Expression of Integrins and MMPs during Alkaline-Burn–Induced Corneal Angiogenesis

Heying Zbang,¹ Chen Li,² and Peter C. Baciu²

PURPOSE. To determine in a corneal alkaline burn model of angiogenesis whether the expression of integrins and MMPs is consistent with a VEGF-induced angiogenic response.

METHODS. Neovascularization in female Sprague-Dawley rats was induced by alkaline cautery of the central cornea. RT-PCR for integrins α1, α2, β1, and β3; the endothelial marker CD31; and metalloproteinases MMP-2 and MT1-MMP was performed on naive corneas and on cauterized corneas 72 and 288 hours after cautery. Analyses of protein and MMP expression were conducted on naive corneas and on cauterized corneas 24, 72, 120, and 168 hours after cautery by immunofluorescence microscopy and gelatin zymography.

RESULTS. RT-PCR indicated a correlation between the induced angiogenic response and the expression of α1 and β1 integrin subunits and MT1-MMP. Immunohistochemical analysis indicated that α1, α2, α5, and β1 integrins and MMP-2 and MT1-MMP were expressed on the newly developing vasculature. The β1 integrin was preferentially expressed on platelets.

CONCLUSIONS. Integrin expression during neovascularization of rat corneas in response to alkaline injury correlates with an angiogenic response that uses the VEGF/α1β1 pathway. MMP-2 and MT1-MMP, but not MMP-9, are expressed in a pattern consistent with their involvement in the angiogenic response.


Angiogenesis in adult tissue is the result of a complex interplay among proangiogenic factors, cell adhesion, and matrix remodeling.¹ Inhibition or disruption of either cell adhesion or matrix-degrading enzymes, many of which belong to the family of matrix metalloproteinases (MMPs), is capable of blocking an angiogenic response.²⁻⁴ The adhesion receptors and MMPs involved in an angiogenic response have been correlated with the presence of select initiating factor(s).⁵⁻⁻⁷ Induction of angiogenesis by bFGF or TNF-α is associated with the selective upregulation of αβ₁ and involvement of MT1-MMP and MMP-2, whereas induction of angiogenesis by VEGF, TGF-β, or PMA, is associated with selective upregulation of αβ₅.⁶⁻⁻⁷ Although these two pathways are well recognized, recent studies suggest that under pathologic conditions the correlation between growth factors and integrin expression is not always maintained. In several instances in which VEGF is present, both αβ₁ and αβ₅ are expressed, and in at least one study it was shown that the functional significance of αβ₅-mediated angiogenesis may reflect the presence of ligand for αβ₅.⁹⁻⁻¹¹ However, not all aspects of angiogenesis are dependent on expression of αβ₁ or αβ₅ integrins. Knockout mice for α₁ and β₁ integrins appear to undergo extensive vasculogenesis and angiogenesis, although in the α₁ null, subtle vascular defects are present, resulting in both embryonic and postnatal death.⁵⁻⁻¹² These results suggest that other integrin family members may compensate for the loss of α₁ or β₁ integrins or may play a more essential role in the angiogenic response. Other members of the integrin family implicated in mediating an angiogenic response include αβ₁, αβ₁, and αβ₁ integrins, which, like α₁ integrins, have also been divided into bFGF-associated (αβ₁) or VEGF-associated (αβ₁, αβ₁) angiogenic events.¹³⁻⁻¹⁹

Recently, the corneal alkaline burn model of angiogenesis has been characterized as having high levels of VEGF present during active vessel growth, suggesting that VEGF is the primary angiogenic factor within this model system.¹⁶ Consistent with this finding, pharmaceutical intervention with αβ₁ antagonists has no effect on the angiogenic response,¹⁷ suggesting that angiogenesis occurs through an αβ₁ adhesion pathway that is consistent with a VEGF-mediated angiogenic response. However, expression of αβ₁ was not established in these studies, and no other potential adhesion receptors have been identified.

The purpose of this study was to characterize the pattern of integrin and MMP expression to determine whether it is consistent with a VEGF-mediated angiogenic response. In agreement with a VEGF-mediated angiogenic response, neovascularization was associated with expression of αβ₁, αβ₁, and αβ₁ integrins as well as αβ₁. Preferential staining of αβ₁ and αβ₁ was seen in the invasive angiogenic front, whereas αβ₁ integrin appeared to be preferentially expressed in regions of vessel maturation. MMP-2 and MT1-MMP were associated with both vessel formation and the robust inflammatory response. These data indicate that the corneal alkaline burn model provides an in vivo model system to examine the role and involvement of αβ₁, αβ₁ integrins and MT1-MMP and MMP-2 in neovascularization of corneal tissue.

MATERIALS AND METHODS

Reagents and Antibodies

5-Bromo-2-deoxyuridine (BrdU) was purchased from Roche Molecular Biochemicals (Indianapolis, IN); DNA extraction reagent (TRIzol), reagent for reverse transcriptase (SuperScript II), and bFGF from Invitrogen (Carlsbad, CA); and gelatin zymography polyacrylamide gels (10%), renaturing buffer, and developing buffer from Novex (San Diego, CA). Primary antibodies were purchased from the following companies and used at the concentrations shown: goat anti-type IV collagen (1:250 dilution; Southern Biotechnology Associates, Inc., Birmingham, AL); rabbit polyclonal anti-integrin α₁, α₂, α₅, and β₁ subunits and anti-CD31 (1:100 dilution for the α subunits 1:500 dilution for the β₁ subunit; Chemicon International, Inc., Temecula, CA); mouse monoclonal anti-rat integrin β₁ chain (1:100 dilution; 5 µg/mL; PharMingen, San Diego, CA); and rabbit polyclonal anti-MMP-2 and MT1-MMP (Chemicon). All secondary antibodies were F(ab’)² fragments conjugated to either tetramethylrhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC; both 1:200 dilution Jackson ImmunoResearch Laboratories, Inc., West Grove, PA).


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From ¹Medimmune, Gaithersburg, Maryland; and ²Allergan, Inc., Irvine, California.

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Animal Model

Female Sprague-Dawley rats, weighing 250 to 300 g, were anesthetized with isoflurane (4% vol/vol) and topical application to the corneal surface with proparacaine 0.1% (Allergan, Inc., Irvine, CA). The alkalineline burn was created by touching the central cornea with the tip of a silver nitrate applicator (75% silver nitrate, 25% potassium nitrate; Grafo; Graham-Field, Inc., Hauppauge, NY) for 2 seconds. At the indicated times, animals were killed and the eyes enucleated for various studies at postinjury intervals ranging from 24 to 288 hours. For immunofluorescence analysis, the eyes were embedded in optimal cutting temperature (OCT) solution and cryosectioned. For wholemount studies, entire corneas were removed and quartered. Experimental animals were treated and maintained in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cryosectioning and Immunofluorescence

The eyes (injured or naive) were sagitally cryosectioned in 8- to 13-μm sections for immunostaining with mouse monoclonal or goat and rabbit polyclonal antibodies. The sections were fixed in 100% acetone for 5 minutes, briefly dried, rehydrated in phosphate-buffered saline (PBS) and incubated in a moist chamber as follows: 5% BSA (Sigma, St. Louis, MO) in PBS for 2 hours, primary antibodies for 2 hours at room temperature, five washes in PBS for 5 minutes each, secondary antibodies conjugated to fluorochromes for 1 hour at room temperature, and five more washes as before. Samples were mounted with fluorescence medium (Fluoromount G; Southern Biotechnology Associates) and observed and photographed with a compound microscope (model E800; Nikon, Tokyo, Japan) equipped with a digital camera (Spot; Diagnostic Instruments, Inc., Sterling Heights, MD). Colocalization of the angiogenesis-related molecules and vascular markers was achieved by using various combinations of mouse, goat, and rabbit primary antibodies. Negative controls for immunostaining were naive serum or purified IgG for each species of primary antibody used, as well as for secondary antibody alone. In all instances tissues were costained with collagen type IV to mark the presence of vessels and to serve as an internal positive control. All control tissues were from corneas harvested 72 hours after injury, because they provided the greatest range of cellularity.

Wholemount Immunofluorescence

Complete fresh corneas were cut in quarters and fixed in 90% methanol and 10% dimethyl sulfoxide (DMSO) for 15 minutes at room temperature, rinsed in 1× PBS three times for 2 minutes each, blocked in 2% BSA in PBS for 4 hours, incubated in primary antibody anti-plexin A1 and Bandeiraea simplicifolia (BS-1) lectin (Sigma) overnight at 4°C, washed 5 times in PBS for 1 hour each, incubated in secondary antibodies conjugated to fluorochromes overnight at 4°C, and washed five times as before. Finally, corneas were flattened and analyzed with either the compound microscope (E800 Nikon) equipped with the digital camera (Diagnostic Instruments, Inc.) or by a confocal microscope (model TCS SP; Leica Microsystems Inc., Exton, PA).

Gelatinase Zymography

For gelatin zymography, MMPs were extracted from corneal tissue sections. For MMP extraction and electrophoresis, corneal sections were obtained by trimming samples separated on 10% SDS-polyacrylamide zymography gels with-
Positive staining on stromal collagen type IV was also observed. This later staining was associated with the angiogenic response (Fig. 1). In contrast, the time points examined and showed no apparent increase correlated with the angiogenic response, as revealed by increased staining was for CD31, a marker for endothelial cells were also examined. Messages for MMP-2 and MT1-MMP were detected only in neovascularized corneas. For analysis of expression patterns, three separate experiments were performed in which naive corneas 72 hours (3 days) and 288 hours (12 days) after cautery. Levels examined included naive corneas and cauterized corneas harvested 72 hours (3 days) and 288 hours (12 days) after cautery. To correlate gene expression relative to vessel growth, levels of CD31, a marker for endothelial cells were assessed in a minimum of three adjacent sections to determine pattern and localization. Nonspecific staining was for the secondary antibody alone. Staining in naive corneas was also observed but to a limited extent that was not readily apparent. No immunoreactivity was seen for β3 integrin (not shown). Localization of integrins to neovessels is shown in Figure 3. Integrins α1, α2, α5, and β3 were seen to stain neovessels as identified by collagen type IV staining in longitudinal sections of developing neovessel (Figs. 3A–L). Integrin β3 appeared to stain individual cells within the lumen (Figs. 3M–O). Positive immunoreactivity for α1, α2, α5, and β3 not colocalizing with collagen type IV was also observed. This later staining was positive on stromal and inflammatory cells. Staining for α1 and α5 was also seen in the corneal endothelium. No stromal staining was observed for β3 integrin.

Table 1. Oligonucleotide Primer Sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Oligonucleotide Sequence</th>
<th>Fragment Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT1-MMP</td>
<td>5'-GTGACAGGCAAGGCGATTCG-3'</td>
<td>446</td>
</tr>
<tr>
<td></td>
<td>5'-TTGGACAGTCAGGGCTCAGC-3'</td>
<td></td>
</tr>
<tr>
<td>MMP-2</td>
<td>5'-ACTCCTGGCAGATCTTTGGC-3'</td>
<td>401</td>
</tr>
<tr>
<td></td>
<td>5'-TAATCTCCGGTGGCTGACACC-3'</td>
<td></td>
</tr>
<tr>
<td>Integrin β3</td>
<td>5'-TTTTGCTAGTTTACACGAGGATGCAACAC-3'</td>
<td>866</td>
</tr>
<tr>
<td></td>
<td>5'-CCTTTTGTAGCGCAGGAGGAGAATCATC-3'</td>
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</tr>
<tr>
<td>Integrin β2</td>
<td>5'-CGATAGGCTGTGAAGGATGATGAAC-3'</td>
<td>854</td>
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<tr>
<td></td>
<td>5'-CAAGGCTTCAGTGAGCCCGAAGAAGAAGAAGA-3'</td>
<td></td>
</tr>
<tr>
<td>Integrin α2</td>
<td>5'-CAACCTTGACTGCTAATAGCCAACAGCAGGAAAAC-3'</td>
<td>728</td>
</tr>
<tr>
<td></td>
<td>5'-CGGAGCTCCWATCGAGYTGCAATTTATGC-3'</td>
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<tr>
<td>CD31</td>
<td>5'-GGCATCGCCAAAAGTGCCAAG-3'</td>
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</tr>
<tr>
<td></td>
<td>5'-CAAGGGGCAATGACACTCCAC-3'</td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td>5'-ATCTGGCAACACCACTCTCTCAATGAGGCTG-3'</td>
<td>837</td>
</tr>
<tr>
<td></td>
<td>5'-CGTCATACCTTCTGCTGCTGTCACATGAC-3'</td>
<td></td>
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W = A or T; Y = C or T.

168 hours after cautery, neovessels extend across the entire cornea from the limbus to the central burn.19

RT-PCR was performed on mRNA extracted from pooled corneas to examine the expression of integrin subunits α1, α2, β1, and β3 and of MMPs MMP-2 and MT1-MMP. Corneal tissues examined included naïve corneas and cauterized corneas harvested 72 hours (3 days) and 288 hours (12 days) after cautery. To correlate gene expression relative to vessel growth, levels of CD31, a marker for endothelial cells were examined. Messages for MMP-2 and β1 and α5 integrins were present at all time points examined and showed no apparent increase correlated with the angiogenic response (Fig. 1). In contrast, the expression of MT1-MMP and α1 and β3 integrin subunits correlated with the angiogenic response, as revealed by increased message correlating with expression of CD31 (Fig. 1).

Association of Integrin Subunits with the Developing Neovasculature

Analysis of integrin expression on neovessels was accomplished by using immunologic reagents that recognized individual integrin subunits. The individual subunits examined included α1, α2, α5, β1, and β3. For analysis of expression patterns, three separate experiments were performed in which naïve corneas 72 hours after harvesting and cauterized corneas 24, 72, 120, and 168 hours after cautery were analyzed. Staining was assessed in a minimum of three adjacent sections to determine pattern and localization. Nonspecific staining at each time point was assessed by the use of naïve serum or purified IgG for each species of primary antibody used, as well as for the secondary antibody alone. Staining in naïve corneas for each of the integrins examined is shown in Figure 2. Most staining was for α1, α2, α5, and β3 and was limited to the corneal epithelium. Stromal staining was also observed but to a limited extent that was not readily apparent. No immunoreactivity was seen for β3 integrin (not shown).

Localization of integrins to neovessels is shown in Figure 3. Integrins α1, α2, α5, and β3 were seen to stain neovessels as identified by collagen type IV staining in longitudinal sections of developing neovessel (Figs. 3A–L). Integrin β3 appeared to stain individual cells within the lumen (Figs. 3M–O). Positive immunoreactivity for α1, α2, α5, and β3 not colocalizing with collagen type IV was also observed. This later staining was positive on stromal and inflammatory cells. Staining for α1 and α5 was also seen in the corneal endothelium. No stromal staining was observed for β3 integrin.

![CD31](image1.png)

![Alpha 1](image2.png)

![Alpha 2](image3.png)

![Beta 3](image4.png)

![Beta 5](image5.png)

![MT1-MMP](image6.png)

![MMP-2](image7.png)

![Beta Actin](image8.png)

**FIGURE 1.** RT-PCR analysis for CD31, MT1-MMP, MMP-2, and integrin chains α2, α5, β1, and β3 from total RNA pooled from four individual corneas representing naïve corneas (lane 1), and cauterized corneas 72 hours (lane 2) and 228 hours (lane 3) hours after cauteration. In naïve and neovascularized corneas, message for α2 and β3 integrins and MMP-2 were detected. Message for α1 and β3 integrins, CD31, and MT1-MMP were detected only in neovascularized corneas.
The punctate staining pattern for \( \beta_3 \) integrin suggests localization within the developing vasculature, potentially on platelets and not endothelium (Figs. 3M–O). Confocal microscopy of whole-mounted corneas indicated that this immunostaining is associated with expression of \( \beta_3 \) on platelets (Figs. 4A, 4B).

To confirm that the staining pattern for \( \beta_3 \) is not associated with neovascularization, we examined, by corneal micropocket assay, the expression of \( \beta_3 \) in tissues in which corneal angiogenesis had been induced by bFGF. Expression of \( \beta_3 \) in the corneal micropocket assay was restricted to the leading part of the vasculature (Figs. 4C, 4D) and was pronounced on endothelial cells (Figs. 4E, 4F). These results are consistent
with previous studies examining \( \alpha_\mathrm{H9251} \), \( \beta_\mathrm{H9251} \), \( \alpha_\mathrm{H9252} \), and \( \beta_\mathrm{H9252} \) expression in neovascularized tissue, but contrast greatly with results in the corneal alkaline-burn model, indicating that \( \beta_\mathrm{H9252} \) expression is principally restricted to platelets.

**Differential Distribution of \( \alpha_\mathrm{H9251} \) and \( \beta_\mathrm{H9251} \) Integrins within the Developing Neovascularure**

Spatial distribution of integrin staining along the developing neovessels is shown in Figure 5. Expression of \( \alpha_\mathrm{H9251} \) integrin within the developing vasculature showed a uniform pattern of staining throughout (Figs. 5A, 5B). Similar to staining for \( \alpha_\mathrm{H9251} \) integrin, staining for \( \beta_\mathrm{H9251} \) integrin was also seen throughout the developing vasculature; however, more pronounced staining was typically seen in distal regions of the neovascularity (Figs. 5C, 5D). Staining for \( \beta_\mathrm{H9252} \) integrin was preferentially seen in more distal regions of the developing neovascularity associated with the invasive front (Fig. 5E, 5F). Staining for \( \alpha_\mathrm{H9252} \) integrin was preferentially observed in regions of vessel maturation, with little or no staining in more distal regions associated with the invasive front (Figs. 5G, 5H).

**Expression of MT1-MMP and MMP-2**

MT1-MMP and MMP-2 form a functional complex on the cell surface involved in the proteolytic modification of the surrounding extracellular matrix. The observed increase in MT1-MMP expression during neovascularization suggests that MT1-MMP and MMP-2 may form a functional complex on endothelial cells, thus promoting the neovascular response. To determine whether the observed staining correlates with MMP-2 activation, processing of MMP-2 was monitored by gelatinase zymography. To correlate MMP-2 processing with vessel growth, corneas were sectioned into

**Correlation of MMP-2 Processing with Angiogenic Responses**

MMP-2 is expressed as a latent 72-kDa inactive proenzyme that is processed to a 62-kDa active form, principally through its association with MT1-MMP. Thus, the presence of the 62-kDa gelatinase activity in gelatin zymography indicates activation of MMP-2. To determine whether the observed staining correlates with MMP-2 activation, processing of MMP-2 was shown in Figure 6. Staining for MMP-2 and MT1-MMP in neovascularized cornea. (A) Localization of MMP-2 and MT1-MMP to neovessels. MMP-2 staining (A–C), MT1-MMP staining (D–F). Arrows: localization of MMP-2 or MT1-MMP to neovessels; (G) staining not associated with neovessels. (B) Composite images of MMP-2 and MT1-MMP showing spatial distribution. (A) Staining for MMP-2; (B) immunofluorescence of MMP-2 with collagen type IV; (C) staining for MT1-MMP; and (D) immunofluorescence of MT1-MMP with collagen type IV. Images are from corneas harvested 72 hours after cautery.
In addition to MMP-2, MMP-9 expression and activation were detected by gelatin zymography. Conversion of MMP-9 from its latent 92-kDa form to its activated 82-kDa form was seen principally in segments 2, 3, and 4, suggesting a correlation with the wound-healing response (Fig. 7B). Expression and conversion of MMP-9 was greatest at 24 hours after cautery and was nearly absent by 120 hours (data not shown). The pattern of MMP-9 expression did not correlate spatially with vessel development.

**Correlation of Expression and Conversion of MMP-2 with In Situ Gelatinase Activity**

The pattern of MMP-2 conversion detected by gelatinase zymography represents both active enzyme and that associated with tissue inhibitors of matrix metalloproteinases (TIMPS) in an inactive complex. To identify endogenously active gelatinase within the cornea, in situ zymography was performed (Fig. 7D). Consistent with the pattern of MMP-2 processing by gelatin zymography, the pattern of gelatinase activity observed reflected a gradient of gelatinase activity with highest levels in the limbal region and lower levels in adjacent tissue within the corneal stroma (Fig. 7D). The pattern of gelatinase activity in the cornea was consistent with regions of neovascularization. Strong punctate gelatinase activity was also seen in the corneal stroma, which probably reflects gelatinase activity on inflammatory cells.

**DISCUSSION**

We examined the pattern of integrin and MMP expression within the corneal alkaline burn model relative to the angiogenic response by RT-PCR, immunohistology, and gelatinase zymography. A summary of the immunohistochimical and zymography analysis for the integrins and MMPs studied are presented in Table 2. Analysis of integrin and MMP expression by RT-PCR demonstrated that expression of CD31, integrins α3, and β3, and MT1-MMP correlated with the angiogenic response. No alteration was detected in the levels of α3 and β3 integrins and MMP-2 that was related to neovascularization. The inability to detect a change in message for α3 and β3 integrins and for MMP-2 probably reflects the existence of abundant message present in naıve tissues within the corneal epithelium for β3, and α3 integrins and within the corneal stroma for MMP-2.

Immunohistochemical analysis of α3, α2, α1, and β3 subunits indicated expression of these subunits within the developing neovessels. The α3 integrin was uniformly expressed within the developing neovasculature, whereas α1 appeared to be more prevalent in regions of vessel maturation. The α3 and β3 integrin subunits showed a preferential localization to the more distal regions of vessel formation associated with the invasive and early remodeling phases of vessel development.

Expression of the β3 integrin subunit was principally restricted to platelets within the developing vasculature. Staining on platelets and not on endothelial cells was confirmed by comparing β3 staining from the corneal burn model with β3 staining induced by bFGF in the corneal microtissues. The presence of a β3-specific band in the RT-PCR analysis may represent the expression of α3β3 on macrophages, which are present as part of the inflammatory response in this model system. Alternatively, the β3 mRNA message detected by RT-PCR may be the result of expression on endothelial cells, which showed a low level of staining localized to the vessel lumen.

Expression of integrin was analyzed in this study with immunologic reagents directed against individual integrin subunits. In most cases, the staining reflected the presence of a
heterodimer pair, because $\alpha_1$, $\alpha_2$, and $\alpha_5$ pair only with the $\beta_3$ integrin subunit, which heterodimerizes with $\alpha_4$ and $\alpha_5$, forming $\alpha_3\beta_3$ and $\alpha_4\beta_3$ heterodimer pairs. We were able to exclude the presence of $\alpha_3\beta_3$, because the primary staining was associated with platelets that express only the $\alpha_4\beta_3$ heterodimer.

The other aspect of angiogenesis studied was the expression of MMP-2 and -9 and MT1-MMP. MMP-2 and MT1-MMP were observed to be associated with the angiogenic response based on both zymographic and immunohistochemical analyses. The correlation between the 62-kDa form of MMP-2 and MT1-MMP immunoreactivity suggests that MT1-MMP is associated with the conversion of the 68-kDa form of MMP-2 to its 62-kDa form in this model system; however, other mechanisms of MMP-2 processing may also be present. Currently, MMP-2 and MT1-MMP are believed to form a functional complex in conjunction with $\alpha_3\beta_3$ and TIMP-2 on the cell surface, which in turn mediates localized pericellular proteolysis of the extracellular matrix, which is essential for endothelial cell migration and invasion. In the alkaline-burn-induced corneal angiogenesis model, $\alpha_3\beta_3$ does not appear to play a major role in mediating the angiogenic response, and thus the role of MT1-MMP and MMP-2 within this model may be outside their association with $\alpha_3\beta_3$. Recently, MT1-MMP has been shown to directly mediate cell migration and adhesion and processing of integrin $\alpha$ chains. This may be an alternate pathway through which MT1-MMP participates in regulation of cellular responses, outside the formation of a complex with $\alpha_3\beta_3$. In addition to MMP-2 and MT1-MMP, we observed increased levels of both the 92- and 82-kDa forms of MMP-9. The temporal and spatial patterns of MMP-9 expression both suggest its association with wound healing and not with neovascularization.

In conclusion, the $\alpha_3\beta_3$ integrin appears to be the principal $\alpha$ integrin associated with endothelial cells within the corneal alkaline burn model of inflammation-mediated angiogenesis. In addition to $\beta_3$, the $\alpha_1$, $\alpha_2$, and $\alpha_5$ integrins showed consistent localization to the developing vasculature bed. Of particular interest was the preferential localization of $\alpha_5$ to more distal regions of the developing vasculature and the preferential expression of $\alpha_5$ integrin to regions of vessel maturation. Both MT1-MMP and MMP-2 were seen in association with the neovascularature, and the conversion of MMP-2 from its 72-kDa latent form to its 62-kDa active form correlated with neovascularization. Based on the characterization of integrin subunit expression, the data suggest that therapeutic approaches to inhibition of corneal angiogenesis in response to an alkaline burn would be best if directed against the $\beta_3$ integrin subunit and not against $\beta_3$. 

### References

13. Senger DR, Claffey KP, Benes JE, Perruzzi CA, Sergiou AP, Detmar M. Angiogenesis promoted by vascular endothelial growth factor: regulation through $\alpha_1\beta_1$ and $\alpha_5\beta_3$. *Proc Natl Acad Sci USA*. 1997;94:13612–13617.


