The Transglutaminase in Vascular Cells and Tissues Could Provide an Alternate Pathway for Fibrin Stabilization

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A thrombin-independent transglutaminase (TG) has been identified in vascular cells and tissues from human, rabbit, rat, porcine, and bovine sources. The vascular TG had several properties that were similar but not identical to the guinea pig liver TG. Both enzymes had similar chromatographic and electrophoretic properties, preferentially cross-linked the α-chains of fibrinogen, and reacted with polyclonal and monoclonal anti-guinea-pig liver TG antibodies. However, the TG from adult bovine aortic endothelial (ABAE) cells exhibited a novel Ca\(^{2+}/Mg\(^{2+}\) dependence for enzymatic activity that was distinct from that of purified guinea pig liver TG. The mol wt of the vascular TG (79 ± 3 kd) determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was slightly lower than the purified guinea pig liver TG (85 ± 9 kd). The TG antigen was detected by immunohistochemical techniques in association with the endothelial and smooth muscle cells of arteries, veins, venules, and capillaries. The TG antigen also codistributed with the fibronectin antigen along the hepatic sinusoids. The ABAE cell TG cross-linked α\(_2\)-plasmin inhibitor to fibrinogen and caused the modified fibrinogen to be 40-fold more resistant to plasminolysis. A thrombin-independent TG in vascular cells of blood vessels could provide an alternate pathway to inhibit fibrinolysis and promote fibrin stabilization.

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**MATERIALS AND METHODS**

Tissue culture dishes were purchased from Falcon Plastics, Oxnard, CA. Tissue culture medium and trypsin were obtained from Gibco (Grand Island, NY), and calf serum and fetal calf serum (FCS) were from Hyclone Laboratories (Logan, UT). \(^{3}H\)Putrescine, Aquasol 2, and Protosil were from New England Nuclear (Boston). Hammerstein casein was purchased from United States Biological Laboratories (Cleveland). BioRad protein assay kit, nitrocellulose membranes, and EnzymeMab reagent were from BioRad, Richmond, CA. Diethylaminoethyl (DEAE)-Sepharacell was obtained from Sigma, St Louis. Human α-thrombin was supplied by Dr John Fenton, II, New York State Department of Health, Albany, NY. Sephadex G-25M, PD-10 columns were from Pharmacia Fine Chemicals, Piscataway, NJ. Factor XIII-free fibrinogen was prepared as previously described. \(^a\) Rabbit aortic and human aortic and venous tissues were generously provided by Dr Per Otto Hagen, Department of Surgery, Duke University. Pig aorta was purchased from the local slaughterhouse. Affinity-purified anti-guinea-pig liver TG antibody\(^a\) is a generous gift from Dr Peter Davies, Department Pharmacology, University of Texas, Houston. Monoclonal antibody (CUB-7401) against guinea pig liver TG\(^a\) was kindly supplied by Dr Paul Birckbichler, Samuel Roberts Noble Foundation, Ardmore, OK. Goat anti-mouse horseradish peroxidase and goat anti-rabbit horseradish peroxidase conjugates were from BioRad. Anti-factor XIII (A-subunit) antiserum for rabbits, rabbit antifibrinogen antiserum, and rabbit anti-human fibronectin antiserum.
were purchased from Calbiochem, La Jolla, CA. Purified human 
$\alpha_2$-plasmin inhibitor was purchased from American Diagnostica, 
Greenwich, CT. Rabbit antiseria to human $\alpha_2$-plasmin inhibitor was 
obtained from American Diagnostica, Greenwich, CT.

**Tissue culture techniques.** ABAE cells and bovine vascular 
smooth muscle (VSM) cells were cultured by previously described 
methods on 10-cm plastic culture plates.11,12 ABAE and VSM cells 
were used between passages 2 and 15. Confluent ABAE cells 
reached a density of 900 cells/mm². ABAE cells were characterized 
by the presence of Weibel-Palade bodies13 and factor VIII-related 
antigen.14 VSM cells were characterized by their typical morphology 
and absence of factor VIII-related antigen. ABAE and VSM cells 
cultured with fibroblast growth factor purified from bovine 
brain.15 Porcine aortic endothelial cells were cultured by previously 
described methods in tissue culture flasks and used during the third 
passage.16 Rabbit vascular (thoracic aorta) smooth muscle cells 
cultured in Dulbecco's modified Eagle's medium (DMEM) containing 
10% FCS.

**Cell harvesting.** Cell monolayers were dissociated by exposure 
(2 to 3 minutes at 22°C) to trypsin. When cells were rounded 
(monitoring by inverted-phase contrast microscope), the trypsin 
solution was removed and 5 mL medium supplemented with 10% 
serum was added to inhibit trypsin. Cells were washed three times 
with 20 mmol/L Tris-HCl, 130 mmol/L NaCl, pH 7.4 (TBS). After the 
third wash, the cells from each dish were resuspended in 0.1 to 
0.2 mL of 50 mmol/L Tris-HCl, 2.5% glycogen, pH 8.5. Phenyl- 
methyl sulfonyl fluoride (PMSF) (0.2 mmol/L) was added immediately 
before sonication to limit proteolysis. In a few experiments, 
cells were removed from tissue culture dishes by scraping with either 
a rubber policeman or a cell scraper after the monolayers were 
waished with media.

**TG assay.** Washed and resuspended cells were sonicated three 
times at 4°C for 15 seconds at 40% output using a model W-220 
sonicator, Heat Systems-Ultrasonics, Farmingdale, NY. The 
protein concentration of the sonicated cells was determined using 
the BioRad Protein Assay. Cell sonicates (10 to 100 μg) were assayed 
for TG activity in presence of either 5 mmol/L CaCl₂ or 10 mmol/L 
EDTA, as described previously.18

**Aagarose gel electrophoresis.** Sonicates of cultured ABAE cells 
were incubated with DEAE-Sepharose in TBS and washed. The 
protein band to the DEAE was eluted with 0.4 mol/L NaCl. The 
eluate was subjected to electrophoresis in gels cast from N,N'-di- 
dimethylacrylamide (0.3%) and agarose (1%) in 75 mmol/L barbital 
buffer containing 1 mmol/L EDTA.19 After electrophoresis, the gels 
were stained for TG activity using monodansylcadaverine.17

**Immunoblotting of ABAE cell TG.** guinea pig liver TG, factor 
XIII, fibrinogen and $\alpha_2$-plasmin inhibitor, antigens after sodium 
dodecyl sulfate-polyacrylamide gel electrophoresis. The ABAE 
cell sonicates or guinea pig liver TG were taken up in a solution 
containing 1% sodium dodecyl sulfate (SDS), 1.5 mol/L urea, 2.5 
mmol/L EDTA, 30 mmol/L Tris, 5% 2-mercaptoethanol, and 
0.001% bromophenol blue, and were boiled for 5 minutes. The 
material was then fractionated by slab-gel electrophoresis in an 8% 
polyacrylamide gel. The gel was stained with Coomassie blue and then 
destained. Purified fibrinogen was electrophoresed under similar 
conditions and served as markers for $\alpha$, $\beta$, and $\gamma$-chains. High- 
and low-mol-wt protein mixtures (BioRad) were electrophoresed simul- 
taneously.

**Identification of fibrinogen cross-linking pattern.** ABAE cell 
sonicate (12.5 μg) was incubated with purified fibrinogen (60 μg) in 
the presence of 25 mmol/L DTT, 1.0 mmol/L calcium chloride at 
22°C for 60 minutes. The reaction was stopped as described in the 
preceding section. SDS-PAGE was performed in an 8% polyacryl- 
amide gel. The gel was stained with Coomassie blue and then 
destained. Purified fibrinogen was electrophoresed under similar 
conditions and served as markers for $\alpha$, $\beta$, and $\gamma$-chains. High- 
and low-mol-wt protein mixtures (BioRad) were electrophoresed simul- 
taneously.

**Identification of TG activity in vascular tissues.** Human saph- 
eneous vein (672 mg) was cut into 2-mm pieces and homogenized five 
times in 3.0 mL of 0.1 mol/L Tris-HCl, pH 8.5, using a Tissumizer 
(Tekmar, Cincinnati) at 80% output for 30 seconds. The homoge- 
inate was sonicated three times for 15 seconds at 60% output 
using a cell sonicator. The sonicated material was centrifuged for 
15 minutes at 10,000 g, and the supernatant was filtered through a 
Millex-HA 0.45 μmol/L filtration unit. All procedures were per- 
formed at 4°C. The final extract, which had a protein concentration 
of 3.72 mg/mL, was kept at 4°C until assayed. The enzyme was 
assayed within 2 hours of homogenization. All other tissues were 
homogenized and sonicated as described above.

**Immunohistochemistry of TG and fibrinectin antigens in human 
tissues.** Immunoperoxidase testing was performed by the standard 
“ABC” method as previously described21 using frozen sections of 
various human and animal tissues. When liver was used, endogenous 
bioin binding was first blocked using excess avidin as described.22 Sections 
were stained with diaminobenzidine and 0.03%

**Distribution of TG in human liver was compared with that of 
fibronecin. In these experiments, frozen sections were cut as above 
and, after preincubation with normal goat serum, were stained with
rabbit antifibrinectin antisera at a dilution of 1:2000, followed by biotinylated goat anti-rabbit IgG, and then avidin and biotinylated horseradish peroxidase. Normal rabbit serum and/or mouse monoclonal antibodies were also substituted as controls. Tissues for these studies came from the frozen bank in the immunopathology laboratory at Duke University Medical Center.

**Enzyme-linked immunosorbent assay for tissue TG.** Enzyme-linked immunosorbent assay (ELISA) for guinea pig liver TG was conducted as described by Birckbichler et al. 

**Effect of TG-catalyzed cross-linking of α2-plasmin inhibitor to fibrinogen on plasminolysis.** Fibrinogen (32 μg) was incubated with confluent ABAE cell sonicate (5 μg cell protein) and α2-plasmin inhibitor (4 μg) in a total volume of 50 μL of 0.02 mol/L Tris-HCl, pH 7.4, containing 130 mmol/L NaCl and 5 mmol/L calcium chloride, for 60 minutes at 37 °C. In some incubations, 10 mmol/L dithiothreitol was also present. Reactions were also performed in the presence of 5 mmol/L EDTA. The reactions were stopped by 1% SDS, 1.5 mol/L urea, 2.5 mmol/L EDTA, 30 mmol/L Tris, 5% 2-mercaptoethanol, and 0.001% bromophenol blue, and boiled for 5 minutes. Samples were separated by SDS-PAGE on a 6% to 15% linear polyacrylamide gradient and subjected to immunoblotting as described previously.

ABAЕ cell sonicate (10 μg) was incubated with fibrinogen (100 μg) and purified α2-plasmin inhibitor (10 μg) in a total volume of 50 μL of 0.02 mol/L Tris-HCl, 0.13 mol/L NaCl, pH 7.4, 5 mmol/L CaCl2, for 30 minutes at 37 °C. Similar reactions were performed either in the presence of 5 mmol/L EDTA or by omitting one of the components from the assay. Fibrinogen was precipitated by proazime sulfate (2 mg/mL), and washed by centrifugation in 0.1 mol/L Tris-HCl, pH 7.4, containing protamine sulfate 2 mg/mL. Fibrinogen was resuspended in 0.02 mol/L Tris-HCl, 0.13 mol/L NaCl, pH 7.4, at a concentration of 0.4 mg/mL and incubated with plasmin (0.005 to 0.16 CU/mL) for 10 minutes at 37°C. Samples were solubilized in 1% SDS, 1.5 mol/L urea, 2.5 mmol/L EDTA 30 Tris, pH 6.8, and electrophoresed on a 4% to 15% gradient SDS-polyacrylamide gel. Gel was stained with Coomassie blue and dried, and the degradation of the fibrinogen was quantitated by scanning densitometry. The absorbance of the fibrinogen bands in the presence of plasmin was divided by the absorbance of fibrinogen in the absence of plasmin and multiplied by 100 to yield the percentage degraded.

**RESULTS**

Enzymatic properties of vascular TG. A thrombin-independent, calcium- and thiol-dependent TG activity was detected in sonicates of ABAE and VSM cells by incorporation of [3H]putrescine into dimethylcasein. The ABAE and VSM TG activity was maximal between pH 8.5 and 9.0. The TG assay was sensitive and detected 40 ng of either purified guinea pig TG, human factor XIIIa, or the TG activity in 1 to 10 μg of ABAE or VSM cell protein. Incubating sonicates of ABAE and VSM cells with 1 mmol/L N-ethylmaleimide or 1 mmol/L iodoacetic acid inhibited 98% of the TG activity. TG activity was not detected in the culture media before or after ABAE cells were cultured for 10 days.

Immunoblotting using polyclonal anti-factor XIII a-chain antisera did not detect the factor XIII antigen in 200 μg of confluent ABAE cell protein. This immunoblotting technique was capable of detecting 50 ng of purified human or bovine factor XIII. Thrombin, which is necessary for the activation of plasma and platelet factor XIII, did not modify the TG activity of ABAE cell sonicates. In contrast to factor XIIIa, which initially catalyzes γ-chain cross-linking of fibrinogen, the vascular TG selectively cross-linked the α-chains of fibrinogen and not the β- or γ-chains (Fig 1).

More than 90% of the TG activity remained in the cytoplasmic fraction of confluent ABAE cells after centrifugation for 60 minutes at 150,000 g. The presence of calcium chloride (5 mmol/L) or EDTA (5 mmol/L) had no effect on the distribution of the TG from disrupted ABAE cells after centrifugation. To determine whether there was latent TG activity in the particulate or soluble fraction of ABAE cell sonicates, each fraction was incubated with 0.5% NP-40 or 0.5% Triton X-100 (Sigma, St Louis) to solubilize the TG. The nonionic detergent treatment did not change the TG activity in either fraction.

Presence of TG in vascular tissues and cells. TG activity was also detected in homogenates from both human and several other mammalian blood vessels (Table 1). Thrombin was not required for the expression of the TG activity from any of the cells or tissues. The TG activity was only detected in fresh sonicates of tissues and cells and was destroyed by freezing at −70 °C unless glycerol (25% vol/vol) was present during freezing. TG activity was also detected in a variety of other cultured vascular cells, including human umbilical vein endothelium (Table 1). Aortic endothelial and smooth muscle cells could be the major source of TG activity in bovine aortic tissues since they had a higher specific activity than the original tissue. The specific activity of confluent ABAE cells remained constant for up to 15 cell passages. However, the specific activity of nonconfluent cells was sevenfold lower (data not shown). Results for all the cells described in Table 1 were from postconfluent cells.

Immunologic characterization of vascular TG. Immunologic similarity between ABAE cell TG and guinea pig liver TG was demonstrated by immunoblotting techniques.

**Fig 1.** Pattern of fibrinogen cross-linking by confluent ABAE cell sonicate (lane A) and purified platelet factor XIIIa (lane B). The mobility of the mol wt standards are shown × 10−3 on the right margin. Purified fibrinogen was incubated with either ABAE sonicate or purified platelet factor XIIIa at 22°C for 1 hour. The incubation mixture was separated by SDS-PAGE as described in the text. Location of the Aα-, Bβ-, and γ-chains of fibrinogen as well as the γγ- and αα dimers are shown on the left margin.
Fig 2. Detection of tissue TG antigen in confluent ABAE cell sonicates by SDS-PAGE and immunoblotting. Confluent ABAE cell sonicate (200 μg) from passage 8 (lane 1) and purified guinea pig liver TG (2 μg) (lane 2) were subjected to SDS-PAGE and Western blotting as described in the text. Monoclonal antibody (CUB-7401) was used to locate the TG antigen on the nitrocellulose membrane. Mobility of the mol-wt standards are shown × 10⁻³ on the right margin. Top arrow, origin; bottom arrow, distance of bromphenol blue dye migration.

Fig 3. Effect of CaCl₂ and MgCl₂ concentration on ABAE cell TG activity. Fifty micrograms of protein from sonicates of ABAE cells was incubated with increasing concentrations of CaCl₂ (-----), MgCl₂ (-----) or MgCl₂ in presence of 0.1 mmol/L CaCl₂ (-----). The amount of [³H]-putrescine incorporated into dimethylcasein was determined after 30 minutes of incubation at 37°C. EDTA (1 mmol/L) was added to each reaction mixture to chelate unbound divalent cations. The CaCl₂ and MgCl₂ concentrations added to the reaction mixtures were 1.0 mmol/L higher than the desired final concentration.

Table 1. Distribution of TG Activity in Cells and Tissues

<table>
<thead>
<tr>
<th>Tissue/Cell</th>
<th>Specific Activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues</td>
<td></td>
</tr>
<tr>
<td>Human abdominal aorta</td>
<td>0.80</td>
</tr>
<tr>
<td>Porcine abdominal aorta</td>
<td>2.64</td>
</tr>
<tr>
<td>Rabbit abdominal aorta</td>
<td>2.15</td>
</tr>
<tr>
<td>Rabbit inferior vena cava</td>
<td>4.10</td>
</tr>
<tr>
<td>Bovine abdominal aorta</td>
<td>1.96</td>
</tr>
<tr>
<td>Cells</td>
<td></td>
</tr>
<tr>
<td>Porcine aortic endothelial cells</td>
<td>2.24</td>
</tr>
<tr>
<td>Porcine VSM cells</td>
<td>1.82</td>
</tr>
<tr>
<td>Rabbit VSM cells</td>
<td>1.30</td>
</tr>
<tr>
<td>Adult ABAE</td>
<td>122.00</td>
</tr>
<tr>
<td>Bovine VSM</td>
<td>36.40</td>
</tr>
</tbody>
</table>

Tissues and cells were homogenized, sonicated and assayed for enzyme activity as described in the Materials and Methods section.

*Specific activity is defined as nanomoles of putrescine incorporated into casein by 1 mg of cell protein in 1 hour at 37°C.

using the culture supernatant from a hybridoma cell line producing monoclonal anti-guinea-pig liver transglutaminase antibody (CUB-7401). The confluent ABAE cell extract revealed a major 79 ± 3-kd band that migrated slightly ahead of the guinea pig liver TG (lane 1; Fig 2). Purified guinea pig liver TG migrated as a single 85 ± 9-kd protein under these conditions (lane 2, Fig 2). Tissue culture media before culturing ABAE cells did not have any detectable TG antigen. However, the culture supernatant did have a very small amount of TG antigen that migrated as a higher mol wt antigen ~150 kd (data not shown). The TG antigen in rabbit VSM, porcine aortic endothelial cells, and an extract of porcine aortic tissue also migrated as an 80-kd antigen. Occasionally, lower mol wt TG antigens were detected in the sonicates of ABAE cells. Inclusion of protease inhibitors in the cell lysis buffer did not completely reduce the appearance of these lower mol wt antigens. The confluent ABAE sonicates did not react with the culture supernatant from a nonreactive hybridoma cell line (CUB-11). The monoclonal antibody (CUB-7401) did not cross-react with either factor XIII, fibrinogen, or fibronectin. The ELISA assay detected 0.3 ng TG antigen in 100 ng cell protein.

Chromatographic and electrophoretic characterization of vascular TG. High-pressure liquid chromatography (HPLC) gel filtration, DEAE column chromatography, and casein-agarose electrophoresis were used to ascertain whether there was a single functional form of TG in ABAE cells. TG activity in confluent ABAE cells eluted as a single peak from the HPLC gel filtration column with the same retention time (RT = 7 minutes) as purified guinea pig liver TG. More than 90% of the ABAE TG activity adsorbed to and eluted from a DEAE-Sephacel column as a single peak at a NaCl concentration (0.35 to 0.38 mol/L), similar to purified guinea pig liver TG.

Identification of TG activity after electrophoretic separation in nondenaturing casein-agarose gels has been used by others to characterize the TG from various tissues. ABAE cell TG partially purified by DEAE-Sephacel chromatography and purified guinea pig liver TG migrated in casein-agarose gels as a single band with an Rf of 0.6 relative to bromphenol blue dye. In contrast, purified factor XIIIa migrated more slowly, with an Rf of 0.1.

Effect of divalent cations on TG activity. We found a unique effect of calcium and magnesium ions on the TG in ABAE cells (Fig 3). A very narrow range of calcium ion
concentration was required for optimum TG activity; and when this range was exceeded, a reduction in TG activity was observed. When magnesium ions were substituted for calcium ions, TG activity was not detected. However, in the presence of suboptimal concentrations of CaCl₂ (0.1 mmol/L), MgCl₂ promoted ABAE cell TG activity (Fig 3). This was consistently observed in five experiments, using both confluent ABAE and VSM cells. The Mg²⁺-dependent increase in TG activity was also studied using partially purified ABAE cell TG obtained from ABAE cells following HPLC gel filtration. Magnesium chloride (1 mmol/L) and 0.5 mmol/L CaCl₂ increased TG activity to 64% of the level achieved by 1 mmol/L calcium chloride. Such an effect was not observed with purified guinea pig liver TG.

Identification and localization of TG antigen in human tissues. When human skin was analyzed by immunohistochemistry, the TG antigen was confined to the blood vessels in the dermis. A section of two blood vessels from the skin showed intense staining along the endothelial portion of a medium-sized vein (Fig 4, arrow). In the vessel of a medium-sized artery, the TG antigen localized mainly to the smooth muscle cell layer (Fig 4). A medium-sized artery showed positive staining with anti-TG monoclonal antibody along the endothelial surface. Staining was also observed along the surface of smooth muscle cells when a cross-section of the vessel was studied (data not shown). Human and rabbit aorta also stained for TG antigens in the endothelium, VSM cells, and interstitial connective tissue of the intima and media (data not shown).

Although TG was purified from guinea pig liver >10 years ago, the location of this enzyme in the liver was never established. We have detected TG antigen lining the sinusoids of human liver (Fig 5) as well as rat and guinea pig livers (data not shown). There was no detectable staining of hepatocytes, and the nonreactive antibody (CUB-11) produced no immunoreactivity (Fig 5). When liver tissues were stained with antifibrinogen antibody, a reaction occurred similar to the pattern observed with the TG, suggesting that both proteins were distributed in the same tissue compartment (Fig 5).

Effect of ABAE cell lysates on plasmin degradation of fibrinogen. The ABAE cell TG cross-linked purified α₂-plasmin inhibitor to fibrinogen (Fig 6A and B). The high-mol-wt complexes (Fig 6A) were not observed when α₂-plasmin inhibitor or CaCl₂ was omitted from the reaction or when the reaction was conducted with 5 mmol/L EDTA. Fibrinogen–α₂-plasmin inhibitor complexes with mol wt of 132, 195, 260, and >260 kd were identified using either antifibrinogen or anti-α₂-plasmin inhibitor antibody. Because these complexes reacted with both antibodies and since the α-chain (67 kd) is the preferred cross-linking site in fibrinogen for the ABAE TG (Fig 1), we believe these complexes to be α₂-plasmin inhibitor cross-linked to the α-chain monomers or α-chain polymers of fibrinogen. The complexes of mol wt ≥ 260 kd represent α₂-plasmin inhibitor cross-linked to α-chain polymers. The exact stoichiometry of α₂-plasmin inhibitor cross-linked to fibrinogen cannot be determined from these results.

ABAE cell sonicates also made fibrinogen resistant to plasmin degradation. In the absence of either TG, calcium ions, or α₂-plasmin inhibitor, plasmin (0.005 CU/mL) degraded 50% of the fibrinogen (Fig 6B). After α₂-plasmin inhibitor was cross-linked to fibrinogen by ABAE cell TG, the plasmin concentration to degrade 50% of the fibrinogen was increased 40-fold (0.20 CU/mL) (Fig 6B). The formation of the cross-linked complexes between fibrinogen and α₂-plasmin inhibitor and the resistance of modified fibrinogen to plasmin degradation were unaffected by dithiothreitol in the cross-linking reaction (data not shown).

DISCUSSION

Although previous investigators have detected TG activity in homogenates of rabbit aorta, the TG was neither biochemically characterized nor immunohistochemically localized. Cultured ABAE and VSM cells contain a thrombin-independent TG with biochemical and immunologic properties similar but not identical to guinea pig liver TG. This protein was biochemically and immunologically distinct from factor XIIIa. Both the vascular and guinea pig liver TG enzymes preferred uncharged polyamines (pH optimum 8.5 to 9.0), required a free sulfhydryl group for activity, reacted with polyclonal and monoclonal antibodies specific for guinea pig liver TG, were predominantly cytoplasmic enzymes, selectively cross-linked the α-chains of fibrinogen, and eluted from DEAE columns under similar conditions. Both enzymes were also inhibited in a noncompetitive manner by GTP.

We observed a novel Ca²⁺/Mg²⁺ effect on TG activity in confluent ABAE cells. With suboptimal concentrations (0.1 mmol/L) of calcium ions, an Mg²⁺-dependent increase in TG activity occurred. To our knowledge, this is the first observation of this type of Ca²⁺/Mg²⁺ effect on TG activity. The Ca²⁺/Mg²⁺ effect was not observed with purified guinea pig liver TG, demonstrating that this was not an artifact of our assay system. Since the Ca²⁺/Mg²⁺ effect was less prominent when the partially purified ABAE TG was stud-
transglutaminase in vascular cells

*Fig. 5. Frozen section of human liver stained by immunoperoxidase with an unreactive antibody (A), and with anti-fibronectin (B), and anti-TG (C). Although hepatocytes do not stain, the edges of the hepatic sinuses stained intensely (S) with both fibronectin and TG. These two proteins also display a similar pattern of stromal localization without epithelial staining in the portal areas (arrowheads).*

ied, Mg$^{2+}$ ions may be modulating ABAE cell TG activity by directly binding to the TG or binding through an intracellular cofactor. A delicate balance between intracellular Ca$^{2+}$/Mg$^{2+}$ in the milieu of the TG may be a mechanism for regulating the intracellular activity of this enzyme. Mg$^{2+}$ ions have been reported as capable of partly replacing the Ca$^{2+}$ requirement of partially purified liver TG.

The mol wt of the ABAE TG as determined by SDS-PAGE was ~5 kd lower than the guinea pig liver TG. The two proteins may be synthesized similarly but processed differently within the cells. Variability in the mol wt of the vascular TG could be due to intracellular proteases. Lacking amino acid sequences, we conclude that the two enzymes are similar but not identical.

The vascular tissues that contained the TG antigen included human aorta, renal capillaries, and hepatic arteries and veins, as well as arterioles and venules in the skin. Using immunohistochemistry, we found that the TG and fibronectin antigens codistribute along the sinusoids of human liver. At the light microscopic level, it is not possible to determine whether the sinusoidal localization of the TG is the result of its presence in sinusoidal endothelial cells, binding to the extracellular matrix, or its presence in the sinusoidal membranes of hepatocytes. Hepatocytes may also synthesize guinea pig liver TG but not have sufficient intracellular antigen to be detected by the monoclonal antibody. Either hepatic endothelial cells or hepatocytes may synthesize the protein and secrete it into the extracellular matrix where it...
may be bound to fibronectin. Fibronectin is a substrate for the plasma TG factor XIII3,26 and the tissue TG.26 The monoclonal antibody used in this study does not cross-react with human factor XIII, fibrogen, fibronectin, collagen, or bovine actin. Fibronectin is synthesized and secreted into extracellular tissues by vascular cells.27 A similar mechanism may account for the TO distribution in vascular tissues. The immunohistochemical results represent the first demonstration of the in vivo distribution pattern of the TO antigen in human tissues. Preliminary electronmicroscopic immunohistochemistry studies suggested that the TO antigen was associated with the hepatic sinusoidal cells and not the hepatocytes (D. Bainton and C.S. Greenberg, unpublished observations).

The vascular TG described in this study is distinct from the plasma, TG factor XIIIa. Neither thrombin-dependent TG activity nor factor XIII-related antigen was detected in the cells and tissue preparations used in the present study. Furthermore, the vascular TG (partially purified or crude cell sonicates) did not cross-react with polyclonal rabbit anti-factor XIII antibody. Finally, in contrast to γ-chain cross-linking of fibrinogen by factor XIIIa, the vascular TG preferentially cross-linked the α-chains to form dimers, mol wt = 132,000. Purified guinea pig liver TG also selectively cross-linked the α-chains of fibrinogen. These results demonstrate that the α-chain of fibrinogen is the preferred substrate for the ABAE cell and guinea pig liver TG. The ABAE cell TG also catalyzed the cross-linking of α2-plasmin inhibitor to the α-chains of fibrinogen. This is the first demonstration that a tissue-derived TG can function to cross-link α2-plasmin inhibitor to fibrinogen. The modified fibrinogen was nearly 40-fold more resistant to plasminolysis as compared with native fibrinogen. We have obtained similar results using the purified guinea pig liver TG (C.S. Greenberg and K.E. Achyuthan, unpublished observations).

The demonstration of a thrombin-independent TG in vascular cells and tissues that can utilize fibrinogen as a substrate provides a mechanism by which insoluble cross-linked fibrinogen could be deposited in normal and atherosclerotic vascular tissues.7 In addition, α2-plasmin inhibitor cross-linked to fibrinogen by the vascular TG might function to protect fibrinogen from plasmin degradation and then lead to accumulation of fibrinogen in the atherosclerotic plaques.7 The vascular TG could play an important role in fibrinolysis and could also provide an alternate pathway to promote fibrin stabilization following vascular injury.

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The transglutaminase in vascular cells and tissues could provide an alternate pathway for fibrin stabilization

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