Mouse Models in Coagulation

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Review Article

Introduction

In the past six years, analysis of numerous murine models, enabled by investigator-designed manipulation of the mouse genome, has generated an explosion of new, and sometimes confounding, data. Mice with complete deficiencies in virtually all the known factors involved in hemostasis have been produced by targeted modification of the genes that encode the proteins that generate and regulate coagulation. Mice with altered factors or altered expression of normal factors have also been produced by transgenic techniques. In this review, we summarize the analyses of these mouse models and compare these results to those obtained from people with deficiencies in the same genes (Table 1). Information is available for all the models, but we will focus on those more completely characterized. We present the models in groups (Fig. 1), which places each factor into a single position within a simplified model of hemostasis. The phenotypes of these genetically modified mice reflect a compilation of functions, some of which do not fit within this simplified model of hemostasis. The phenotypes of these genetically modified mice reflect a compilation of functions, some of which do not fit within this simplified model of hemostasis. For example, Factor X (FX) not only acts in concert with factor V (FV) to foster clot formation but also acts in concert with tissue factor pathway inhibitor (TFPI) to limit clot formation. Additionally, we know that many factors have functions outside hemostasis; for example, a cleaved fragment of antithrombin III (ATIII) serves as an anti-angiogenic factor (1). Moreover, as shown remarkably by the analysis of these mouse models, many of these proteins also have a critical role in normal embryonic development (Fig. 2). Finally, genetic background significantly influenced the phenotype of some models, and we specify when this variable was examined.

To provide a structure to consider analysis of these mice, we divided the hemostasis proteins into three categories: those that promote the generation of thrombin, those that participate in clot formation, and those that curtail the generation of thrombin. To place these proteins in context, we begin with a brief overview of coagulation. Coagulation is initiated when injury to the blood vessel exposes tissue factor (TF) to circulating factor VII (FVII), resulting in the formation of TF/FVII complexes. This complex activates factor X (FX), either directly or indirectly through FIX. Activated FX (FXa) converts prothrombin to thrombin. Thrombin catalyzes multiple events in coagulation by activating the platelet integrin αIIbβ3, which mediates fibrinogen- and von Willebrand factor (vWF)-dependent platelet interactions, and by transforming soluble fibrinogen molecules into a fibrin fiber matrix. Thrombin further amplifies its own generation by activating factors (FXI, FX, FIX) and cofactors (FV and FVIII) that promote TF-independent production of the active protease FXa. Anticoagulation is achieved through the inhibition of the various steps of thrombin generation. TFPI forms a complex with TF, FVIIa and FXa to terminate the initiating step. Protein Z forms a complex with FXa and serves as a cofactor in the inhibition of FXa activity. Thrombomodulin (TM), an endothelial cell surface-bound protein, and thrombin complex to activate protein C (PC). With its cofactor protein S (PS), activated protein C (APC) degrades FVa and FVIIIa and thus limits generation of thrombin. The serpin, ATIII, forms complexes with FXa and thrombin to directly inhibit these proteases.

Promoting Thrombin Formation

Tissue factor, Factor VII, Factor X, Factor V, Prothrombin, Factor IX, Factor VIII, Factor XI

Tissue Factor (TF): TF is a cell-surface protein that is not normally found on endothelial cells lining the blood vessel but on cells immediately below the endothelial layer. Following vascular injury, the newly-exposed, subendothelial TF binds to circulating FVII to form the TF/FVII protease complex that activates FX and FIX. People with TF deficiencies have never been described, suggesting this protein is essential for life. In fact, three independent groups had difficulty producing TF -/- mice by gene targeting, indicating embryonic lethality in the absence of TF (3-5). TF -/- embryos arrested development between E8.5 and 10.5 (3) and had pools of red blood cells in the yolk sac cavity (3-5). The embryos lacked the large vitelline vessels that connect the yolk sac and embryonic vasculogenesis, and no blood flow was seen in the yolk sac vessels (4). Thus, TF is essential for the assembly of functional vascular structures and the maintenance of vascular integrity in the yolk sac, although the role of TF in vasculogenesis remains unclear. Importantly, the penetrance of the severe TF -/- phenotype varies with genetic background, as a low percentage of embryos survive to birth on a mixed background containing 129SvJ and C57BL/6. Related experiments demonstrated that low levels of TF activity, approximately 1% of normal, were sufficient to rescue the TF -/- mice from embryonic lethality (6).

Studies with transgenic models have elucidated which of two domains within TF is essential for normal development. The extracellular

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domain of TF interacts with FVII to generate FXa and thrombin, and the intracellular domain has recently been implicated in signaling (reviewed in (7, 8)). The transgenic addition of human TF lacking the intracellular domain rescued the TF -/- embryos, suggesting that the signaling activity of the TF intracellular domain is not required for embryogenesis (8, 9). This conclusion was directly demonstrated with a human TF transgene coding for a protein with a mutation in the active site of the extracellular domain; the proteolytic activity of TF was required for rescue of embryonic lethality (9). These results suggest that TF's role in activating proteases, which ultimately results in thrombin generation, is essential for embryogenesis. The function of the intracellular domain remains unclear.

The once simple model of TF function in clot initiation has become quite complex recently. TF expression can be upregulated by inflammatory and angiogenic factors, and TF is implicated in tumor metastasis (reviewed in (7, 10, 11)). Additionally, a ligand for the intracellular domain (actin binding protein 280) has recently been documented (12); further studies with this ligand may identify pathways outside of coagulation in which TF participates. Thus, future studies with the TF mouse models will surely elucidate novel roles for TF beyond the initiation of blood coagulation.

### Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Gene Mutation</th>
<th>Human deficiency</th>
<th>Embryonic Lethal?</th>
<th>Murine Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Promoting Thrombin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF -/-</td>
<td></td>
<td>Partial and complete deficiencies unknown</td>
<td>Yes (E8.5-10.5)</td>
<td>Lacked vascular integrity, red blood cell pools in yolk sac cavity</td>
</tr>
<tr>
<td>FVII -/-</td>
<td></td>
<td>Variable bleeding, thrombosis reported rarely</td>
<td>No</td>
<td>No vascular defects, death caused by hemorrhage in neonates, no survival beyond 24 days</td>
</tr>
<tr>
<td>FX -/-</td>
<td></td>
<td>Variable bleeding; Deficiencies &lt;1% extremely rare</td>
<td>Partial (E11.5-12.5)</td>
<td>No vascular defects, death caused by hemorrhage in neonates, no survival beyond 20 days</td>
</tr>
<tr>
<td>FV -/-</td>
<td></td>
<td>Mild bleeding, complete deficiency unknown</td>
<td>Partial (E9.5-10.5)</td>
<td>Vascular defects, death caused by hemorrhage in neonates, limited survival beyond 2 hours</td>
</tr>
<tr>
<td>FII -/-</td>
<td></td>
<td>Severe bleeding, complete deficiency unknown</td>
<td>Partial (E9.5-11.5)</td>
<td>Inconsistent reports of vascular defects; neonatal hemorrhage, no survival beyond several days</td>
</tr>
<tr>
<td>FIX -/-</td>
<td></td>
<td>Hemophilia B; severe bleeding, spontaneous joint bleeds</td>
<td>No</td>
<td>Hemorrhagic swelling of feet, death from internal bleeding after injury and normal fighting</td>
</tr>
<tr>
<td>FVIII -/-</td>
<td></td>
<td>Hemophilia A; severe bleeding, spontaneous joint bleeds</td>
<td>No</td>
<td>Spontaneous bleeding only after partial tail amputation</td>
</tr>
<tr>
<td>FXI -/-</td>
<td></td>
<td>Mild bleeding</td>
<td>No</td>
<td>Tendency for increased bleeding time, prolonged APPT</td>
</tr>
<tr>
<td><strong>Clot Formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fbg -/-</td>
<td></td>
<td>Severe bleeding associated with complete deficiency</td>
<td>No</td>
<td>Neonatal hemorrhage, cannot support pregnancy, strain influences survival</td>
</tr>
<tr>
<td>γA5 Fbg</td>
<td></td>
<td>Never recorded</td>
<td>No</td>
<td>Impaired platelet aggregation, increased bleeding time</td>
</tr>
<tr>
<td>V/F IV Fbg</td>
<td></td>
<td>Venous thrombosis in heterozygotes</td>
<td>No</td>
<td>Heterozygotes appear normal, neonatal hemorrhage in homozygotes</td>
</tr>
<tr>
<td>Elevated Fbg</td>
<td></td>
<td>Correlated with various pathologies, thrombosis</td>
<td>No</td>
<td>No gross defects, normal histology in lung, liver, spleen and kidney</td>
</tr>
<tr>
<td>NF-E2 -/-</td>
<td></td>
<td>Never recorded</td>
<td>No</td>
<td>Absolute thrombocytopenia, hemorrhage in neonates, less than 10% survive to adulthood</td>
</tr>
<tr>
<td>vWF -/-</td>
<td></td>
<td>Type III vWD; severe bleeding, reduced FVIII levels</td>
<td>No</td>
<td>Prolonged APPT, reduced FVIII, neonatal hemorrhage, GI bleeding</td>
</tr>
<tr>
<td><strong>Curtailing Clot Formation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TPPI -/-</td>
<td></td>
<td>May be thrombotic (121), complete deficiency unknown</td>
<td>Yes (E9.5-11.5)</td>
<td>Liver fibrin deposition, rarely intravascular thrombosis</td>
</tr>
<tr>
<td>TM -/-</td>
<td></td>
<td>Thrombosis, complete deficiency unknown</td>
<td>Yes (E9.5)</td>
<td>Overall retardation in growth, maternal environment negatively affects embryonic survival</td>
</tr>
<tr>
<td>TM Pro/Pro</td>
<td></td>
<td>Never recorded</td>
<td>No</td>
<td>Fibrin deposition in lungs, heart, spleen, and liver; phenotype worse upon thrombogenic challenge</td>
</tr>
<tr>
<td>PC -/-</td>
<td></td>
<td>Disseminated intravascular coagulopathy (DIC) as neonates</td>
<td>No</td>
<td>DIC by E12.5, no survival beyond 24 hours after birth</td>
</tr>
<tr>
<td>FV Leiden</td>
<td></td>
<td>Thrombosis</td>
<td>No</td>
<td>APC resistance, fibrin deposition, strain influences survival</td>
</tr>
<tr>
<td>AT III -/-</td>
<td></td>
<td>Thrombosis, complete deficiency unknown</td>
<td>Yes (E15.5-16.5)</td>
<td>Subcutaneous and intracranial hemorrhaging, thrombotic damage to embryonic heart and liver, DIC</td>
</tr>
<tr>
<td>PZ -/-</td>
<td></td>
<td>May be thrombotic (103), complete deficiency unknown</td>
<td>No</td>
<td>No overt phenotype</td>
</tr>
</tbody>
</table>

The once simple model of TF function in clot initiation has become quite complex recently. TF expression can be upregulated by inflammatory and angiogenic factors, and TF is implicated in tumor metastasis (reviewed in (7, 10, 11)). Additionally, a ligand for the intracellular domain (actin binding protein 280) has recently been documented (12); further studies with this ligand may identify pathways outside of coagulation in which TF participates. Thus, future studies with the TF mouse models will surely elucidate novel roles for TF beyond the initiation of blood coagulation.

**Factor VII (FVII):** FVII is the only known ligand for TF's essential extracellular domain. Therefore, the most significant finding from FVII -/- mice is their phenotypic difference from the TF -/- mice. The FVII
mice on the same genetic background as the TF -/- mice were born in a normal Mendelian pattern, demonstrating that FVII deficiency did not affect survival to term (13). The FVII -/- mice had normal vascular development. These data are consistent with the maternal transfer hypothesis that predicts that the loss of a membrane-bound molecule would be more detrimental to the organism than the loss of a soluble one. The investigators speculated that maternal FVII might circulate in the embryo and obscure the FVII -/- phenotype. However, the procoagulant activity of the embryos’ murine FVII was undetectable (<0.05% of adult FVII activity), and human recombinant FVII did not cross the placenta beyond supraphysiological plasma levels (13). Thus, maternal transfer may not explain the phenotypic differences between FVII -/- and TF -/- mice. It remains possible, however, that a critical threshold level, which was below detection in FVII -/- embryos, was sufficient for proper development. Other data do suggest that very low levels of some hemostatic factors are sufficient to support normal development (6, 14). Conversely, it is possible that while TF is important in generating thrombin, it has other roles in development independent of clot initiation. Support for this independent function comes from the observation that TF is abundantly expressed in human embryos prior to accumulation of FVII protein (15).

The majority of FVII -/- neonates died from intra-abdominal bleeding within 24 h of birth, and the remaining mice died within 24 days from intra-cranial hemorrhage. The neonatal bleeding observed in the FVII -/- mice seems a good model for the human deficiency. FVII-deficient patients, none of whom totally lack FVII, have variable degrees of severity of bleeding and, rarely, thrombotic tendencies (16). The massive hemorrhage in FVII -/- mice indicates that the human and mouse proteins have similar functions.

**Factor X (FX):** Circulating FX is activated either by TF/VIIa or FIIa/FVIII. Active FX binds to FV to form the procoagulate complex FXa/FV that activates prothrombin to thrombin, and this is the only known activator of thrombin. Since the loss of thrombin in mice is partially lethal (discussed below), it was expected that the absence of FX would also be lethal. Indeed, approximately one-third of the FX -/- mice died in utero by E12.5 (17). The majority of the null embryos were resorbed. Those remaining showed signs of bleeding, including

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**Fig. 1** Proteins involved in hemostasis. For simplicity, each protein is placed under one of three categories: factors that promote thrombin formation, factors that participate in clot formation, and factors that curtail thrombin formation.

**Fig. 2** Mouse development. This succinct description of murine embryogenesis emphasizes vascular development because several murine models are defective in this specific process (for a review of mouse development, see (2)). The gestation period is between 19–20 days. Implantation of the blastocyst occurs at embryonic day (E) 4.5 and shortly thereafter, gastrulation begins. By E7.5 the three germ layers of the embryo have been formed, and the tissues required for embryomaternal interactions are developing. These tissues go on to form the placenta and the extraembryonic yolk sac. Blood islands are first seen in the yolk sac; these islands give rise to both blood cells and endothelial cells. The yolk sac vasculature is developed at E8 and primitive red blood cells are contained within the yolk sac vessels. Meanwhile, intraembryonic vessels form as the yolk sac vasculature develops, and at E8.5 the two vascular beds fuse. By E9, the heart is formed and beats regularly, circulating red blood cells through the yolk sac and embryonic vasculature. Vascular development progress, and major and minor vessels form. Organogenesis is prominent between days E10-E14, bone formation begins between E14-E15, and the fetus continues to grow and develop until birth at E19.
intracranial hemorrhage, but no vascular defects were seen in the yolk sac. The cause of the developmental block between E11.5 and E12.5 remains unresolved. The FX -/- embryos that survived to term died within 20 days, due to severe bleeding.

**Factor V (FV):** Homozygous disruption of the FV gene also resulted in partial embryonic lethality (18). At E9.5, a subset of FV -/- embryos showed multiple anomalies such as overall growth and developmental delay, defects in cardiac muscle, focal hemorrhage, and collapsed vascular channels in the yolk sac with fewer blood islands than normal. The authors concluded that partial lethality at E9.5-10.5 was likely a result of impaired vasculogenesis in the yolk sac. The FV -/- mice that survived to birth (approximately half) died within 2 h due to massive intra-abdominal hemorrhage. When <0.1% FV was expressed with a FV transgene in FV -/- mice, some survived to adulthood without hemorrhage, demonstrating that quite low levels of FV may be sufficient for hemostasis (14). As discussed in the introduction, activated protein C inactivates FVa to limit thrombin production. A prevalent mutation in FV affecting the efficiency of this inactivation is associated with clinically significant thrombosis in the population and is known as FV Leiden. Targeted replacement of the FV gene with the mouse homolog of FV Leiden is described under the section on anticoagulation.

**Prothrombin (FII):** Similar to disrupting the activators of prothrombin (FX and FV), the homozygous deletion of the prothrombin gene resulted in partial embryonic lethality between days E9.5-11.5 (19, 20). In the FII -/- embryos, the vessels were not filled with fetal blood cells and free blood was found in the yolk sac cavity (19). These observations suggest a loss of vascular development, but further ultra-structural examination of the cell layers comprising the yolk sac showed no abnormalities in E9.5 FII -/- embryos. In contrast, Xue et al. observed no bleeding in the yolk sac, yet found signs of abnormal vascular development (20). Although these differences remain unresolved, genetic variation may explain the differing phenotypes as mice in the first study were of 129/Black Swiss background and mice in the latter study were C57BL/6J. Nonetheless, both studies showed that the loss of FII resulted in a partial embryonic lethal phenotype with at least 50% of FII -/- embryos dying between E9.5 and E11.5. Furthermore, FII -/- mice that survived to term (approximately 20-25%) died within a few days of birth, due to massive hemorrhagic events.

The partial lethality associated with the FII -/- mice and several other coagulation factors involved in the activation of prothrombin (TF -/-, FX -/-, and FV -/-) suggests that active thrombin is essential for development. These factors may represent the most direct pathway to produce low levels of thrombin in the embryo. The similarities in some of the phenotypes associated with their loss (vascular defects) suggest that active thrombin is essential in vasculogenesis. However, it is unclear, then, why FVII -/- mice do not have an analogous phenotype and why FX -/- mice do not also have vascular defects. Phenotypic differences might be explained if certain players in hemostasis have more (or less) important roles in the embryo than those currently defined for the adult. Embryonic differences are evident in studies of the temporal expression of hemostasis-related proteins in murine embryos (21) and studies using a FVII inhibitor in embryos (13). Further analyses of these mouse models should provide insights into the function of the coagulation factors in developmental processes.

**Factor IX (FIX) and Factor VIII (FVIII):** Circulating FIX is activated by TF/FVIIa and binds to circulating factor VIII (FVIII) to form the FIXa/FVIIIa protease complex that activates FX. As in humans, the FVIII and FIX genes are both located on the X chromosome in mice. The FIX -/- mice had hemorrhagic swelling of the feet or footpads due to spontaneous bleeding (22). Normal fighting between littermates often led to massive hemorrhage and death. Partial amputation of the tail was fatal if the wound was not cauterized. Splenomegaly, a sign of anemia, was often observed upon autopsy. In contrast to FIX -/- mice, the FVIII -/- mice had a milder bleeding disorder (23, 24). These mice did not have spontaneous bleeding; a bleeding phenotype was only observed after partial tail amputation, which was fatal in roughly 2/3 of the affected males examined.

In humans, FVIII deficiency (hemophilia A) and FIX deficiency (hemophilia B) have nearly identical clinical presentations (25). The symptoms vary from person to person, but severe bleeding and spontaneous joint bleeds are common. Bi et al. suggest that the difference between the relatively mild phenotype in FVIII -/- mice and hemophilia A may reflect an inherent difference between the human and mouse proteins, since only 2 of 66 anti-human factor VIII monoclonal antibodies cross-react with mouse FVIII (24). Nevertheless, these mouse models should be useful to explore the feasibility of gene-therapy for humans, as successful studies have been reported with both murine hemophilia models (26-28). The non-essential roles of FVIII and FIX in human and murine development correlate with the observation that there is little or no expression of FVIII and FIX early in murine development, when other factors that generate thrombin are highly expressed (21). Additionally, expression of the factors in human embryos suggests that both factors play less important roles in the embryo than the adult (29). Since these factors serve to amplify thrombin when large amounts of the protease are needed, they are likely not involved in the direct pathway used to generate thrombin during embryogenesis for vasculogenesis.

**Factor XI (FXI):** Factor XI is activated by thrombin and, as an active protease, it further enhances thrombin production by activating FIX. The amplification role of FXI is somewhat analogous to the functions of FVIII and FIX, and so it might be predicted that the loss of FXI would not result in developmental arrest. Indeed, the FXI -/- mice were viable, and homozygous females were able to deliver pups. The mice had prolonged APTTs and normal prothrombin times (PTs) (30), as expected. A bleeding time assay from tail transections indicated that the median bleeding time was slightly longer in the FXI -/- mice than heterozygous or wild type mice. Because murine and human FXI proteins were found to be quite similar (31), it is not unanticipated that humans with a deficiency in FXI have comparable mild phenotype, with variable symptoms mainly related to bruising and post-operative bleeding (32).

Studies have suggested that FXI has a role outside of clot formation, and may serve to protect clot dissolution (33, 34) and play a role in thrombosis (35). These studies suggest that the loss of FXI could have a protective effect against thrombosis. Indeed, when mice with a combined FXI and protein C (PC) deletion were generated, the loss of FXI improved the survival of thrombotic PC neonates (discussed in more detail below). Future crosses of the FXI -/- mice with other models of spontaneous thrombosis will likely define FXI’s role in fibrinolysis and thrombotic disorders.

**Discussion/Conclusion**

The embryonic lethality in TF -/-, FV -/-, FII -/-, and FX -/- mice strongly suggests that thrombin generation is critical for normal development. Complete deficiency of TF, FV or FII has never been reported in humans, and cases with less than 1% FX activity are extremely rare (6, 18, 36, 37). However, individuals with low levels of FV, FX or FII have been reported and in these cases there are variable degrees of bleeding. The symptoms are all mild relative to the fatal bleeds ob-
served in the null mice that survived to birth. As mentioned above, together the results indicate that low levels of these factors are necessary and sufficient for life.

Is it their role in coagulation that makes these proteins essential for life? Two clues indicate that it is not. First, mice lacking even the most essential components of a clot, fibrin (38, 39) or platelets (40) have no developmental defects (discussed in more detail below). Second, vascular anomalies were observed in several models, indicating problems independent of coagulation. These findings support the hypothesis that the generation of thrombin is important for vascular development during embryogenesis. Further, it has been speculated that the generation of thrombin may serve as a “leak detector” for developing blood vessels (41). For example, if a vessel is not patent, then TF from underlying, non-endothelial cells may contact FVII in plasma to initiate thrombin generation. This thrombin might serve to signal through a receptor on a nearby endothelial cell that the vessel is not fully developed and result in local morphological responses by the endothelium.

Other in vivo evidence also suggests that thrombin signaling through endothelium may be essential to vascular development. The loss of the PAR-1 thrombin receptor, expressed on endothelium, causes a partial embryonic lethal phenotype with abnormal bleeding and yolk sac vasculature (42, 43). Of the four known PARs, both PAR-1 and PAR-2 are expressed on endothelial cells. As PAR-1 is highly expressed in endothelium by E 9.5, it is likely the major mediator of thrombin signaling in vascular cells (44, 45). In fact, when PAR-1 was re-expressed only in the endothelium of PAR-1 -/- embryos, there was a decrease in embryonic lethality, suggesting that the vascular defects in PAR-1 -/- embryos were due to its loss in endothelial cells and not from the perivascular mesenchyme (43). The partial embryonic lethality of the PAR-1 -/- embryos suggests that other PARs, possibly PAR-2, may be able to compensate for the loss of PAR-1. For instance, in the presence of TF and FXa, picomolar concentrations of FVIIa can activate PAR-2 and to a lesser extent, PAR-1 (46). It is possible that the combined loss of both PAR-1 and PAR-2 would have severe consequences for vasculogenesis. The joint loss of PAR-1 and FV results in a more lethal phenotype than the loss of either gene alone; Griffen et al. propose that these data suggest that the PAR-1 and FV pathways interact, yet other targets and agonists in these pathways remain ambiguous (43). Therefore, it is likely that while thrombin signaling through PAR-1 is important in vascular development, there may be other prototases in the cascade that signal through the PARs or yet unidentified receptors.

In vitro studies also provide evidence for a bridge between coagulation and vascular development. The regulation of vascular endothelial growth factor (VEGF), the primary growth factor involved in both vasculogenesis and angiogenesis, and PAR signaling have been linked; this regulation was shown to involve TF, FVII, FXa, and thrombin (47). Studies demonstrating that VEGF can upregulate TF and thrombin expression or conversely, can be regulated by these proteins, indicate the complexity of the interactions between the hemostatic proteins and vascular-specific proteins (48-54). Combined, the insights from the in vivo and in vitro data reinforce the opinion that the hemostatic proteins, particularly those that regulate thrombin levels, constitute far more than an independent system to control the generation of fibrin.

Not all factors that serve to promote thrombin generation play roles in embryogenesis. FVIII, FIX, and FXI are not essential to generate small amounts of thrombin; rather, these factors serve to enhance or magnify its generation. Therefore, it is not unanticipated that the loss of these factors did not cause developmental problems. Nonetheless, variable bleeding phenotypes were associated with the loss of these proteins, as predicted from human phenotypes. Defects in hemostasis were also observed in those mice that survived development. The disparity between early developmental defects and later death secondary to hemorrhage is most clearly evident in mice lacking factors X, V, and II; some embryos escape the developmental bottleneck, but these “survivors” die from massive bleeding in the peri- /postnatal period. These results suggest that the arrest of bleeding in embryos in utero is either not essential until the time of birth or is controlled differently in embryos than neonates. Little is still known about hemostatic factors in embryos but evidence that birth is a traumatic situation requiring an intact hemostatic system is demonstrated by the improved long-term survival of embryos lacking platelets if delivered by cesarean section (40).

**Clot Formation**

**Fibrinogen, Platelets, von Willebrand Factor**

**Fibrinogen:** Fibrinogen is composed of two copies of three polypeptides, Aα, Bβ-, and γ, encoded by three genes. Homozygous disruption of one gene, either Aα or γ, resulted in total loss of fibrinogen expression (38, 39). Embryonic development was normal, and, surprisingly, most homozygous mice survived to adulthood. About one third of Aα -/- females could not support pregnancy due to fatal vaginal bleeding. Aα +/- females were normal. In vitro studies with plasma from Aα -/- mice demonstrated complete loss of platelet aggregation. Nevertheless, following ferric-chloride injury to arterioles, in vivo platelet deposition and thrombus initiation were normal (55). Unlike normal controls, where vessel occlusion occurred at the site of ferric-chloride injury, vessel occlusion in Aα -/- mice was embolic and occurred downstream from the site of injury, implying that fibrinogen is essential in platelet-vessel wall interactions.

While mouse models of fibrinolytic deficiencies are beyond the scope of this review, it is of interest to mention the plasminogen deficient mice in the context of fibrinogen deficiency. Since plasmin (activated form of plasminogen) is the major protease responsible for lysing insoluble fibrin, the Plg -/- mice represent a situation in which excess fibrin causes a severe phenotype. Plg -/- mice developed normally to birth, but multiple symptoms appeared with maturity (56-58). They had rectal prolapse, fibrin deposition in the liver, runting, and they had severely delayed lysis of induced pulmonary emboli. The Plg -/- mice also had impaired wound healing (59, 60) and conjunctival lesions, analogous to the ligneous conjunctivitis seen in plasminogen deficient humans (61). When Plg -/- mice were bred onto a fibrinogen null background, the mice were phenotypically similar to fibrinogen null mice (62), and the pathologies associated with the Plg -/- mice were corrected. These studies suggested that the major physiological role of plasminogen was to degrade fibrin. Nonetheless, a subsequent study of toxic liver injury in these doubly deficient mice demonstrated that persistent, centrilobular lesions characteristic to the Plg-/- mice were not corrected by the loss of fibrinogen (63), indicating a function for plasmin independent of fibrinolysis.

Mice with specific fibrinogen alterations have been generated by targeted replacements in the γ-chain gene. A five-residue truncation of the C-terminal end of this chain (γΔ5) removed a site identified as critical for fibrinogen mediated platelet aggregation (64). In homozygous mice, bleeding time from a nail bed injury was indefinite, while only minimal bleeding was found from a skin injury. These results indicate that platelet aggregation at injury sites in large vessels requires normal fibrino-
gen, and that this C-terminal γ-chain segment is essential for fibrinogen-platelet interactions. Homozygous females were able to support pregnancy, suggesting that platelet aggregation is not essential for implantation. Mice heterozygous for this deletion were normal. Mice with a two amino acid deletion in the γ-chain (γA319,320) were generated to model the human dysfibrinogen Vlissingen/Frankfurt IV (65). As expected from studies of recombinant human fibrinogen with this deletion, homozygous mice had a severe bleeding diathesis and most animals died as neonates with abdominal hemorrhage (KAH and STL, unpublished observation). Mice heterozygous for this deletion appeared normal. Transgenic mice with elevated levels of fibrinogen have also been generated. Although elevated fibrinogen is a strong risk factor for atherothrombotic disease in people, under normal laboratory conditions these mice appeared no different from their littermates (66).

p45 NF-E2: The deletion of the p45 subunit of the transcription factor NF-E2 demonstrated its necessity in platelet production, resulting in mice with absolute thrombocytopenia (40). As intriguing as the ability of embryos to develop to term without fibrinogen, NF-E2 -/- mice also survived to birth lacking this primary component of hemostasis. Nonetheless, hemorrhage was common in newborns, and less than 10% of liveborn homozygotes survived to adulthood. As stated above, cesarean section delivery significantly decreased bleeding in newborns. Adult homozygous animals were fertile and females successfully supported pregnancy (S. Orkin, personal communication). In contrast to mice, a lack of platelets due to a genetic mutation is likely not compatible with human life. There are no reports of humans born with absolute thrombocytopenia, although a severe reduction in platelet count can be a consequence of a pathological condition (e.g. HIV infection) or a side effect of certain pharmaceuticals.

Von Willebrand Factor (vWF): In the circulation, vWF is associated with FVIII and protects FVIII from rapid degradation; vWF is also found in the α-granules of platelets and the Weibel-Palade bodies of endothelial cells, and these sources likely contribute to vWF-mediated platelet adhesion and aggregation. vWF bridges platelets with the subendothelium through platelet membrane glycoproteins GPIbα (part of the Ib-IX-V complex) and αIIbβ3. Homozygous disruption of the vWF gene led to complete loss of vWF antigen and a 4-5 fold reduction in FVIII antigen (67). Embryonic development was normal and the mice were viable, although bleeding was not controlled following tail transection. The vWF -/- mice were fertile, and pregnancy was normal with no prenatal or post partum bleeding complications. About 10% of the mice had spontaneous and sometimes fatal neonatal bleeding. Gastrointestinal bleeding was seen in some older animals. Heterozygous mice did not have these bleeding phenotypes.

Evidence for a suggested link between vWF and P-selectin, a protein involved in leukocyte rolling and extravasation, has been supported utilizing the vWF -/- mice. It had been previously shown that P-selectin and vWF are stored in the same places (α-granules and Weibel-Palade bodies), and that deletion of P-selectin disrupted hemostasis, as demonstrated by a prolonged bleeding time (68). Furthermore, P-selectin is involved in platelet adhesion and rolling mediated through GPIbα (69, 70). A recent study examined leukocytes in vWF -/- mice and found a 70% decrease in leukocyte rolling in stimulated mesenteric venules viewed by intravital microscopy and a significant decrease in leukocyte recruitment utilizing inflammatory models of meningitis and wound-healing (71). Interestingly, the loss of vWF affects the Weibel-Palade body structure, thereby leading to altered storage of P-selectin in vWF -/- endothelial cells (71). To examine the loss of vWF in chronic inflammation, the vWF -/- mice were bred onto an atherosclerotic-prone background (LDR receptor deficiency). When placed on an atherogenic diet, the lesions in the doubly-deficient animals were smaller than LDLR -/-, vWF +/- at earlier timepoints (8 and 22 weeks) but not at a later timepoint (37 weeks) (72). While P-selectin expression of LDLR -/-, vWF -/- mice was slightly different than the LDLR -/-, vWF +/- mice, the authors suggest that it is unlikely that this alone leads to the protective phenotype observed. Rather, due to the observations that fewer monocytes were recruited and that lesions were smaller in areas of disturbed flow (branch points) the authors discuss the possibility of a complex role for vWF involved in 1) flow dependent direct and indirect recruitment of monocytes and platelets and 2) shear-stress regulation of endothelial cell gene expression.

Discussion/Conclusion

In mice, some deficiencies in factors that participate in clot formation were associated with phenotypes that could be anticipated from human studies. Cases of afibrinogenemia have been reported (73, 74) and these patients have severe bleeding disorders, as was seen in the mouse models. Women with low or undetectable levels of fibrinogen have difficulty maintaining pregnancy without clinical intervention (75), similar to fibrinogen null mice. Mice lacking vWF essentially recapitulated the analogous human disease; complete deficiency of vWF, or Type III von Willebrand disease (vWD), is associated with life-threatening hemorrhage and hemarthroses, and reduced FVIII levels (76). In some instances, the studies in mice gave insight into a possible basis for previously confounding pathologic consequences. For example, pulmonary emboli have been observed in afibrinogenemic patients (77, 78), in seeming contradiction to severe bleeding, but in agreement with the ferric-chloride injury studies in Ao -/- mice, which showed embolic thrombus formation (55). As humans with an absolute deficiency in platelets have never been identified, the ability of mice lacking platelets to develop to term and survive is remarkable.

These mouse models suggest that some aspects of clot formation are essential in pregnancy. For example, uterine bleeding is evident halfway through gestation in female mice deficient in fibrinogen (38) or with variant fibrinogen incapable of forming fibrin (γA319,320) (KAH and STL, unpublished data). This bleeding coincides with the maternal blood source becoming linked with the fetus through the choioallantoic placenta. The loss of fibrinogen lacking the domain essential for platelet aggregation or absolute thrombocytopenia is compatible with pregnancy, suggesting that fibrin (not fibrinogen) may be an essential coagulation component for placental function. Nevertheless, the loss of integrin β3, a component of the platelet receptor αIIbβ3, also has consequences on pregnancy; 77% of β3 -/- females experience intrauterine hemorrhage, occurring within the labyrinth of the placenta and under Reichert’s membrane (79). The difference in ability to support pregnancy between mice lacking platelets and those lacking a platelet receptor component may be explained by the expression of β3 in other cell types. Therefore, while the loss of some hemostatic factors such as TF, FV, FX, and FII, directly affects embryonic development, loss of others such as fibrinogen affect the placenta that supports this development.

Technology to generate mutant mice is always improving, as are the methods for studying them. One significant breakthrough has enabled the monitoring of thrombus formation in vivo in real time. Arteriole thrombus formation induced by ferric-chloride can now be followed by intravital microscopy. Early experiments by Ni et al. showed that the vessel injury is thrombin dependent (80). Multiple studies with this method have helped to identify molecules that operate in vivo to promote platelet plug formation and stability. For example, less stable thrombi, that embolized 100% of the time, were generated in fibrinogen
null mice, indicating the necessity of fibrinogen/fibrin interactions between platelets and the vessel wall (55). In vWF -/- mice, platelet deposition was decreased and thrombus initiation was delayed (55). In contrast to fibrinogen deficiency, these thrombi were stable on the vessel wall, but the adhesive properties of the platelets in these mice were insufficient to terminate blood flow in high shear stress channels of nearly occluded vessels, as with thromb in wildtype mice. Surprisingly, vWF-independent thrombus formation persisted in the absence of fibrinogen (Aα -/-, vWF -/- mice), although the thrombi were more fragile than in either single deficiency (55). This finding suggests a novel pathway for platelet adhesion/aggregation that does not require vWF or fibrinogen (55). It is likely that at least one other ligand of β3 may be responsible for the residual thrombus formation in the Aα -/-, vWF -/- mice because thrombus formation is ablated in β3 -/- mice (80). Interestingly, this real time model has also been useful in identifying a state of accelerated platelet adhesion and thrombus formation. Mice lacking the platelet membrane glycoprotein V (GPV -/-) have 2.5 fold more platelets interacting with the vessel wall in the first minute, and the time required for appearance of the first clot is significantly decreased. Thrombi from the GPV -/- mice emolized more frequently and were noted to be much larger in size than the emboli from fibrinogen null mice. GPV is a member of the complex that binds vWF, yet interactions of GPV -/- platelets with vWF were normal. The authors conclude that an increased responsiveness to thrombin caused the observed phenotype. In sum, this particular model of thrombus formation and numerous others, provide new and informative information while analyzing the mutant mice in vivo.

Clot formation likely plays a major role in atherothrombotic diseases. Following atherosclerotic plaque rupture, thrombus formation on the damaged vessel can occlude blood flow through the vessel. Studies with mouse models have provided some insight into the role of clot formation, and in particular the role of fibrinogen, in promoting this pathological condition (81). The development of atherosclerotic lesions in mice mediated by the loss of apolipoprotein E (ApoE) has been examined in fibrinogen -/- mice (82). Lesions developed to the same extent in the ApoE -/- mice and the ApoE -/-, fibrinogen -/- mice, though fibrinogen was absent from the lesions in the doubly-deficient mice. This result, which suggests that fibrinogen has no role in atherosclerotic disease, must be interpreted with caution (81). As elevated fibrinogen is the known risk factor, a more compelling experiment would examine atherosclerosis in the context of elevated fibrinogen. In recent experiments with transgenically induced elevated fibrinogen, we found that diet-induced lesion development was not altered in transgenic mice relative to their normal littermates, indicating that fibrinogen has no influence on the development of small lesions (Gulledge et al., in preparation). In a different model of atherosclerosis, wherein mice express human apolipoprotein (a), a role for fibrinogen was demonstrated (83). Diet-induced atherosclerosis was markedly reduced in apo(a)+, fibrinogen -/- mice relative to apo(a)+ littermates, suggesting that fibrinogen has a role in lesion development in the presence of apo(a). Future studies will likely further examine the influence of platelets and vWF on the development of atherothrombotic disease in mouse models.

**Curtailing Thrombin Formation**

*Tissue Factor Pathway Inhibitor, Thrombomodulin, Protein C, Factor V Leiden, Antithrombin III, and Protein Z*

Tissue Factor Pathway Inhibitor (TFPI): The phenotype of mice with a functional disruption of the TFPI gene was similar to that of TF -/- mice: no embryos survived to birth due to intra-yolk sac hemorrhage and depletion of blood cells in the yolk sac and placental vessels. This vascular insufficiency led to wasting and death between E9.5 and E11.5 (84, 85). Some TFPI -/- embryos survived beyond E12.5, and these embryos had deposition of fibrin in the liver and, rarely, intravascular thrombosis (84). When TFPI +/- mice were bred to FVII +/- mice, the reduced expression of FVII rescued the embryonic lethality associated with the loss of TFPI (86). Although massive hemorrhage was still a problem shortly after birth of these mice, survival to birth was dramatically increased with the loss of either one or both copies of the FVII gene. The authors reasoned that the loss of TFPI alone leaves the activity of TF/FVII unrestrained, so removing the activity (by reducing FVII levels) restored development.

**Thrombomodulin (TM):** The complete loss of TM function in mice results in early post-implantation growth arrest of TM -/- embryos, followed by the rapid and complete resorption of the conceptus (87, 88). Failure of TM null embryos occurred at a very early stage in development, and in fact preceded the establishment of cardiovascular function within the embryo. The analysis of mutant embryos at the time of failure suggested that the death of TM null embryos was not associated with overt thrombosis of embryonic blood vessels, or maternal blood vessels supplying the placenta. When only the cytoplasmic domain of TM was disrupted, expression of the remaining gene product was sufficient for early development of the mice (89).

Placenta-specific restoration of TM expression via the tetraploid embryo aggregation technique rescued TM -/- embryos from early abortion and developmental arrest (90). This finding, together with the analysis of TM expression in the developing embryo, showed that the critical site of TM function was not the vascular endothelium, but non-endothelial placental tissue (trophoblast and/or parietal endoderm). Yet, TM -/- mice died during the second half of pregnancy (day 12.5-16.5 p.c.) from a lethal hemorrhagic diathesis associated with focal thrombosis in the liver and brain and subcutaneous, intracranial bleeding. It was concluded that the early death of TM -/- embryos is caused by a placental defect that probably does not involve intravascular thrombosis or vascular dysfunction. In contrast, the secondary phenotype observed in TM -/- embryos rescued by tetraploid aggregation, which closely resembles the phenotype of PC -/- mice described below, was likely caused by a consumptive coagulopathy. While the midgestation coagulopathy of TM -/- mice is consistent with the anticoagulant function of TM in endothelium, the precise role of TM function in the placenta is as yet unknown.

In contrast to the embryonic lethal phenotype seen with a complete loss of TM function, a selective disruption of TM cofactor function was compatible with embryonic development. These mice (TM<sup>pro/pro</sup> mice) were generated by targeted replacement of the TM codon for Gla404 with a codon for Pro (88, 91). In mice homozygous for this substitution, the expression of TM was diminished and thrombin-dependent activity of TF/FVII unrestrained, so removing the activity (by reducing FVII levels) restored development. Following atherosclerotic plaque rupture, thrombus formation on the damaged vessel can occlude blood flow through the vessel. Studies with mouse models have provided some insight into the role of clot formation, and in particular the role of fibrinogen, in promoting this pathological condition (81). The development of atherosclerotic lesions in mice mediated by the loss of apolipoprotein E (ApoE) has been examined in fibrinogen -/- mice (82). Lesions developed to the same extent in the ApoE -/- mice and the ApoE -/-, fibrinogen -/- mice, though fibrinogen was absent from the lesions in the doubly-deficient mice. This result, which suggests that fibrinogen has no role in atherosclerotic disease, must be interpreted with caution (81). As elevated fibrinogen is the known risk factor, a more compelling experiment would examine atherosclerosis in the context of elevated fibrinogen. In recent experiments with transgenically induced elevated fibrinogen, we found that diet-induced lesion development was not altered in transgenic mice relative to their normal littermates, indicating that fibrinogen has no influence on the development of small lesions (Gulledge et al., in preparation). In a different model of atherosclerosis, wherein mice express human apolipoprotein (a), a role for fibrinogen was demonstrated (83). Diet-induced atherosclerosis was markedly reduced in apo(a)+, fibrinogen -/- mice relative to apo(a)+ littermates, suggesting that fibrinogen has a role in lesion development in the presence of apo(a). Future studies will likely further examine the influence of platelets and vWF on the development of atherothrombotic disease in mouse models.
cumb to massive thrombosis at a young age (93). The penetrance of disease reaches 100%, and the mice experience large vessel arterial and venous thrombosis in their extremities, as well as in internal organs, and eventually die from a consumptive coagulopathy. The disease can be prevented by treating animals with anticoagulants, demonstrating that unfettered coagulation activation is the predominant pathophysiological mechanism underlying the disease.

Protein C (PC): PC -/- mice developed normally to term, yet perished at birth (94). Clotted plasma fibrinogen was undetectable in neonatal PC -/- mice, suggesting a severe consumptive coagulopathy as the cause of death. Symptoms of thrombosis, including fibrin deposition in the brain and liver, bleeding in the brain, and focal liver necrosis, were visible in E12.5 embryos. This phenotype is similar to that observed following tetraploid rescue in TM -/- mice, a coincidence indicating that TM and APC have related functions in embryogenesis. The thrombotic diathesis became progressively more severe until birth; at E17.5, 22% of all pups were PC -/- and by birth only 8% were PC -/-.

As delivery by cesarean section did not prolong survival, it is unlikely that the trauma of birth precipitated death of PC -/- mice. PC -/- mice did not develop thrombosis, but did have significantly reduced levels of plasma fibrinogen consistent with increased coagulopathy. An attempt to rescue PC -/- mice with a superimposed deficiency of FVII yielded a surprising result: the coagulopathy of PC -/- was augmented by the loss of FVII (95). That is, the unrestrained generation of thrombin expected with loss of APC was not compensated by the reduced generation of thrombin expected with the loss of FVII.

In contrast, the PC -/- coagulopathy was compensated by the loss of FXI. PC -/-/FXI -/- mice survived the early lethality observed in the PC -/- neonates, the oldest mice living to 3 months of age (96). Growth of these mice was retarded and, on sacrifice or natural death, all had massive systemic fibrin deposition with concomitant hemorrhage and fibrosis. Thus, although the PC -/-/FXI -/- mice survived the lethal perinatal coagulopathy seen in the PC -/- neonates, they nonetheless succumbed to overwhelming thrombotic disease in later life. Thus, limiting thrombin generation through FXI was not sufficient to completely compensate for the loss of the APC-mediated arrest of thrombin generation. It would be of interest to see if the PC -/- coagulopathy could be compensated by loss of either APC substrate, FV or FVIII.

FV Leiden: FV Leiden is the most common inherited risk factor for thrombophilia in humans (97, 98). FV Leiden, which has an R506Q missense mutation, is resistant to inactivation by APC. Targeted mutagenesis of the mouse factor V gene to introduce the R504Q mutation (99) produced mice with resistance to APC. The plasma APC resistance ratio was decreased from 2.1 ± 0.3 in normal mice to 1.5 ± 0.1 in heterozygous (FvQ+/+) and to 1.3 ± 0.1 in homozygous (FvQ/Q) mice. Adult FvQ/Q mice appear healthy, and reproduce normally. Bio- synthesis, processing and clearance of FV were not altered by this mutation in mice. Nevertheless, fibrin deposition was seen in the lung, heart, spleen, kidney and brain, suggesting chronic, low-grade thrombin generation in the vascular beds of these organs. Despite this latent prethrombotic state, the histopathology of FvQ/Q mice is unremarkable except for occasional focal hepatic fibrosis. Similar to the TM Pro/Pro mice, the genetic background had a profound effect on the phenotypic penetrance of the FvQ/Q mutation: on a C57Bl/6J background, FvQ/Q mice develop normally and survive, while on a mixed 129Sv-C57Bl/6J background, about 50% of FvQ/Q mice suffer from fatal disseminated intravascular thrombosis in the perinatal period. The genetic modifiers that enhance thrombosis in the mixed background remain to be identified.

Antithrombin III (ATIII): The antithrombin III gene was disrupted by targeted deletion of exon II (100). ATIII +/- mice exhibit -50%

Discussion/Conclusion

Similar to the factors that promote thrombin generation, the phenotypes associated with factors that curtail thrombin generation can be divided into two categories: those associated with developmental deficiencies and those associated with hemostasis problems, here with thrombosis. A clear distinction between these two groups is difficult in some cases, as the loss of either PC or ATIII led to embryonic thrombotic complications and severely affected the ability of embryos to develop. The TFPI -/-, TM -/- and ATIII -/- mice did not survive to birth. Complete deficiencies of these factors have not been reported in humans, indicating that they have a critical role in human embryogenesis. Analysis of the mouse embryos showed evidence for unchecked coagulation, as would be anticipated for these factors that curtail thrombin generation. Mice deficient in TFPI, the regulator of TF activity, display vascular abnormalities closely resembling the phenotype observed in FV -/-, FVIII -/-, FII -/-, and TF -/- mice. While the role of the TFPI in vasculogenesis is still ambiguous, these results nevertheless suggest that TF controls vascular development by converting dormant signaling molecules into agonist proteases capable of interaction with specific cell-surface receptors. Further, the rescue of the TFPI -/- mice by reduced levels of FVII is consistent with the conclusion that defective development is associated with unchecked thrombin generation. The more complex developmental phenotypes seen with TM -/- mice suggest that TM has a role in development in addition to the regulation of thrombin generation.

PC -/-, FV Leiden and TM ProPro mice had phenotypes of thrombosis. Both the PC -/- and the factor V Leiden models recapitulate the major hallmarks of human disease associated with these gene defects. The perinatal pathology observed in protein C knockout mice closely resembles the neonatal disseminated intravascular coagulation and purpura fulminans in newborns with minimal protein C levels (104, 105). Similar to the situation in humans, the phenotype associated with the factor V Leiden mutation varied. This mutation was compatible with long-term survival in mice on a C57Bl/6J genetic background, with

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only sporadic spontaneous thrombosis. In contrast, with significant contribution from the 129Sv mouse strain, factor V Leiden mice had severe thrombosis. With the first human gene mutation in TM only discovered in 1995 (106), little is known about inherited TM deficiency; data suggest a correlation with reduced TM expression and thrombotic disease in humans (107-110). The phenotype of the TMProPro mice suggests that disruption of TM cofactor function would be associated with thrombosis in people. Like FV Leiden, expressivity of thrombosis would be expected to vary.

In all mice where the proteins curtailing thrombin formation are completely disrupted, fatal hemorrhage associated with consumptive coagulopathy occurs in midgestation, roughly the same time that fatal bleeding occurs with deficiencies of the proteins that promote thrombin formation. The site and severity of hemorrhage in individual organs is rather distinct in these mice, suggesting that organ specific differences in the control of blood coagulation (91, 111, 112) are already established during intrauterine development. Disruption of the pathways that curtail thrombin formation, including the TM-PC system, ATIII, PZ, and TFPI, has shown that thrombin levels must be effectively controlled. Of these proteins, only TFPI deficiency is associated with vascular defects in the yolk sac or the embryonic vasculature. It therefore appears that uncontrolled thrombin formation is not detrimental to yolk sac blood vessel formation/formation and does not impair other morphogenetic differentiation processes. The placental defects in TM-/- mice might be a special case. Further investigations of these mice are warranted, as the placental abnormalities in mice with minimal TF (113) could potentially lead to novel insights about the pathogenesis of fetal growth retardation and other pregnancy complications in females with familial or acquired thrombophilia.

Additional investigations of thrombophilic mice with heterozygous gene defects also appear warranted, as current work suggests that these investigations will provide insight into the molecular basis of vascular bed-specific differences in the regulation of hemostasis. These animals also provide good experimental means to define the genetic and epigenetic risk factors that modify the phenotypic manifestation of inherited defects in coagulation factor/regulator genes. The demonstration that deficiency of protein Z is a thrombotic risk factor in factor V Leiden mice has preceded the discovery that protein Z may be a risk factor in humans. This represents a nice example to show that results obtained in mice might facilitate the rational design of studies in human populations. One can also envision development of “sensitized” screens in these deficient mice to quantitatively assess suspected risk factors or modifiers of thrombotic disease under precisely controlled environmental conditions. It appears safe to predict that an increasing number of studies of the in vivo role of hemostasis in complex processes like atherosclerosis, cancer, inflammation, aging, and reproduction will utilize many of the mouse models described here.

Conclusion

The observation that some, but not all, hemostasis proteins are essential regulators of morphogenetic processes during early embryogenesis has provided a novel link between developmental biology and blood coagulation. To date, the correlation between hemostasis and embryonic development is seen only with proteins that control thrombin levels, and not with the proteins that participate in clot formation.

Murine models have been revolutionary tools in modern science, and there are some clear advantages to using these models. We can do experiments in mice that would not be feasible in humans, and we can control genetic background and most environmental factors. Nevertheless, we must remember that animal model studies have limitations. As summarized here, some murine factors have different roles than the comparable human factors. These differences may limit interpretation of some experiments, but they should not take away from the numerous discoveries in defining new roles for proteins and confirming the roles of other proteins in specific systems. These differences can provide us with subtle clues that apply globally to various biological functions. All in all, the analysis of mice altered by gene targeting has not produced evidence for dramatic differences in hemostasis between mice and men. In fact, coagulation appears qualitatively very similar in both species (114). Nevertheless, analysis of these pathways in a different species has provided a fresh look at hemostasis, and allowed us to ask new questions. We are looking forward to Zebrafish!

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