analysis, and zymography with densitometric analysis, and were still significant when the MMP and TIMP levels were compared with total aqueous protein levels. An additional increase of aqueous MMP-12 in PEX samples was suggested by semiquantitative Western blot analysis. In aqueous samples from patients with POAG, total levels of MMP-2 and -3 and TIMP-1 were also increased, compared with levels in patients with cataract, but the differences were no longer significant after BC. Despite the total increase, the activated form of MMP-2 was significantly decreased in both PEX and POAG groups, as demonstrated by zymography and activity assays and the ratio of the total to activated MMP-2 increased from 66.6 in cataract samples to 333.3 in PEX samples and 250.0 in POAG samples. The ratio of total MMP-2 and its principal inhibitor TIMP-2 was balanced in normal cataract samples, but was increased in PEX syndrome and POAG samples and decreased in PEXG samples, resulting in a stoichiometric excess of TIMP-2 over MMP-2.

In contrast, serum levels of MMPs and TIMPs did not show any significant differences between the groups, apart from markedly decreased MMP-9 levels in patients with PEX or POAG. Serum levels of TIMP-1 were increased in PEX compared with cataract samples, but the difference was also not statistically significant.

Functional Significance of MMPs and TIMPs in Human Aqueous Humor

Normal tissue homeostasis requires a balanced interaction of MMPs and TIMPs, and the ratio of enzyme to inhibitor is normally 1:1. Any disturbance in the balance may result in excessive or insufficient matrix degradation and matrix accumulation. An excess of proteases over inhibitors or an excessive MMP activity is associated with abnormal matrix degrada-
tion, as seen in inflammatory diseases. In contrast, an excess of inhibitors over proteases or reduced MMP activity may lead to abnormal matrix accumulation, as observed in chronic fibrotic disorders and scarring. In fibrotic liver disease, for example, expression of MMP-1 is downregulated, whereas expression of MMP-2 and TIMP-1 and -2 is generally enhanced. Similar findings have been reported in patients with scleroderma and in hypertrophic scar tissue. Significantly higher levels of TIMP-1 and MMP-2 have also been found in the vitreous of patients with proliferative vitreoretinopathy. However, evaluation of the biological significance and functional proteolytic activity of MMPs requires data on endogenously active rather than total protein levels and on MMP-to-TIMP ratios as well.

The MMPs and TIMPs present in the aqueous humor may participate in the remodeling of the extracellular matrix in tissues bordering the ocular chambers. In ocular tissues, a role for MMPs and TIMPs has been suggested in trabecular meshwork matrix turnover, which is critical to the regulation and maintenance of aqueous humor outflow and has been implicated in the pathogenesis of POAG. In both POAG and PEXG, accumulation of abnormal extracellular material in the trabecular meshwork, either in the form of plaque material or PEX material, may be responsible for increased outflow resistance and chronic elevation of pressure. The decreased aqueous levels of endogenous MMP-2 activity may contribute to the abnormal matrix accumulation found in the juxtacanalicular meshwork of POAG eyes. Correlating results have been reported by a previous study, demonstrating increased MMP-2 (gelatinase-A) activity in aqueous humor from patients with POAG by means of zymography only.

In eyes with PEX syndrome and PEXG, the excess de novo production of various extracellular matrix components results in a progressive accumulation of an abnormal fibrillar material in most anterior segment tissues, including the juxtacanalicular region of the trabecular meshwork. The findings of this study suggest that both the reduced levels of activated MMP-2 and the significantly increased concentrations of TIMP-1 and -2 leading to a stoichiometric excess of TIMP-2 over MMP-2 in aqueous humor from PEXG eyes are causally related to inappropriate matrix degradation and progressive matrix accumulation. A decreased degradability of PEX material from increased cross-linking processes preventing access of proteases or MMP activators may further play a role. Although the differences were generally more pronounced in patients with PEXG, they were also evident in patients with PEX syndrome only, arguing against a decisive influence of previous laser treatment or glaucoma medication on aqueous MMP and TIMP levels.

Increased deposition of matrix components may provide a signal for increased MMP synthesis. Therefore, the significantly increased levels of total (i.e., biologically inactive) MMP-2 and -3 in PEXG samples cannot be causally related to pathogenesis, and upregulation of total MMPs is more likely to be a consequence of matrix remodeling and accumulation than a cause. However, the enzymes are obviously not activated, and an appropriate matrix turnover is compromised.

Source and Regulation of MMPs and TIMPs in Human Aqueous Humor

The origin of MMPs in normal aqueous humor is not known, but they may be produced by surrounding cells and tissues, such as the corneal endothelium or the trabecular meshwork. The elevated levels of MMPs and TIMPs present in the aqueous humor of PEX and POAG eyes may be produced by anterior segment tissues or may be derived from breakdown of the blood–aqueous barrier, which is a consistent feature of at least eyes with PEX syndrome. Correspondingly, significantly increased protein concentrations were measured in the aqueous humor of PEX eyes in this study. Correlation of enzyme and inhibitor levels with total aqueous protein concentration, which is a marker of barrier breakdown, suggests a passive influx from the blood rather than local synthesis. Total aqueous protein concentration appeared to correlate with total MMP-2 in all groups except patients with PEX syndrome, and with MMP-3 and TIMP-1 in patients with PEXG only. TIMP-2 did not correlate with protein concentration at all. Despite significantly increased protein levels, patients with PEX syndrome did not show any correlation between protein concentration and any of the enzymes or inhibitors studied. These findings support the active upregulated production of MMPs and TIMPs by anterior segment tissues rather than passive derivatives from the circulation. Regardless of the source, altered aqueous levels of any of these factors may have potentially adverse pathologic effects.

MMPs and TIMPs are regulated at the transcriptional level by various growth factors and cytokines (e.g., TGF-β). TGF-β1, in particular, has been shown to downregulate the expression of MMP-1 and -3, and to upregulate the expression of MMP-2 and TIMP-1 and -3, whereas expression of MMP-2 and TIMP-1 and -3 is enhanced. However, the repressive effects of TGF-β1 on MMP mRNA expression were not apparent in aged cells. The combined effect is to prevent the destruction of the newly formed matrix and explains why elevated levels of TGF-β are associated with fibrosis within the eye and elsewhere in the body. The increased levels of TGF-β1 and TGF-β2 in the aqueous humor of PEXG and POAG eyes, respectively, may be involved in the upregulation and increased levels of MMPs and TIMPs in these patients. Reduced oxygen tension, as measured in the aqueous humor of PEXG eyes, may be another regulatory factor, because the TIMP-1 promoter contains a hypoxia response element. In conclusion, the findings of this study suggest that complex changes in the local MMP-TIMP balance and reduced MMP activity in the aqueous humor may promote the abnormal matrix accumulation characteristic of PEX syndrome and may be causally involved in the pathogenesis of both PEXG and POAG. As the importance of MMP-TIMP involvement, in particular in PEX syndrome and PEXG, becomes increasingly apparent, these enzymes and inhibitors may become targets for pharmacotherapeutic intervention.

References