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A hypothesis: Factor VII governs clot formation, tissue repair and apoptosis

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Summary A hypothesis: thrombin is a ''Universal Enzyme of Energy Transduction'' that employs ATP energy in flowing blood to activate biochemical reactions and cell effects in both hemostasis and tissue repair. All cells possess PAR-1 (thrombin) receptors and are affected by thrombin elevations, and thrombin effects on individual cell types are determined by their unique complement of PAR-1 receptors. Disruption of the vascular endothelium (VE) activates a tissue repair mechanism (TRM) consisting of the VE, tissue factor (TF), and circulating Factors VII, IX and X that governs localized thrombin elevations to activate clot formation and cellular effects that repair tissue damage. The culmination of the repair process occurs with the restoration of the VE followed by declines in thrombin production that causes Apoptosis (''programmed cell death'') in wound-healing fibroblasts, which functions as a mechanism to draw wound edges together. The location and magnitude of TRM activity governs the location and magnitude of Factor VIII activity and clot formation, but the large size of Factor VIII prevents it from penetrating the clot formed by its activity, so that its effects are self-limiting. Factors VII, IX and X function primarily as tissue repair enzymes, while Factor VIII and Factor XIII are the only serine protease enzymes in the ''Coagulation Cascade'' that are exclusively associated with hemostasis.

The multiple effects of thrombin

Thrombin is a powerful, multi-functional enzyme whose effects are numerous and diverse [1,2]. It plays a central role in the coagulation process, where it energizes the conversion of Soluble Fibrin to insoluble fibrin including the conversion of Factor X to Factor XIII, the addition of plasminogen and fibronectin cross-links to soluble fibrin by Factor XIII to produce the three-dimensional structure of insoluble fibrin (IF), a natural polymer, and plasminogen stabilization to prevent plasmin attack on the structure of IF, the final product of the ''Coagulation Cascade''. Defects in Factors VII, IX and X and medications such as warfarin that impede thrombin production, plus drugs such as thalidomide that obstruct thrombin effects [3], all inhibit wound healing, tissue maintenance, and coagulation, indicating that thrombin is essential for all these processes [4–6].

More than 95% of thrombin production occurs in the aftermath of clot formation [7], and this may be explained by its role in tissue repair, which involves cellular activity. All animal cells thus far tested possess PAR-1 (thrombin) recep-

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tors that proliferate in the presence of elevated thrombin levels and cause changes in cell size [8], morphology [9,10], metabolism, intracellular caspases and cytoskeleton [11], migration (chemotaxis) [12], mitosis [13], collagen production [14], angiogenesis [15], inflammation [16], and other effects related to tissue repair [1,11,17–19]. Differences in the type and number of PAR-1 receptors determine the specific response of individual cell types to thrombin [1,20]. Thrombin elevations are essential for the activity and survival of some cell types, notably wound-healing fibroblasts [21,22], but may cause death in other cell types, such as neurons [23,24]. Abnormal thrombin elevations may provoke pathological cell proliferation, and extreme thrombin elevations can be lethal to cells [13,23,25,26]. All known thrombin effects require the presence of calcium and ATP [27,28]. The simplest explanation is that thrombin utilizes the energy stored in ATP in flowing blood to activate a wide variety of biochemical reactions and cell activities via a common mechanism that has yet to be characterized, but may be similar to an intracellular mechanism [29]. Many details of thrombin action remain unclear at this time, but available evidence suggests that thrombin is a ''Universal Enzyme of Energy Transduction'' in flowing blood that converts ATP in the presence of Ca^{+} to cell and enzyme effects [30].

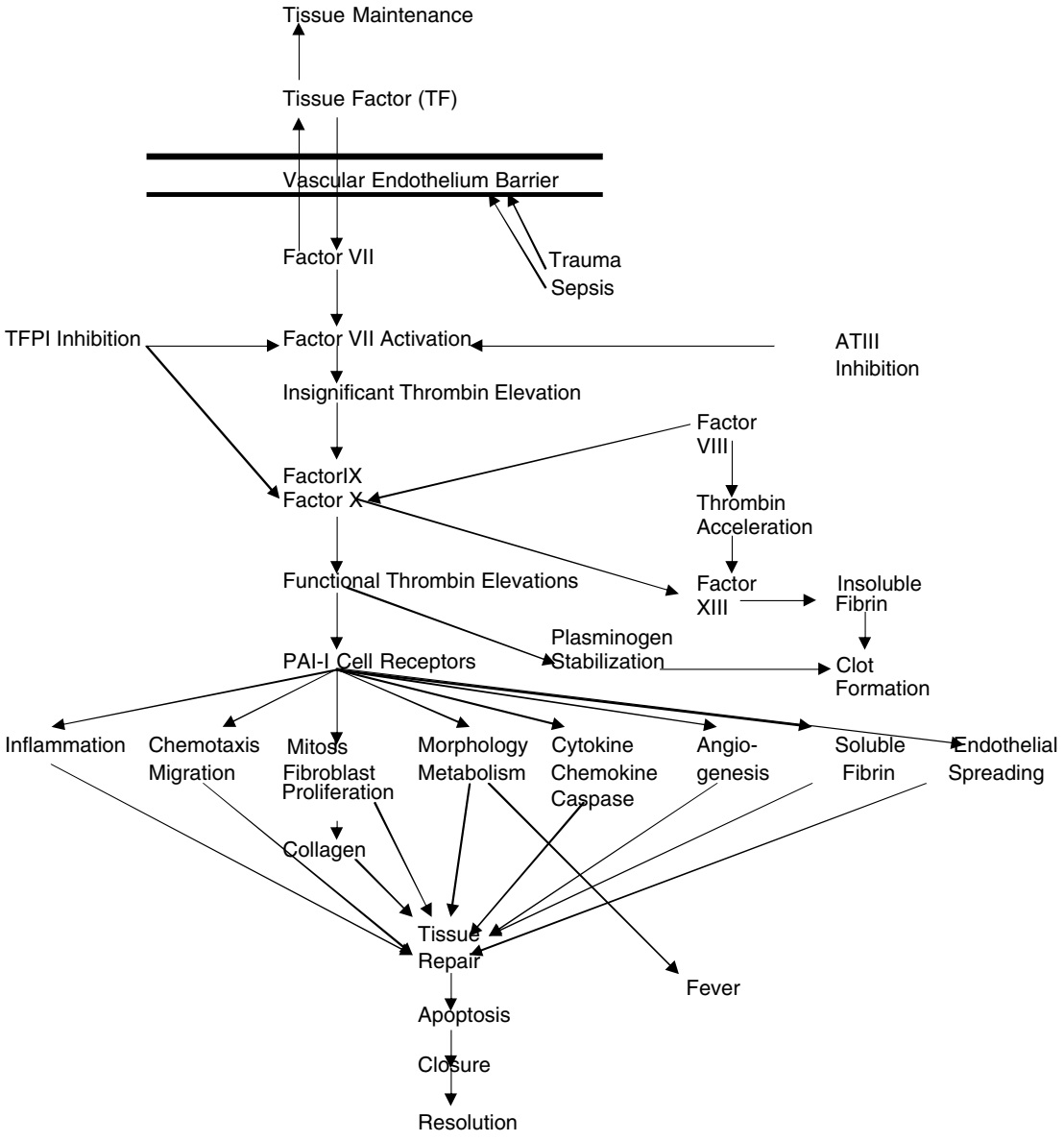

Figure 1 A diagram of the tissue repair mechanism.

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The tissue repair mechanism

In the adult vertebrate body, physical integrity is continuously maintained by a tissue repair mechanism (TRM) consisting of the vascular endothelium (VE), tissue factor (TF), and circulating Factors VII, IX and X that governs localized thrombin elevations. The cellular effects of these thrombin elevations explain all aspects of tissue repair and maintenance. The VE is a one-cell-thick membranous layer that lines the inner walls of all blood vessels and constitutes the sole component of capillary walls. It functions as an ''insulator'' that inhibits contact between TF, a glycoprotein ubiquitous in extravascular tissues, and Factor VII, a serine protease enzyme in flowing blood [31]. Disruption of the VE by trauma, sepsis and other factors increases contact between Factor VII and TF, causing Factor VII ''activation'' and the production of very small amounts of thrombin that is known as the ''initiation'' phase of blood coagulation [32]. During the subsequent ''propagation'' phase Factors IX and X enzymatically amplify the Factor VII thrombin production to functional levels that induce and sustain cell-based TRM activity. The magnitude and location of VE disruption therefore governs the magnitude and location of TRM activity.

The VE barrier is imperfect, so that even in the absence of VE disruption there is continuous ''leakage'' of small amounts of TF through the VE into circulating blood and ''penetration'' of small amounts of Factor VII from flowing blood into extravascular tissues. The ''leakage'' of TF into flowing blood causes ''background'' levels of systemic Factor VII activation and potentially harmful thrombin amplification by Factors IX and X that is quenched by the actions of tissue factor pathway inhibitor (TFPI) [33,34] and stoichiometric ATIII [35,36]. This explains the continuous systemic presence of low levels of activated Factor VII that enables Capillary Gate function [37] The ''penetration'' of Factor VII into extravascular tissues exposes it to TF that causes its activation and the production of small amounts of thrombin that induce slow and continuous cell ''turnover'' and collagen ''replenishment'' that accounts for tissue maintenance. The TRM is shown in diagram form in Fig. 1.

Thrombin and the TRM

Thrombin elevations induce inflammation that loosens cell connections to facilitate the movement of tissue repair cells into damaged tissues [38], increase tissue repair cell mitosis, metabolism and motility [39], and cause chemotaxis of platelets, fibroblasts, neutrophils, leukocytes, monocytes, polymorphonucleocytes and macrophages towards the location of highest thrombin levels [40] that explains how these wound-healing cells move into sites of tissue damage. They stimulate intracellular caspases and cytoskeleton structure [11], phagocytic activity of macrophages, blast transformation of lymphocytes [41], the production of cytokines, chemokines, growth factors, and adhesion molecules by the vascular endothelium [1,42], and endothelial cell spreading and migration [19]. They induces angiogenesis [43] and the proliferation of vascular smooth muscle cells to provide perfusion to cells engaged in the tissue repair process [20]. They cause proliferation of airway smooth muscle cells, an effect that is opposed by glucocorticoids [44]. They cause proliferation of wound-healing fibroblasts [45], myoblasts [46] and osteoblasts [21] and sustain their elevated mitosis, metabolism and collagen production during the tissue repair process to enable granulation tissue to fill wound defects. Thrombin elevations thereby explain all known tissue repair activities.

Thrombin, apoptosis and resolution of the repair process

Elevated thrombin levels are necessary for fibroblast viability and proliferation and to inhibit apoptosis in these cells [21,45–47]. During the ''resolution'' phase of tissue repair, the restoration of VE integrity causes thrombin levels to decline towards normal, whereupon fibroblasts and other tissue repair cell types cease their activities and die in vast numbers. This event known as ''apoptosis'' (programmed cell death) [45,48]. Apoptosis is usually assumed to be controlled via a DNA/RNA signaling pathway, but there is no convincing evidence of such a mechanism. Apoptosis is therefore best explained by declines in thrombin levels that undermine fibroblast viability. In the context of wound healing, it functions as a mechanism that draws wound edges together.

The role of factor VIII

TRM activity remains normal in hemophiliacs, who produce soluble fibrin in abundance and retain the ability to repair and maintain tissue, though

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they lack the ability generate Insoluble Fibrin to form clots and activate capillary hemostasis. If Factor VIII is present, its effects synergize with exposed TRM activity to accelerate thrombin production that activates platelets and enzymatically converts Factor X to Factor XIII to rapidly generate insoluble fibrin to enable clot formation and capillary hemostasis [37,49]. However, the large physical size of Factor VIII prevents it from penetrating the clot formed by its own activity, so that its activity is self-limiting. The continued operation of the TRM associated with the ongoing tissue repair process explains why the majority of thrombin generation occurs after clot formation is complete. Factors VII, IX and X, which are much smaller in size, readily penetrate the formed clot and interact with underlying exposed TF to sustain thrombin generation at lower rates adequate to maintain TRM activity and stabilize plasminogen to maintain clot integrity [50]. The location and magnitude of VE disruption determines the location, magnitude and duration of TRM activity, and TRM activity determines the location and size of clot formation [51].

Tissue repair, as opposed to hemostasis, is the primary function of Factors VII, IX, X. Factors VIII and XIII are the only enzymes in the classical ''Coagulation Cascade'' whose effects are primarily associated with hemostasis, which includes clot formation and Capillary Hemostasis [37,49].

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