A major complication of treatment of hemophilia A is the formation of inhibitory antibodies against the therapeutic factor VIII (FVIII) protein, which occurs with an overall incidence of 20% to 30% of patients. Using hemophilia A mice and in vitro assays, Qadura et al find that a plasma-derived FVIII product containing von Willebrand factor (VWF) activates dendritic cells (a critical antigen-presenting cell for the initiation of a specific immune response) quite differently than recombinant FVIII.1 Similarly, when injected into the hemophilia mice, the cytokine profiles of responding T cells differed substantially after administration of the 2 FVIII formulations, suggesting that different subsets of T-helper cells were being stimulated. The response to recombinant FVIII had a bias toward Th1 response but also involved production of the regulatory cytokine IL-10, while plasma-derived protein caused mostly a Th2 response and up-regulation of TGF-β, a cytokine with immune-suppressive properties. In addition, plasma-derived FVIII promoted FoxP3+ Treg responses and caused lower-titer inhibitor and total antibody (IgG) formation compared with recombinant FVIII.1

A controversial debate over potential differences in the immunogenicity of FVIII products has been ongoing for years. Plasma-derived products may vary in purity and VWF content. Recombinant products produced in mammalian tissue culture are of high purity, do not contain VWF, and may be full-length or B domain–deleted FVIII. Some studies had claimed a higher propensity of recombinant products to cause inhibitor formation, which, however, may have been influenced by the retrospective nature of such analyses and has not been substantiated by prospective studies.2

Questions that fuel the discussion of immunogenicity of factor products include the safety of switching treatment of a patient from one product to another, matching of products to specific patient populations, and the role of VWF in immune responses.3,4 Similar discussions are ongoing about which product and dose to use in immune tolerance induction (ITI) protocols. Different hemophilia treatment centers report diverse experiences. For example, a recent report documents a higher success rate using a plasma-derived, VWF-containing product compared with recombinant FVIII in elimination of inhibitors by ITI.3 It has been hypothesized that VWF could prevent binding of inhibitors by masking epitopes on the C2 domain or otherwise interfere with antibody binding. Qadura et al propose an alternative mechanism, namely antigenic competition for processing by antigen-presenting cells. Interpretation of these animal data are somewhat complicated by murine immune responses to human VWF that may not occur in patients. Nonetheless, this new study provides the hemophilia research community with the exciting perspective of having an animal model available that allows studies that address immunogenic differences between FVIII products, doses, and treatment protocols. In addition, such studies can determine involvement of T regulatory cells in controlling immune responses, as evidence is mounting that active suppression of antibody formation by this subset of T cells is a critical component of maintaining tolerance to coagulation factors.5,6

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Comment on van der Meijden et al, page 881

Thrombosis in flowing blood

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In this issue of Blood, van der Meijden and colleagues report on the mechanisms by which collagen exposure in flow-dependent circulation contributes to thrombus formation.1

In their report, van der Meijden et al show that 2 independent summating mechanisms for thrombin generation initiated by fibrillar type I collagen lead to platelet and coagulant thrombus formation. Type I collagen added to citrated human plasma upon recalcification shortens thrombin generation times (TGT). TGT are blocked by the addition of corn trypsin inhibitor (CTI) but not in the presence of inactivated factor VIIa (FVIIai), a tissue factor pathway inhibitor. TGT are also blocked in factor XIX– or XI–deficient plasma. Under conditions of whole blood flow, addition of CTI (but not FVIIai treatment), reduced platelet adherence to collagen, and combined CTI and JAQ1 glycoprotein VI (GPVI)–blocking antibody treatment further decreased platelet adhesion and thrombin generation. Platelet activation by GPVI was mediated through LAT and PLCγ2, which are established signaling pathways for GPVI.1 These investigations show that collagen exposure in flowing blood results in at least 2 pathways for thrombus formation, one directly through platelet adhesion and activation, and another indirect mechanism via thrombin generation in response to contact activation.
Nieswandt et al first established the crucial role of GPVI adhesion of platelets to collagen under conditions of flowing blood, suggesting that GPVI is essential for platelet activation by collagen under physiologic conditions.² Sarratt et al demonstrated that inhibition or absence of αIβ₃ or GPVI results in defective adhesion to collagen under flow.³ Although GPVI is a low-affinity collagen receptor compared with αIβ₃, it may dominate as a response entity to exposed collagen because it does not require prior activation and is required to then activate high-affinity integrins that mediate firm adhesion to the extracellular matrix. However, platelet activation by other agonists, such as thrombin, ADP, thromboxanes or VWF adherence to GPⅡb, also results in integrin activation and likely regulates platelet adherence in flowing blood under physiologic conditions⁴⁻⁶ (see figure). Platelet adherence to collagen is known to lead to tissue factor–mediated thrombin generation.⁷ In the present study, JAQ1 blocked thrombin generation in both contact- and tissue factor–activated plasma and CTI inhibited platelet adhesion to collagen.¹ These data indicate that collagen-induced thrombin generation is a product of both platelet and contact pathway activation and that exogenously produced thrombin is required for maximal platelet adhesion to collagen under flow. This latter point was confirmed in a recent publication, which shows reduced platelet adherence and a 50% decrease in mean thrombus area of blood anticoagulated with CTI versus heparin flowed over collagen.⁵

The novel finding in the report by van der Meijden et al is the demonstration that factor XII autoactivation on type I fibrillar collagen contributes to platelet adherence and thrombin generation in flowing blood. The present work rediscovers older studies, recognizing that fibrillar collagen supports factor XII binding and autoactivation.⁸⁻¹⁰ It should also be noted that new investigations indicate that certain aggregated proteins also initiate factor XII autoactivation.¹¹ Interest in this in vivo mechanism for thrombin generation waned more than 30 years ago when it was reported that highly purified collagen did not lead to factor XII autoactivation.¹¹ The molecular mechanism for autoactivation is not known, but a recent study using sum frequency generation spectroscopy indicates that factor XII binding to negatively charged surfaces results in a molecular rearrangement of the zymogen elaborating enzymatic activity.²² New interest in contact activation in vivo arose when Renne et al reported that mice lacking factor XII have delayed times to arterial thrombosis.¹³ Efforts have been made to explain the mechanism(s) for reduced thrombin generation in these animals that may arise from diminished contact activation around a developing thrombus. RNA, sulfatides, and polysomes present in developing platelet thrombi result in factor XII autoactivation.¹⁴⁻¹⁵ It is important to note that investigations of kininogen or bradykinin B2 receptor knockout mice also show delay in arterial thrombosis studies and, in the latter case, the mechanism has nothing to do with reduced in vivo contact activation.¹⁶⁻¹⁷

Factor XII's function in vivo is more than an activity prompted by exposure of collagen, platelet components, or aggregated protein that leads to thrombin generation in the intravascular compartment. Factor XII has been recognized as a growth factor leading to ERK1/2 activation.¹⁸⁻¹⁹ The mechanism and importance of this activity is being characterized. Regardless, the present report moves the factor XII field forward and shows that contact activation in vivo in high-flow circulation contributes to thrombin generation, platelet activation, and development of arterial thrombosis. This investigation supports the observation that factor XII and other contact proteins influence arterial thrombosis risk independent of hemostasis.¹³⁻¹⁶⁻¹⁷

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Comment on Pucci et al, page 901

Judging a proangiogenic cell by its cover

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In a murine tumor microenvironment, TEMs display distinct proangiogenic functions and a gene expression signature that is closer to nontumor “resident” blood monocytes and embryonic macrophages than TAMs, suggesting the existence of a novel lineage of proangiogenic cells.

Three paradigms for neangiogenesis have long been recognized: vasculogenesis, angiogenesis, and arteriogenesis.1 Vasculogenesis is the de novo formation of blood vessels from angioblast precursors that are principally mesoderm derived. The angioblasts migrate into tissues where they self-assemble into primitive endothelial cell tubes with lumens. These simple lumens coalesce to form a primitive capillary plexus. Angiogenesis is the formation of new vessels via sprouting of endothelium from these primitive capillaries or later from other preexisting vessels. During embryogenesis, the primitive capillary plexus is subsequently remodeled into mature vessels via a complex network of genetic specification cues, tissue metabolic demands, responses to biomechanical changes in tissues, and hemodynamic forces.2 Commensurate with these adaptations, various mesenchymal-derived cells are recruited in supportive roles to form tunable vascular structures capable of systemic regulation of blood flow. Arteriogenesis is both the process of arterial vessel formation and subsequent arterial collateral formation that plays a role in overall enhancement of blood flow delivery to tissues and organs. The molecular regulation of these fundamental processes is becoming increasingly understood; however, the specific cellular components engaged in each stage of these processes are less well-defined.

While numerous tissue stromal cells play important vascular–supportive roles during angiogenesis and arteriogenesis, both circulating and resident hematopoietic cells are now recognized as important components in the initiation of vessel formation. A vast array of hematopoietic cell subsets have been purported to promote neangiogenesis, ranging from hematopoietic stem cells to committed erythroid or myeloid progenitor cells.3 The role of certain monocyte and macrophage subsets in promotion of tumor angiogenesis has been recently highlighted,4 but remains controversial with respect to whether these cells give rise to functional endothelium; a point further complicated by the shared cell-surface expression of a variety of molecules, including the receptor Tie2, between endothelial cells and some hematopoietic cell types.

The major murine myeloid cell types implicated in tumor angiogenesis can be classified as mast cells, tumor–associated macrophages (TAMs), Tie2–expressing monocytes (TEMs), neutrophils, and myeloid–derived suppressor cells (MDSCs). TEMs circulate in peripheral blood before being recruited to tumor microenvironments where they promote angiogenesis and tumor growth. These activities cannot be substituted by other myeloid subsets when TEMs are depleted.5 The exact relationship between the proangiogenic TEMs and other tumor–associated myeloid cells has not been clarified nor has an established relationship between the circulating TEMs and other blood monocyte subsets been found.

In this issue, Pucci and colleagues compare the gene expression signature of murine tumor–derived TEMs, TAMs, MDSCs, and circulating blood monocytes.6 The authors employ standard flow–cytometric cell-sorting techniques on samples from tumor–bearing transgenic mice to identify and isolate myeloid subsets and to distinguish the cells from tumor or host endothelium (see figure). Custom or premade TaqMan low–density qPCR arrays were probed with transcribed products of mRNA isolated from the various cell subsets. The mRNA expression profiles of TEMs and TAMs were highly related and clearly distinct from that of endothelial cells. However, TEMs could be distinguished from TAMs (and all other tumor–associated myeloid subsets) with a skewing toward significantly higher expression of a panel of gene transcripts frequently identified in M2 alternatively activated macrophages (cells with an antiinflammatory phenotype and enhanced angiogenic and wound–healing capacity). While both TEMs and TAMs displayed defective responses to inflammatory stimuli, TEMs were the most responsive to interleukin–4 (M2 response) at least with respect to transcription of certain “M2” genes. The TEM mRNA