Evidence for TIMP-1 Protection Against
P. aeruginosa–Induced Corneal Ulceration
and Perforation

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PURPOSE. To determine the biological significance of individual endogenous tissue inhibitors of metalloproteinases (TIMPs) in protection against tissue destruction using a Pseudomonas aeruginosa–induced model of corneal ulceration.

METHODS. Corneal TIMP-1, -2, and -3 mRNA levels were compared between young adult (resistant) and aged (susceptible) mice challenged with P. aeruginosa. Resistant mice that demonstrated greater amounts of an individual TIMP were treated with polyclonal antibody (pAb) to that TIMP. To determine whether TIMP neutralization exacerbated P. aeruginosa–induced corneal disease, TIMP pAb– and normal rabbit serum (NRS)– (control) treated mice were examined macroscopically and histopathologically after infection. Corneal neutrophil (PMN) myeloperoxidase (MPO) levels also were examined in these mice.

RESULTS. Greater amounts of TIMP-1 mRNA only were found in corneas of resistant versus susceptible mice after P. aeruginosa challenge. Systemic treatment of resistant mice with TIMP-1 pAb resulted in corneal perforation by 5 to 7 days after infection (PI). Histopathologic evaluation of corneal tissues from TIMP-1 pAb– versus NRS-treated mice confirmed that TIMP-1 pAb treatment resulted in extensive stromal dissolution. This treatment also was associated with loss of epithelium within the central cornea. Both the histopathology and PMN MPO enzyme assays also showed an increase in corneal PMN number following TIMP-1 pAb treatment.

CONCLUSIONS. These studies provide evidence that, after P. aeruginosa infection, adequate endogenous expression of TIMP-1 in cornea protects against extensive corneal tissue destruction. The protective effects of TIMP-1 may be multifactorial. In addition to directly protecting extracellular matrix components from active matrix metalloproteinases, TIMP-1 may either directly or indirectly influence recruitment of PMNs into infected cornea. Finally, TIMP-1 also may affect wound healing and resurfacing of the corneal epithelium. (Invest Ophthalmol Vis Sci. 1999;40:3168–3176)

Corneal ulceration is often observed after ocular exposure to infectious agents such as Pseudomonas aeruginosa or herpes simplex virus, after chemical or thermal injury to the cornea, or in association with diseases such as rheumatoid arthritis or vitamin A deficiency.1–6 The condition is characterized by dissolution of the extracellular matrix (ECM) components of the corneal stroma, often leading to extensive corneal scarring and perforation. Despite the nature of the corneal insult, the sequence of events leading to ulcer formation in the different infectious and noninfectious conditions are remarkably similar.7–59 Previous reports have shown that the presence of a persistent or recurring epithelial defect precedes stromal destruction.10 Likewise, animal studies have demonstrated that loss of basement membrane (BM) underlying the epithelium occurs after epithelial defect formation, but before stromal involvement, suggesting that loss of BM constituents may be a regulating step for initiating stromal degradation.11,12 In addition, infiltration of neutrophils (PMNs) into affected corneal tissue is closely associated with corneal stroma dissolution.12–14

In ulcerating cornea, destruction of corneal ECM components (BM constituents and stroma) has been attributed largely to the action of a family of proteolytic enzymes [matrix metalloproteinases (MMPs)] that collectively have the ability to degrade virtually all components of the ECM. These assumptions were based primarily on the ability of different broad-spectrum MMP inhibitors to block corneal tissue loss or destruction either in vivo or in vitro.15–19 In contrast, only a few studies have correlated overall increased expression of one or more MMP with BM destruction or stromal loss.18,20

MMP activity can be controlled by interaction with tissue inhibitors of metalloproteinases (TIMPs), the principal natural inhibitors of the MMPs.21,22 To date, four members of the TIMP family of inhibitors, TIMP-1, -2, -3, and -4, have been cloned and sequenced from a number of animal species.23 All the TIMPs inhibit active MMPs by binding to the MMP active site, forming tight, noncovalent complexes. In addition, strong interactions between the latent form of MMP-2 and TIMP-2 and MMP-9 and
TIMP-1 have been described. As an auxiliary means of control, most cells that secrete a MMP also produce at least one form of TIMP; however, the expression of MMP and inhibitor are often independently or reciprocally regulated.21

Little has been done to examine the expression and/or participation of endogenously produced TIMPs in corneal disease. Both TIMP-1,-2, and -3 mRNA and protein have been detected in normal and diseased corneal tissues.20,24–26 In these studies, differences in TIMP expression or MMP/TIMP ratios were shown in normal versus diseased human corneas, yet the biological significance of these important data could not be tested. We recently showed that expression of TIMP-1, -2, and -3 mRNA were independently regulated in corneal tissue of outbred mice infected with *P. aeruginosa.*21 As an extension of this work, our current studies have compared TIMP-1, -2, and -3 mRNA expression in mice shown to be either resistant (cornea heals) or susceptible (cornea perforates) to *P. aeruginosa* challenge to determine whether adequate expression of one or more of the TIMPs protects the cornea of resistant mice from irreversible tissue destruction. Resistant mice that demonstrated increased expression of an individual TIMP were injected with a TIMP-specific polyclonal antibody (pAb) to evaluate the in vivo contribution of the MMP inhibitor to the resistance phenotype.

**Methods**

**Infection of Mice**

Young adult (8 weeks) and aged (12 months) BALB/cBy (BALB/c) mice (Charles River Laboratories, Kingston, NJ) were used for these studies. Before corneal infection, mice were lightly anesthetized with isoflurane (Aerrane; Anaquest, Madison, WI) and placed beneath a stereoscopic microscope at ×40 magnification. The central cornea of the left eye was scarified with three 1-mm incisions using a sterile 26-gauge needle. Random eyes were routinely examined histologically to ensure that wounds penetrated only the epithelial basal lamina and that the wounds healed. Before corneal infection, mice were administered with three 1-mm incisions using a sterile 26-gauge needle. Young adult (8 weeks) and aged (12 months) BALB/cBy (BALB/c) mice (Charles River Laboratories, Kingston, NJ) were used for these studies. Before corneal infection, mice were lightly anesthetized with isoflurane (Aerrane; Anaquest, Madison, WI) and placed beneath a stereoscopic microscope at ×40 magnification. The central cornea of the left eye was scarified with three 1-mm incisions using a sterile 26-gauge needle. Random eyes were routinely examined histologically to ensure that wounds penetrated only the epithelial basal lamina and that the wounds healed. Before corneal infection, mice were administered with three 1-mm incisions using a sterile 26-gauge needle.

Eyes were examined macroscopically 24 hours after infection (PI) and/or at times described below to ensure that all mice were similarly infected and to monitor the course of disease in infected mice, respectively. All animals were treated humanely and in full compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Ocular Response to Infection**

After *P. aeruginosa* corneal infection, ocular disease was graded using the following established scale:29: 0, clear or slight opacity partially covering the pupil; +1, slight opacity fully covering the entire anterior segment; +2, dense opacity partially or fully covering the pupil; +3, dense opacity covering the entire anterior segment; and +4, corneal perforation. To observe eyes whose lids were sealed, mice were anesthetized with isoflurane and sterile phosphate-buffered saline was applied to the lids to permit their careful partial opening without inducing corneal perforation. A mean clinical score was calculated for each group of mice to express disease severity. This was done by summation of the scores for each group divided by the total number of mice scored at each time point. For each of the experiments described below [young versus aged and TIMP-1 pAb- versus normal rabbit serum (NRS)-treated] ocular disease grades were determined using two different groups of mice (*n* = 5 mice/experimental group/experiment) to ensure reproducibility of the data. Data from a single representative experiment (young versus aged and TIMP-1 pAb- versus NRS-treated) are shown in the results.

**Quantitation of Corneal TIMP-1, -2, and -3 mRNA Levels**

RNase protection assays were used to quantitate corneal levels of TIMP-1, -2, and -3 mRNA as described previously.27 For these studies, corneal tissue was collected from young adult and aged mice before and at 12 hours, 1, 3, and 5 days after corneal challenge with *P. aeruginosa.* Immediately after collection, corneas were flash-frozen in liquid nitrogen and stored at −70°C until extraction of RNA. Six corneas were pooled for an individual sample. Total RNA was extracted from corneal tissues using RNazol B (Tel-Test, Friendsville, TX) according to the recommendations of the manufacturer. As described before,27 5 μg of total RNA from each sample was hybridized overnight at 56°C to 300 pg of the 32P-labeled TIMP-1 and -3 riboprobes, whereas 25 μg of total RNA was used for the TIMP-2 assays. Similarly, various concentrations of unlabeled sense-strand standard (1.6–50 pg) was hybridized to the same amount of the respective TIMP-1, -2, or -3 riboprobes. After hybridization, samples were digested with 1000 U of T1 nuclease (Gibco-BRL, Gaithersburg, MD). Nuclease-protected fragments were resolved on a 4.5% urea containing sequencing gel. Protected bands were observed by exposing the dried gel to x-ray film and quantitated using a MDX Persen Densitometer (Sigma II and Image Quant Densitometric software ( Molecular Dynamics, Sunnyvale, CA). This experiment was performed at least three times using three different groups of mice. Single values for TIMP-1, -2, or -3 mRNA were obtained from each of the individual pooled samples. The data from the three experiments were combined to determine whether statistical differences in TIMP-1, -2, or -3 expression existed between young and aged mice. Results are reported as atomoles of TIMP mRNA per microgram of total RNA (± SEM).

**Recombinant TIMP-1 Protein Generation and TIMP-1 pAb Production**

Purified murine recombinant TIMP-1 (rTIMP-1) was expressed in *Escherichia coli* using the QIAexpress system from Qiagen (Chatsworth, CA). A cDNA for the entire coding region (minus the signal sequence) of murine TIMP-1 was generated by polymerase chain reaction (PCR) using plasmid DNA containing 825 bp of both coding and noncoding regions of the TIMP-1 gene as the template (plasmid provided by Dylan Edwards, University of Calgary, Alberta, Canada). PCR primers were engineered with BamHI (+ strand) and HindIII (− strand) restriction sites to facilitate ligation into the PQE30 expression vector. At the N terminus of the coding region of the TIMP-1 cDNA, a 6X-His affinity tag coding sequence from the PQE30 vector was added. The ligation product was transformed into *E. coli* M15 (pREP). Clones were sequenced using the Sequinase Version 2 kit (US Biochemicals, Cleveland, OH) to verify insertion of the proper DNA sequence and in frame orientation within the PQE30 plasmid. Recombinant protein was gener-
ated by inducing bacterial cultures with isopropylthiogalacto-
side (IPTG) and purified from bacterial cell lysates using a resin
column (Sephrose CL-6B) to which nitrilotriacetic acid (NTA)
charged with Ni\(^{2+}\) ions was bound. The Ni\(^{2+}\) ion binds the
6XHis–tagged protein with high affinity and allows purifica-
tion of proteins to >95% homogeneity.\(^{30}\)

The column purified protein was analyzed on a 12% SDS-
polyacrylamide gel. Gel slices containing the rTIMP-1 protein
were excised and used for the immunization of rabbits. pAb to
murine rTIMP-1 was generated by Great Lakes Biomedical
Research (Romeo, MI). The specificity of the TIMP-1 pAb was
determined by Western blot analysis. For these studies, 100 ng
of the rTIMP-1 protein or supernatant from a \(P.\ aeruginosa\)
infected corneal tissue homogenate were diluted in Laemelli
sample buffer containing 2-mercaptoethanol. Proteins in the
individual samples were resolved on 12% SDS-polyacrylamide
gels. After electrophoretic transfer to nitrocellulose, blots were
blocked in BSA (Tris-buffered saline [TBS] containing 0.5%
Tween 20, 3% skim milk powder, and 2% bovine serum albu-
min [BSA]). The blocking step was followed by incubation of
an individual blot overnight at 4°C with the TIMP-1 pAb (1:
5000 dilution in TBS with 10% BSA). The control blot was
incubated similarly in preimmune, NRS. Blots were washed in
TBS-Tween 20 and incubated at room temperature for 2 hours
with a peroxidase-labeled goat anti-rabbit IgG (1 mg/ml) (Am-
ersham, Arlington, IL). After a final series of TBS-
TWEEN 20 washes, blots were developed by chemilumines-
cence as specified by the manufacturer (Amersham).

TIMP-1 pAb Treatment

A group of five young adult BALB/c mice were injected intra-
peritoneally with 0.2 ml serum containing the TIMP-1 pAb at 1
and 3 days before and at 1 and 3 days after corneal \(P.\ aerugi-
nosa\) challenge. Control young adult BALB/c mice \((n = 5)\)
were similarly treated with 0.2 ml NRS. Each of the pAb
treatment experiments were performed in duplicate to ensure
reproducibility of the data. Data for representative experi-
ments are presented in the results.

Histopathology

For histopathologic analysis, whole eyes were enucleated from
three mice from each experimental group (TIMP-1 pAb– versus
NRS-treated) at 3 days PI. Enucleated eyes were immersed
immediately in PBS, rinsed, and placed in a fixative containing
1% osmium tetroxide, 2.5% glutaraldehyde, and 0.2 M Soren-
son’s phosphate buffer (pH 7.4), (1:1:1) at 4°C for a total of 3
hours. Eyes were transferred into fresh fixative after 1.5 hours.
Eyes were then dehydrated in graded ethanol and embedded in
Epon-araldite as described previously.\(^{28}\) Thick sections (1.5
\(\mu\)m) were cut, stained with a modified Richardson’s stain, and
observed. Representative sections were photographed with a
Zeiss Axiophot photomicroscope equipped with bright field
optics using Ilford pan F film (Moberley, Cheshire, UK).

Quantitation of PMN in Corneal Tissues

A myeloperoxidase (MPO) assay was used to quantify the
number of PMN infiltrating the cornea after infection.\(^{31,32}\) At 3
and 5 days PI, individual corneas \((n = 3/group/time)\) were
collected from TIMP-1 pAb– and NRS-treated mice. Corneas
were excised at the limbus with a sterile razor blade, and
noncorneal tissue was removed by dissection. After collection,
ICR mice, the central cornea of inbred aged mice was not completely obscured by corneal opacity by 24 hours PI. In these mice, a dense ring of opacity was observed at the limbus, whereas the central cornea typically displayed faint opacity indicative of a delayed infiltration of PMN into that area. Although this response is not defined within the conventional grading scale described by Hazlett et al., aged mice were assigned a grade of 1 because of the faint opacity within the central cornea. Young adult mice displayed faint opacity across the entire anterior segment (1) at 1 day PI. Although young adult mice did not progress past a +2 ocular disease grade throughout the experiment, all the aged mice showed +4 ocular disease grades (corneal perforation) by 7 days PI. Significant differences between young adult and aged mice were found from 3 to 7 days PI (P < 0.006, 0.011, and 0.001 at 3, 5, and 7 days PI, respectively).

**TIMP mRNA Levels during *P. aeruginosa* Corneal Infection**

Corneal TIMP mRNA levels were quantitated in resistant (young adult) and susceptible (aged) mice before and after *P. aeruginosa* challenge to determine whether differences in the regulation of one or more of the TIMPs could account, in part, for the disparate response to infection. TIMP-1, -2, and -3 mRNA levels were measured in corneal tissues collected before and at 12 hours, 1, 3, and 5 days PI. The data for three individual experiments were combined and are shown in Figures 2 to 4. While TIMP-1 mRNA transcripts were not found in uninfected corneal tissues from either young adult or aged mice, TIMP-1 mRNA was detected in both experimental groups.
by 12 hours PI (Fig. 2). Peak TIMP-1 mRNA expression was detected in both experimental groups at 1 day PI. TIMP-1 mRNA levels were significantly greater in corneas from young adult versus aged mice from 1 to 5 days PI ($P = 0.001, 0.01$, and 0.041 at 1, 3 and 5 days PI, respectively). Alternatively, low levels of TIMP-2 message were found in both young adult and aged mice before and after corneal challenge (Fig. 3). No significant differences in TIMP-2 expression were detected between young adult and aged mice at any of the times tested. Constitutive TIMP-3 mRNA expression was detected in both experimental groups of mice (Fig. 4). Corneal challenge with *P. aeruginosa* showed a slight decrease in TIMP-3 levels from 12 hours to 5 days PI. However, similar to the TIMP-2 data, no significant differences in corneal TIMP-3 mRNA levels were found between young adult and aged mice.

**Generation of rTIMP-1 Protein and TIMP-1 pAb**

As notable differences in only TIMP-1 expression were found between young adult versus aged BALB/c mice, we next focused our attention on determining if these differences were biologically relevant. To do this, murine rTIMP-1 protein was generated using a bacterial protein expression and purification system (see Materials and Methods). The rTIMP-1 protein was used to generate a murine TIMP-1–specific pAb. Figure 5 shows bacterial cell lysates (IPTG induced and uninduced) analyzed by SDS-PAGE. A 21-kDa protein, corresponding to the molecular weight of nonglycosylated murine TIMP-1, was detected only in lysates from bacterial cultures induced with IPTG. After affinity column purification of rTIMP-1, a single 21-kDa band was observed (Fig. 5). pAb generated against the recombinant protein reacted specifically with the 21-kDa protein in *P. aeruginosa*–infected corneal homogenates (Fig. 6). The predicted molecular weight of glycosylated murine TIMP-1 is approximately 28 kDa.

**TIMP-1 pAb Treatment**

Because resistant young adult mice expressed greater amounts of TIMP-1 mRNA, we next tested whether systemic injection of the TIMP-1 pAb would exacerbate *P. aeruginosa*–induced corneal disease. For these studies, TIMP-1 pAb or NRS were administered to young adult mice at 1 and 3 days before and at 1 and 3 days after *P. aeruginosa* corneal challenge. The ocular response to infection was examined from 1 to 7 days PI. Mean clinical scores for the TIMP-1 pAb- and NRS-treated mice were calculated and are shown in Figure 7. Both experimental groups initially (1 day PI) displayed similar ocular disease grades. Although mice given NRS did not progress past a +2 ocular disease grade over the time period examined, corneal perforation was evident by 5 to 7 days PI in mice treated with...
TIMP-1 pAb. Significant differences between TIMP-1 pAb– and NRS-treated mice were observed between 3 and 7 days PI ($P = 0.0039$, $0.008$, and $0.0001$ at 3, 5, and 7 days PI, respectively). Figure 8 shows slitlamp photomicrographs of representative eyes photographed at 3 days PI. By 3 days PI, mice treated with TIMP-1 pAb showed dense corneal opacity covering the entire anterior segment of the eye (Fig. 8A). In addition, all mice treated with TIMP-1 pAb showed limbal blood vessel ingrowth into the peripheral cornea and extensively swollen eye lids. In contrast, mice treated with NRS showed dense corneal opacity covering only the central cornea (Fig. 8B). Neither blood vessel ingrowth nor lid swelling was apparent in NRS-treated mice at this or any other time after infection.

Corneas from TIMP-1 pAb– or NRS-treated mice also were examined histopathologically at 3 days PI and marked differences were noted between the two experimental groups (Fig. 9). TIMP-1 pAb–treated mice exhibited centrally thinned corneas (approximately $1/2$ of normal), denuded epithelium in the central cornea and numerous free bacteria and intact PMNs throughout the stroma (Figs. 9A, 9B). PMNs filled the anterior chamber and were associated with the surface of the ocular lens. In the cornea, Descemet’s membrane and the endothelium were denuded from limbus to limbus. In the peripheral cornea, epithelium was present. PMN were frequently seen as well as a few mononuclear cells. In control (NRS-treated) mice, the central cornea was not thinned or degraded (stromal collagen intact) as extensively and epithelium was intact centrally (Figs. 9C, 9D). PMN were observed in the superficial half of the stroma and free bacteria in the stroma were also plentiful. Descemet’s membrane and the endothelium were intact and few PMN were present in the anterior chamber. In the peripheral cornea, the epithelium was intact, and PMNs were observed in the superficial stroma. Lastly, as treatment of mice with TIMP-1 pAb was qualitatively associated with a greater number of PMN in infected corneal tissue, we used an established MPO assay to quantitate PMN number in infected corneal tissue collected from TIMP-1 pAb– and NRS-treated mice. These data are presented in Figure 10. Significantly greater amounts of MPO activity were detected in corneas from TIMP-1 pAb– versus NRS-treated mice at both of the
Likewise, Kenney et al. described an increase in the ratio of immunostaining in ulcerating versus normal human corneas. In another study, Riley et al. demonstrated increased MMP-1 and decreased TIMP-1 expression in various disease models. In this regard, topical application of either recombinant TIMP or MMP inhibitors prevented or delayed corneal ulceration in various corneal ulcerative models. Accordingly, Schultz et al. recently reported data that complements our current studies. Their work suggested that the decreased TIMP-1 mRNA and protein expression in cutaneous wounds from aged versus young individuals was associated with the reported impaired wound healing in the aged.

Because differences in only TIMP-1 mRNA levels were detected between resistant and susceptible mice, the remainder of this study focused on determining if systemic neutralization of TIMP-1 protein exacerbated corneal disease pathology in resistant mice and on the initial characterization of the effects of this treatment. Gipson et al. recently used a similar TIMP neutralization approach and found increased influx of PMN into lung tissue and intensification of lung injury after TIMP-2 pAb versus preimmune serum treatment. As predicted, treatment of resistant mice in the present study with the TIMP-1 pAb converted these mice to the susceptible phenotype. Corneal perforation was evident in TIMP-1 pAb- versus NRS-treated mice by 5 to 7 days PI, similar to that observed with susceptible aged mice (Figs. 1, 7).

Histopathologic evaluation of corneas from TIMP-1 and NRS-treated mice corroborated the macroscopic findings (Fig. 9). By 3 days PI, the epithelium in TIMP-1 pAb-treated mice was centrally denuded and the stromal layer was thinned to approximately ½ that of normal. Alternatively, the epithelium in NRS-treated mice was present from limbus to limbus and the stroma was not degraded as extensively. These data are in accordance with previous studies that showed a loss or defect in regeneration of the epithelium preceded stromal collagen dissolution. In addition to inhibition of BM degrading MMP activity, it has also been suggested that endogenous TIMPs may influence healing of the corneal epithelium by enhancing spreading and proliferation of the epithelial cells.

In the experiments described herein, both the histopathology and PMN MPO assays demonstrated a significantly increased number of PMNs in corneas of mice treated with the TIMP-1 pAb (Figs. 6, 7). The presence of a large number of PMNs also has been associated with stromal collagen degradation in various corneal ulcerative models. Accordingly, Schultz et al. demonstrated reduction of PMN influx into alkali-burned corneas and ultimately prevention of corneal ulceration after treatment with a synthetic MMP inhibitor. The mechanism by which TIMP-1 prevents corneal PMN influx after P. aeruginosa challenge remains unknown. However, based on the data reported herein as well as in previous studies, various possibilities may be considered for future testing. In this regard, PMNs, on activation, have the ability to release MMP-8 (PMN interstitial collagenase) and MMP-9 (gelatinase B) from secondary granules. PMN collagenase can directly degrade stromal collagen and generate collagen peptide fragments that are chemotactic for PMN. Inhibition of MMP-8 activity by TIMP-1 may therefore help to control this.

**DISCUSSION**

During normal wound healing processes, it has been suggested that an appropriate balance exists between ECM degradation and deposition, such that the overall outcome is maintenance of integrity and function of the affected tissue. Alternatively, in pathologic states, this balance is thought to be altered in a manner that promotes progressive ECM degradation or extensive deposition of fibrotic tissue. In ulcerative corneal disease, there is evidence to suggest that alteration of the ratio of MMPs:TIMPs plays a role in progressive stromal degradation. In this regard, topical application of either recombinant TIMP or synthetic MMP inhibitors prevented or delayed corneal ulceration in various disease models. In another study, Riley et al. demonstrated increased MMP-1 and decreased TIMP-1 immunostaining in ulcerating versus normal human corneas. Likewise, Kenney et al. described an increase in the ratio of MMP-2/TIMP-1 in corneas of keratoconus patients. Although suggesting a general role for MMPs and TIMPs in these diseases, neither study was able to directly examine the participation of individual endogenously produced TIMPs in protection against corneal destruction. Therefore, to address this problem, we used a resistant (cornea heals) versus susceptible (cornea perforates) model of P. aeruginosa-induced corneal disease to identify those TIMPs that are involved in corneal wound healing. In this model, young adult (8 weeks) BALB/c mice restore corneal clarity within 2 weeks after ocular P. aeruginosa challenge, whereas aged mice undergo corneal perforation within 7 days PI (Fig. 1).

When TIMP-1,-2, and -3 mRNA levels were examined in young adult and aged inbred mice before and after P. aeruginosa corneal challenge, we found that all three of the TIMPs were independently regulated in corneal tissue (Figs. 2–4). These data, using a well-defined inbred mouse model system, confirm and extend our previous results using outbred young adult Swiss-ICR mice. Based on promotor regions of the TIMP genes and previous gene induction studies, these results are not surprising. In the current studies, TIMP-1 mRNA was not expressed in uninfected corneas of either experimental group under the conditions tested. However, after corneal challenge, significantly greater amounts of TIMP-1 were detected in corneas of young adult and aged mice from 1 to 5 days PI. Ascroft et al. recently reported data that complements our current studies. Their work suggested that the decreased TIMP-1 mRNA and protein expression in cutaneous wounds from aged versus young individuals was associated with the reported impaired wound healing in the aged.

![Figure 10](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932899/)
cyclic response. Likewise, it has been suggested that PMNs use MMP-9 to traverse endothelial BM and extravasate into inflamed tissues. Finally, TIMP-1 may reduce PMN infiltration into the cornea by blocking the shedding of the L-selectin adhesion molecule present on activated PMN. It has been suggested that release of L-selectin from PMNs after the initial attachment to the endothelium facilitates entry into subendothelial tissues. Shedding of the L-selectin molecule was shown to be inhibited by synthetic MMP inhibitors. Furthermore, a study by Pfister et al. showed that a synthetic MMP inhibitor directly affected PMN chemotaxis.

In summary, we have investigated the role of TIMP-1, -2, and -3 in a P. aeruginosa-induced model of ulcerative corneal disease. Because differences in the expression of only TIMP-1 could be detected between resistant and susceptible mice, we focused our study on this TIMP. These studies, using a systemic TIMP-1 pAb treatment protocol, are the first to show that expression of adequate endogenous levels of TIMP-1 in cornea after P. aeruginosa challenge is associated with protection against extensive stromal destruction and corneal perforation. The data also strongly suggest that TIMP-1 may be protective in several phases of the ulcerative process, including epithelial resurfacing, BM and/or stromal ECM loss, and PMN infiltration.

References

15. Paterson CA, Wells JC, Koklitits PA, Higgs GA, Docherty AJ. Recombinant tissue inhibitor of metalloproteinase suppresses alkali-


