Tissue Factor as a Link Between Wounding and Tissue Repair

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The initial phase of wound repair involves inflammation, induction of tissue factor (TF), formation of a fibrin matrix, and growth of new smooth muscle actin (α-SMA)-positive vessels. In diabetes, TF induction in response to cutaneous wounding, which ordinarily precedes increased expression of vascular endothelial growth factor (VEGF) and α-SMA transcription, is diminished, though not to a degree causing excessive local bleeding. Enhanced TF expression in wounds of diabetic mice caused by somatic TF gene transfer increased VEGF transcription and translation and, subsequently, enhanced formation of new blood vessels and elevated blood flow. Furthermore, increased levels of TF in wounds of diabetic mice enhanced wound healing; the time to achieve 50% wound closure was reduced from 5.5 days in untreated diabetic mice to 4.1 days in animals undergoing TF gene transfer (this was not statistically different from wound closure in nondiabetic mice). Thus, cutaneous wounds in diabetic mice display a relative deficiency of TF compared with nondiabetic controls, and this contributes to delayed wound repair. These data establish TF expression as an important link between the early inflammatory response to cutaneous wounding and reparative processes. Diabetes 54:2143–2154, 2005

Physiologic wound healing involves a complex interaction between cells, mediators, growth factors, and cytokines. The cascade of events starts with activation of the procoagulant pathway and recruitment of inflammatory cells and is followed by a phase of cellular proliferation and tissue repair/resolution of the injury. Recent studies (1–3) indicate that tissue factor (TF), the major initiator of the extrinsic coagulation cascade, is involved in all phases of the host response to wounding, implying a likely central role for TF in wound healing.

TF is an immediate early gene. TF transcription, which is upregulated when cells start to divide (4,5), is controlled by a variety of transcription factors associated with the inflammatory response such as SP-1, activator protein-1 (AP-1), and nuclear factor-κB (NF-κB) (6–11). As might be expected, TF expression is also closely linked to inflammation (6,7,9) and hypoxia (10), both conditions also associated with wounding. The physiologic function of rapid induction of TF, a member of the class II cytokine receptor family, in response to a range of stimuli has been less clear. The major function of TF is believed to be related to initiation of the procoagulant pathway (11–14). In fact, as TF is plentiful in the subendothelial and, especially, subcutaneous spaces, contact of blood with these tissue surfaces, a part of the hemostatic process, rapidly results in clot formation. Thus, hemostasis does not require de novo TF synthesis, implying that immediate early upregulation of TF is important in processes other than hemostasis (1,11–15).

Consistent with this view, recent studies have pointed to functions of TF that are quite distinct from its role in coagulation. Depending on the genetic background, TF−/− mice not only display severe defects in hemostasis but also die at an early embryonic stage for reasons other than bleeding (16–18). Defective vitello-embryonic vasculature (16) in TF−/− mice suggests a link between TF and angiogenesis (19). Recent studies (20–22) have shown that TF, either directly or indirectly (via binding of factor VIIa and activation of the coagulation cascade) (23,24), triggers a number of cellular responses. The latter include de novo synthesis of vascular endothelial growth factor (VEGF) (24–26); recruitment of signal transduction pathways, in part through changes in cytosolic calcium (27); transient tyrosine phosphorylation (28); mitogen-activated protein kinase activation (29); and gene transcription (24).

While most of these data were obtained using cells stably transfected to overexpress TF, fewer studies have addressed the importance of TF-dependent gene regulation. In this context, one example is the positive correlation between TF and VEGF (1,25,26), consistent with the concept that low levels of TF might be associated with diminished VEGF expression. These observations led us to
explore pathophysiologically relevant situations in which decreased cell proliferation and angiogenesis might be explained by a relative impairment in local TF induction.

Delayed healing of cutaneous wounds in diabetes is associated with reduced VEGF expression (30) and impaired recruitment of smooth muscle cells (31). Thus, such wounds in diabetic mice might provide an ideal setting to determine whether local induction of TF might be linked to the subsequent proliferative and angiogenic response. Furthermore, the inflammatory response, characterized by cytokine expression and recruitment of immunocompetent inflammatory cells (32) and critical for initiating wound healing, has been shown to be impaired in diabetes (33,34). We reasoned that reduced expression of inflammatory cytokines after cutaneous wounding of diabetic animals might result in reduced cytokine-dependent induction of TF (8,35–37). If this proved to be true, then reduced TF in diabetic wounds might, in turn, be followed by decreased angiogenesis, diminished recruitment of smooth muscle cells, and delayed wound healing in diabetics. We have tested these concepts in a murine model of diabetes using somatic cell transfer of the TF gene to drive local upregulation of TF.

**Table 1.** Primers used in RT-PCR experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
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<tbody>
<tr>
<td>mGAPDH-F</td>
<td>5′-TGAAGCTCGTTAGAGGATGGAGATT GG-3′</td>
<td>5′-CATGAGCCCATGAGGCCCCACAC-3′</td>
</tr>
<tr>
<td>mGAPDH-R</td>
<td>5′-CATGAGCCCATGAGGCCCCACAC-3′</td>
<td>5′-TCCCTCTTGACCAGAGACCTTGT-3′</td>
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<tr>
<td>mTF-41-F</td>
<td>5′-GCCGTTACCCTACCTCGCCTCCCTCGG-3′</td>
<td>5′-TTTCCCTTGGGAGAGGACCTTGT-3′</td>
</tr>
<tr>
<td>mTF-47-R</td>
<td>5′-TTTCCCTTGGGAGAGGACCTTGT-3′</td>
<td>5′-AACACCTTTCTAAACCTCGCCT-3′</td>
</tr>
<tr>
<td>hTF 1567-U</td>
<td>5′-AAATGTCCTGGCTCAGAATC-3′</td>
<td>5′-AAATGTCCTGGCTCAGAATC-3′</td>
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<tr>
<td>mVEGF164-F</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
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<tr>
<td>mVEGF164-R</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
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<tr>
<td>α-SMA-F</td>
<td>5′-GACCTCATGACCTTCGTGCTTCTTTGGG-3′</td>
<td>5′-GACCTCATGACCTTCGTGCTTCTTTGGG-3′</td>
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<tr>
<td>α-SMA-R</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
<td>5′-CCATGACCTTCGTGCTTCTTTGGG-3′</td>
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**LaCz staining.** Transfection with a reporter-plasmid expressing β-galactosidase (pβ-gal-Gal; Promega, Heidelberg, Germany) was performed as described above. α-gal immunohistological staining was performed according to the protocol provided by the manufacturer. Briefly, the wound sample was sectioned and fixed in 37% formaldehyde fixing solution for 2 h at 4°C. After washing twice in PBS, the sample was incubated in staining solution (20 mg/ml X-Gal) for 2 h at 37°C for colorimetric development.

**Western blotting for luciferase expression.** Transfection with a reporter-plasmid expressing luciferase (pGL3-luc; Promega) was performed as described above. Wounds were harvested and immediately frozen in liquid nitrogen. The snap-frozen skin was trimmed of any adhering fat and subcutaneous tissue. The sample was homogenized in 1 ml of lysis buffer (50 mmol/l Tris-HCl [ph 8.0], 150 mmol/l NaCl, 0.02% sodium acid, 1% Triton X-100, 1 mmol/l leupeptin, 2 mmol/l benzamidin, 20 μg/ml soybean inhibitor, 1 μg/ml aprotin, and 0.5 mmol/l phenylmethylsulfonyl fluoride). Hair and other insoluble material were removed by centrifugation at 4,000 rpm for 2 min, before the supernatant was subjected to four freeze-thaw cycles, followed by a 20-min incubation on ice with several vortex steps (10 s each) and a final centrifugation for 5 min at 15,000 rpm. Protein concentration in the supernatant was determined using the bichinonic acid assay kit according to the manufacturer’s instructions (Pierce Biotechnology, Rockford, IL). A total of 20 μg of total protein extracts were separated onto 10% SDS-PAGE, followed by electrophoretic transfer to nitrocellulose membranes. Membranes were incubated with a primary antibody for luciferase (1:1,000; Promega) for 60 min at RT. After washing (2 × 10 min in TBS, 0.05% Tween), the secondary antibody (horseradish peroxidase–coupled donkey IgG, 1:5,000; Santa Cruz, Heidelberg, Germany) was added, and incubation was continued for 30 min at room temperature. Membranes were washed 4 × 10 min as above. Immunoreactive proteins were detected with the enhanced chemiluminescence–Western blot system (Amersham-Pharmacia, Freiburg, Germany) and subsequent autoradiography for 10 min.
TABLE 2
Primers used in real-time PCR experiments

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer 5′-3′</th>
<th>Reverse Primer 5′-3′</th>
</tr>
</thead>
<tbody>
<tr>
<td>mGAPDH</td>
<td>5′-AACGACCCTCTCATGGAC-3′</td>
<td>5′-TGCAAGCATGATACCAAGAC-3′</td>
</tr>
<tr>
<td>mGAPDH</td>
<td>5′-TCCACCGATCTAGCCGAC-3′</td>
<td>5′-GCCACCTGTCGAGATGTTG-3′</td>
</tr>
<tr>
<td>mTF41-F</td>
<td>5′-GAGGCTCGAGCCGAG-3′</td>
<td>5′-CCAAGAGAGAAGAGTGAC-3′</td>
</tr>
<tr>
<td>mTF41-R</td>
<td>5′-GATAAAATGGTGCCGACCAGA-3′</td>
<td>5′-GACCAACCTTTTTTATCTCCC-3′</td>
</tr>
<tr>
<td>Exo-mTF-1600F</td>
<td>5′-GACGCCAAGATTTTTTATTTGACCAGA-3′</td>
<td>5′-GACGCCAAGATTTTTTATTTGACCAGA-3′</td>
</tr>
<tr>
<td>mTF-1820-R</td>
<td>5′-GCCACCAGATCCACGAGA-3′</td>
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Quantitative real-time RT-PCR. Real-time PCR was performed on a LightCycler (Roche, Mannheim, Germany), as described by Galiano et al. (40). First, 3 μl of cDNA, 20 pmol of each primer, and 125 units Taq polymerase (Promega) were used in each reaction at a final volume of 50 μl. The primers used are summarized in Table 2. Cycling conditions were (95°C, 5 min); (94°C, 1 min; 50°C, 2 min; 72°C, 2 min); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); (94°C, 1 min; 50°C, 3 s; 72°C, 2 s); and (94°C, 1 min; 50°C, 3 s; 72°C, 2 s). The expected PCR products were 191 bp for mTF, 189 bp for VEGF, and 218 bp for α-SMA. The expected PCR products were 191 bp for mGAPDH, 230 bp for mTF, 189 bp for VEGF, and 218 bp for α-SMA. To provide the required standards for real-time PCR, these fragments were cloned using the TOPO TA cloning kit, amplified using the PCR purification kit (Qiagen), and verified by sequence analysis. Serial dilutions of each purified product were made and standard curves obtained on a real-time PCR cycler (Qiagen), and verified by sequence analysis. The resulting standard curves were exported into the RelQuant software (Version 1.01, Roche) to establish individual standard curves (coefficient file), allowing evaluation of the final data including normalization to GAPDH and PCR efficiency correction.

Statistics. Values are expressed as means ± SD. Student’s t test was used to determine statistical significance. The antibody-stained sections were scored based on the strength of the brown product present. The designations used were no staining (−), light or weak staining (+), strong staining (−−), and intense staining covering most of the cellular tissue and matrix (−−−). Consecutive sections that stained without antibodies were used as negative controls for specific staining. Staining was analyzed independently by J.C., A.B., and M.K.

RESULTS

Responses of nondiabetic and diabetic mice to cutaneous wounding. When wound healing was studied, nondiabetic and diabetic NOD mice were subjected to a standardized procedure for cutaneous wounding. The time needed to achieve 50% closure of the wound increased from 3.6 ± 0.98 days in nondiabetic to 5.5 ± 1.92 days in diabetic mice (P = 0.002). Wound tissue was recovered at different time points, and the expression profile of TF mRNA was determined (Fig. 1A, B top row, C, and D). Rapid induction of TF (~3.2-fold) occurred in nondiabetic mice (P = 0.046 at 1 h, 0.027 at 3 h) (Fig. 1C), while no significant induction occurred in diabetic mice (Fig. 1D).

After the increase in TF transcripts, VEGF induction occurred in nondiabetic mice (Fig. 1E), rising approximately twofold above the baseline within 12 h, however, without reaching statistical significance. Levels of VEGF transcripts were maintained for 24 h, thereafter slowly decreasing at day 7, a time when wound healing was almost complete. In contrast, tissue from diabetic mice subjected to cutaneous wounding displayed a small, but not significant, reduction in VEGF transcripts not before 12 h after wounding (Fig. 1F) and further increased at day 3 when the wound in the diabetic animal was ~47% closed. Thus, TF and subsequent VEGF gene expression in wounds from nondiabetic animals could be contrasted with lack of TF and VEGF induction in diabetic animals within the first hours of wounding.

Similar results were observed when the transcription of α-SMA mRNA was analyzed (Fig. 1A, B, G, and H). Within 1 h of wounding, nondiabetic mice displayed an apparent, but not significant, induction of α-SMA mRNA (P = 0.25), while upregulation of α-SMA transcripts was absent in diabetic mice. These data are consistent with the hypothesis that attenuation of the inflammatory response in diabetic wounds (33,34), possibly decreasing the content of proinflammatory cytokines, may contribute to the blunted induction of TF and angiogenesis.

Somatic TF gene transfer induces expression of TF transcripts in wounds of diabetic mice. To monitor efficacy of somatic gene transfer, wounds were transfected with human TF (hTF) plasmid DNA instead of mouse TF
(mTF) plasmid to avoid cross-reactivity with endogenous mTF transcripts. These experiments demonstrated that the transfected plasmid was still transcribed in wounds 7 days after transfection (Fig. 2A). Next, wounds were transfected with plasmids overexpressing a luciferase reporter gene. Wounds were harvested on consecutive days (days 0–12), and luciferase expression levels of luciferase were determined in a course of 0–12 days by Western blot analysis. Strong luciferase gene expression was observed between day 1 and day 3 (Fig. 2B) and, to a lesser extent, was still evident until day 7. To circumvent the limitations of Western blot analysis in its sensitivity...
and the inability to localize the expressed antigen at the cellular level (e.g., to a subset of transfected cells), immunohistochemistry was performed after delivering a plasmid carrying LacZ into the wound. Expression of LacZ was still evident 7 days later (Fig. 2C). The above evidence confirmed that topical somatic gene transfer is an effective method to allow gene expression in wounds of mice.

Thereafter, somatic gene transfer was used to elevate TF expression in diabetic wounds, in order to assess the relationship between TF and impaired VEGF and α-SMA induction. Wounds in diabetic mice were harvested 1 day after somatic gene transfer with either vector alone or TF cDNA cloned into pcDNA3. Somatic gene transfer using the TF overexpressing plasmid resulted in increased levels of TF (Fig. 3A, P < 0.004). This change in expression of TF transcripts, following transfer of the TF cDNA, was paralleled by induction of VEGF (P = 0.014) and α-SMA mRNA (P = 0.14) in diabetic mice compared with mice treated with vector alone. Studies in nondiabetic mice displayed a much smaller increase in TF, VEGF, and α-SMA transcripts (data not shown). Real-time PCR with samples randomly chosen at time points day 1 and day 7 from vector and TF transfected wounds confirmed these results (Fig. 3B), although it failed to show a significant VEGF induction at a marginal level.

To further study the effect of TF expression on VEGF and α-SMA, levels of TF antigen expression were determined on day 2, and subsequent VEGF and α-SMA antigen were analyzed on day 7 in diabetic animals (Fig. 4). Normally, the intact granular cell layer expresses TF, whereas the basal layer of the epidermis and dermal connective tissue is weak to negative (42). In diabetic wounds treated with the TF expression vector, TF antigen was more prominent at the migrating epidermal tip compared with vector controls. This suggests successful transfection and overexpression of TF in keratinocytes. In addition, the number of fibroblasts and inflammatory cells positive for TF antigen increased in the wounded area of mice treated with the TF expression vector compared with the neighboring skin, where they were mostly negative (Fig. 4A).

Seven days after TF gene transfer, VEGF expression was higher in the diabetic wound than in neighboring tissue (Fig. 4B). A significant increase in VEGF antigen (P = 0.017) was observed in the TF-transfected wounds compared with wounds treated with vector alone (Fig. 4B). In diabetic wounds exposed to TF expression vector, VEGF-positive cells appeared to be distributed according to a gradient of antigen expression, with highest levels at the wound edge and decreasing signal intensity toward the...
wound center (Fig. 4B, middle). This pattern of VEGF expression was not observed in vector control–treated wounds. In the latter case, there was no significant increase in VEGF antigen either at the wound edge or in the center (Fig. 4B, top). Cells expressing VEGF in TF-transfected diabetic wounds were keratinocytes, fibroblasts, and smooth muscle cells. Increased immunostaining for VEGF antigen at the edge of TF-transfected diabetic wounds corresponded to sites of increased staining for TF antigen (Fig. 4A). Upregulation of TF, by somatic gene transfer into diabetic wounds, also increased expression of α-SMA antigen (Fig. 4C). Immunostaining of TF-transfected wounds with α-SMA antibody showed cells expressing α-SMA antigen to be mostly concentrated around vessels. About 49% more α-SMA positive vessels were seen in TF-transfected wounds (Fig. 4C, bottom) than in vector-transfected wounds (Fig. 4C, top) (P = 0.037). Furthermore, fibrin/fibrinogen-deposition was increased in wounds from mice transfected with TF (Fig. 5). Since the antibody used did not discriminate between fibrin and fibrinogen, the wounds were extensively washed before staining to remove residual fibrinogen. The reactive material represented at least in part fibrin, since we observed striking differences in fibrin/fibrinogen deposition between the vector- and TF-transfected wound that correspond to the data obtained for TF, VEGF, α-SMA, and macroscopic wound healing in the differently transfected mice. Thus, overexpression of TF results in enhanced VEGF and α-SMA transcription and expression and increased fibrin deposition in diabetic wounds.

Overexpression of TF results increases vascularization of diabetic cutaneous wounds. The functional significance of the local TF delivery was shown by injecting ink into the vascular space to highlight dermal blood vessels (Fig. 6A). In both vector- and TF-treated diabetic wounds, the density of vessels was high at the wound edge, forming a vascular rim around the wound periphery. TF-transfected diabetic wounds (Fig. 6A, bottom) showed a more prominent protrusion of vasculature to the wound center, as well as an apparent elevation of vessels at the center, compared with vector-transfected wounds from the same mouse (Fig. 6A, top). Hematoxylin-eosin staining of the wounds from diabetic animals treated either with vector (Fig. 6B, top) or TF (Fig. 6B, bottom) demonstrated...
an increase in the number of vessels per microscopic field in TF-transfected mice. Furthermore, the density of blood vessels in TF-transfected wounds (Fig. 6C) was 65% higher ($P = 0.035$) than in vector controls. To analyze blood flow through the wound area, microspheres were injected into the left ventricle. After dissolving the wound tissue, the TF-treated diabetic wounds displayed 92% more beads ($P < 0.05$) per gram of tissue than vector-treated wounds (Fig. 6D).

**TF gene transfer accelerated wound healing in diabetic mice.** The effect of enhanced TF expression in wounds was not only evident at the level of induction of
VEGF and α-SMA, and an increase in newly formed vessels, but also with respect to macroscopic appearance of the wound and accelerated closure (Fig. 7A–C). To evaluate the effect of TF on wound healing in situ, wound diameters in nondiabetic mice or diabetic mice either treated with vector or TF were measured daily in anesthetized mice. The boundary of the migrating tip of the neoepidermis was taken as wound edge. Wound size on a given day was compared with the original area determined immediately after skin injury. Wound closure reflected the area covered by the neoepidermis as a result of reepithelialization. Transfer of TF resulted in more rapid closure of diabetic wounds compared with vector-treated control wounds. After 3 days, TF-treated wounds displayed a significantly (P = 0.02) accelerated reduction in wound size compared with vector-treated controls (both of which were placed on the back of the same diabetic mouse) (Fig. 7A).

When the median time required to reach 50% wound closure was determined, this time was reduced from 5.5 days in vector-treated diabetic mice to 4.1 days in TF-treated animals (Fig. 7C) (P = 0.048). Wound closure in diabetic mice subjected to TF gene transfer was almost comparable to that observed in nondiabetic control mice: the time lag before 50% wound closure in diabetic TF-treated mice was 4.1 days and did not differ significantly from 3.6 days in nondiabetic animals (P = 0.321). These observations suggested that increased local expression of TF in diabetic wounds drives processes relevant to tissue repair, potentially linking early steps in the inflammatory phase to the proliferative and resolution stages.

DISCUSSION
Previous reports (33,34) have shown that the inflammatory phase of wound repair is delayed/diminished in diabetes. The results reported herein indicate that early induction of TF in wounds is an essential component of the host response and is also attenuated in a murine model of diabetes. This relative paucity of TF in diabetic wounds appears to result from decreased expression of TF in keratinocytes, fibroblasts, and smooth muscle cells rather than simply reflecting decreased influx of TF-bearing mononuclear phagocytes, leading us to hypothesize that
conditions leading to TF induction in the wound are suboptimal. Elevating TF expression in diabetic wounds by local somatic gene transfer increased VEGF, α-SMA synthesis and fibrin deposition, enhanced formation of neovascularure, and, ultimately, normalized wound closure. Thus, the induction of TF in the early phase of the host response to wounding appears to link the initial inflammatory phase to later stages of the healing process. These observations add to a growing body of literature indicating that dysregulation of TF, because of excessive expression, as in septicemia; disseminated intravascular coagulation (43–45); atherosclerosis (46,47) and thrombosis (48); or deficiency, as in embryonic development in TF−/− mice (16–18,49), has implications for pathologic and/or physiologic processes. The time course of TF induction early after wounding in order to maximally enhance reparative mechanisms provides insight into a physiologic context in which TF functions as an immediate early gene. Expression of TF must occur rapidly after wounding in order to effectively orchestrate subsequent events in repair, including expression of VEGF and α-SMA, and neovascularization.

Somatic gene transfer using TF accelerated wound healing in diabetic but not in nondiabetic (data not shown).
mice despite increasing VEGF levels in each case. This suggests that there is a narrow window of TF and VEGF levels critical for triggering physiologic events, such as wound repair. However, it is important to note that the situation in the diabetic wound is complex, as it has been shown in ob/ob mice that leptin reconstituted VEGF expression in the wound but failed to restore the angiogenic response (50).

Factors underlying impairment of the inflammatory response in diabetic wounds remain to be elucidated. Several studies (33,34) have noted blunted induction of inflammatory cytokines after wounding in diabetes. This is surprising, since sustained activation of the proinflammatory transcription factor NF-κB is a characteristic feature of peripheral blood mononuclear cells derived from patients with diabetes (51). However, the situation after local wounding differs significantly from that in the systemic circulation. Namely, brisk and prominent upregulation of TF expression is required to promote wound repair, whereas long-term low-level activation of circulating mononuclear phagocytes has been observed in the intravascular space. Insight into a potentially significant negative regulator of inflammation in diabetes is derived from a recent in vitro study (52) in which high glucose induced NF-κB activation and expression of endothelial nitric oxide (NO) synthesis. Increased levels of intracellular NO apparently mediated inhibition of endothelial cell migration. Since endothelial TF expression has also been shown to be inhibited in the presence of NO (53), one might speculate that hyperglycemia-induced NF-κB activation might cause generation of NO, which would serve to inhibit TF expression.

It is likely that the effects of TF on wound healing extend beyond enhanced expression of VEGF. For example, induction of α-SMA is likely to contribute to the stability of the newly formed vessels and prevent apoptosis of the growing endothelial cell layer, as previously shown for pericytes (54). In this regard, it is noteworthy that TF has also been shown to affect migration of smooth muscle cells (55) and mononuclear phagocytes (56). Furthermore, TF might also contribute to wound repair by promoting formation of a provisional fibrin matrix facilitating epidermal cell migration (57). Consistently, the effects of TF transfection on VEGF expression, α-SMA induction, and fibrin deposition described here are minor and do not always reach statistical significance, while wound healing (Figs. 6 and 7) is significantly enhanced. Therefore, we hypothesize that the beneficial effects of TF are mediated by an interplay of different processes (VEGF expression, recruitment of α-SMA-positive cells, and fibrin deposition) and not by promoting expression of a single gene.

Although physiologic upregulation of TF in wounds is transient, its effects on the reparative process appear to be long lasting. Generation of a fibrin-rich matrix supporting the cellular response, at the level of cell migration and sequestration of growth factors, is one example of a mechanism through which an early burst of TF following wounding could modulate subsequent events important for healing. This may explain why even though relatively few cells expressed TF after somatic gene transfer (e.g., principally keratinocytes), there was a strong effect on the overall cellular response.

In vivo, the multiple contributions of TF to wound healing are difficult to separate. In addition to hemostasis, TF-mediated activation of the procoagulant cascade might also enhance wound healing through paracrine/hormonal effects of factor Xa (58,59), thrombin (60), and other products of coagulation that have been shown to impact on VEGF and platelet-derived growth factor generation (23,24). Keratinocytes form an activated epithelium that is critical for wound resurfacing (61). In vitro, factor VIIa induces TF-bearing keratinocytes to express genes associated with inflammation, proliferation, angiogenesis, cellular reorganization, and migration. All of these are central to wound healing. Our studies further suggest an important role of keratinocytes in wound healing because of their constitutive expression of TF. Furthermore, expression of VEGF by keratinocytes provides a potential situation in which TF can regulate angiogenesis at the level of the keratinocyte. In this context, TF has been linked to keratin expression in tumor cells (62). Finally, wound repair involves both dermal contraction and epithelial migration, both of which occur in the hemostatic and inflammatory responses (61). Whether delayed wound healing in diabetes involves either of these mechanisms, both of which can be modulated by TF, is not clear. The latter demands for further studies identifying the molecular mechanisms underlying the metabolic regulation of local TF expression in diabetic wounds and to answer the question, whether good glycemic control per se might influence TF expression. Our results clearly indicate that the relative lack of TF induction at the site of diabetic wounds contributes to the impaired reparative response. We propose that upregulation of TF at such local sites, potentially by somatic gene transfer, may enhance the closure of diabetic wounds, at least in settings analogous to our acute murine model.

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