Perivascular tissue factor is down-regulated following cutaneous wounding: implications for bleeding in hemophilia

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Healing of skin wounds is delayed in hemophilia B (HB) mice. HB mice do not bleed excessively at wounding, yet rebleed hours to days later. Tissue factor (TF) expression is up-regulated by inflammatory cytokines and has been linked to angiogenesis. We hypothesized that impaired thrombin generation in HB leads to impaired TF expression following injury. Punch biopsies were placed on wild-type (WT) and HB mice. Tissues from wound sites were immunostained for TF. Blood vessels are normally surrounded by a coat of pericytes expressing TF. Surprisingly, within a day after wounding TF disappeared from around nearby vessels; returning after 8 days in WT and 10 days in HB mice. The granulation tissue filling the wound during healing also lacked TF around angiogenic vessels. Thus, perivascular TF expression is down-regulated during wound healing. This may prevent thrombosis of neovessels during angiogenesis but renders hemophiliacs vulnerable to hemorrhage during healing.

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Results and discussion

Immunostaining of unwounded skin from WT and HB mice showed strong TF staining in the squamous epithelium and around dermal vessels (left panel, Figure 1) as previously reported in human tissues.9,10,19 To our surprise, TF was absent from around vessels near wounds in both WT and HB mice by 1 day after wounding. A section from 2 days after wounding is shown in the right panel of Figure 1. Over time TF expression returned around dermal vessels, as is plotted in the lower panel of Figure 1. By day 8, TF again surrounded all vessels near the wound bed in WT mice. However, reexpression of TF was delayed in the HB mice, with full expression occurring only after 10 days.

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The defect produced by a punch biopsy is filled by a highly vascular stroma called “granulation tissue.” The vessels within the granulation tissue also did not express TF in either WT or HB mice during healing. In contrast to the reappearance of TF around dermal vessels, the newly formed vessels in the granulation tissue lacked TF through the 20 days of the experiment. The lack of TF at the site of a biopsy wound is illustrated in the left panel of Figure 2 from an 8 day old wound. The inset shows a high-power view of a vessel within the granulation tissue demonstrating its lack of TF staining. Strong TF staining (brown color) can be seen in the overlying squamous epithelium above the granulation tissue (right panel). The pattern of staining was consistent in 15 skin samples for TF and 23 for desmin, and was the same for WT and HB mice.

Figure 1. TF immunostaining around dermal vessels near sites of wounding. (Top panels) TF antigen is indicated by the brown color. Representative vessel profiles from WT animals are shown. Normal TF distribution around a dermal vessel is shown in the upper left, and 2 days after wound placement in the upper right. Original magnification 1000×. (Bottom panel) TF expression around vessels within 2 high-power (40×) fields of the wound bed was scored for 3 or 4 mice per time point, and means with SD are plotted. The SD values are 0 for days 0, 8, 10, and 12 for WT mice and 0, 10, and 12 for HB mice because all vessels were surrounded with a TF coat at these times. *P < .05 vs HB.

Figure 2. TF is absent from around vessels in granulation tissue even though pericytes are present. Antigen is indicated by the brown color. Representative sections from WT at day 8 after wounding are shown. TF was not present around vessels in the granulation tissue (left panel), since the intensity of staining was no greater than negative control sections stained with nonimmune rabbit IgG. The squamous epithelium above the granulation tissue does stain for TF. Vessels in the granulation tissue were surrounded by pericytes, as indicated by a layer of desmin staining around the vessels (right panel). The pattern of staining was consistent in 15 skin samples for TF and 23 for desmin, and was the same for WT and HB mice. Original magnification 100× and 1000× for insets.
squamous epithelium. Squamous epithelium stained for TF throughout the healing process, serving as a cellular positive control.

TF around vessels is primarily expressed by pericytes; myofibroblast-like cells that surround the endothelium of capillaries and the muscular layer of arterioles and venules. Therefore, we explored whether the pericytes themselves disappeared from around vessels after wounding. We used the expression of desmin, an intermediate filament found in cells of muscle origin, to identify pericytes. We found that dermal vessels retain their surrounding pericytes after wounding, even while TF is absent (data not shown).

Ensheathment by pericytes stabilizes small vessels. Pericytes also have a role in the process of angiogenesis. Pericytes have been visualized at the tip of growing capillary sprouts, apparently serving as guiding structures. We initially thought that the lack of TF around neovessels in the granulation tissue might reflect a lack of pericyte ensheathment. However, the developing blood vessels in granulation tissue were surrounded by pericytes throughout the healing process (Figure 2 right panel).

At this point we can only speculate on the mechanism of TF disappearance around preexisting vessels. We favor the hypothesis that TF loss might occur by proteolytic shedding. TF is not homologous to coagulation proteins, but is a member of the cytokine receptor superfamily. Soluble forms of many cytokine receptors are formed by regulated proteolytic release from the cell surface. Growth hormone receptor, for example, is structurally related to TF and is shed from cell surfaces by cleavage near the transmembrane domain in response to cell-signaling events. Such regulated shedding could account for the rapid and complete loss of TF antigen from pericytes.

TF antigen could also be removed from pericyte surfaces by endocytosis. While we expect TF antigen would still be detectable within pericytes under these conditions, it might be degraded and the fragments no longer recognized by our antibody. It is also possible that a dramatic conformational change in the TF molecule could reduce its reactivity with our antibody reagent. This is unlikely, because the polyclonal antibody we used was raised to the entire extracellular domain of TF and even recognizes denatured and reduced TF on Western blots.

Mature vessels that provide sites for angiogenic sprouting exhibit vasodilatation, increased permeability and detachment of supporting cells. Endothelial sprouts migrate into the healing wound, acquire lumens and fuse with existing the vasculature. We propose that a lack of TF around angiogenic vessels during wound healing may reflect a mechanism to prevent thrombosis of newly formed, delicate and leaky vessels. This leads to an increased risk of bleeding from granulation tissue that is not a significant liability in normal individuals. However, in hemophilia the absence of TF near wound sites could lead to repeated hemorrhage in and near the wound site, even after the surface defect has been healed.

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Authorship

Contribution: A.G.M. and K.Y. carried out immunostaining and interpreted results; H.R.R. interpreted data; D.M.M. designed research and interpreted data; and M.H. designed research, reviewed histology slides, interpreted data, and wrote the paper.

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