GATA1 N-terminal domain) and hypothesis-generating (identification of GATA1 N-terminal domain regulated pathways) results. It will be important to determine the features of the N-terminal domain of GATA1 that are required for normal erythropoiesis. Identifying the proteins that interact with the N-terminal domain of GATA1 will be critical to our understanding of how GATA1 mutations that result in the deletion of the N-terminal domain cause hematologic diseases. Ling et al have made an impressive start in answering this question.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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THROMBOSIS AND HEMOSTASIS

Comment on Aymonnier et al, page 1632, and de Maat et al, page 1658

Serpin targets in hemostasis/kinin formation

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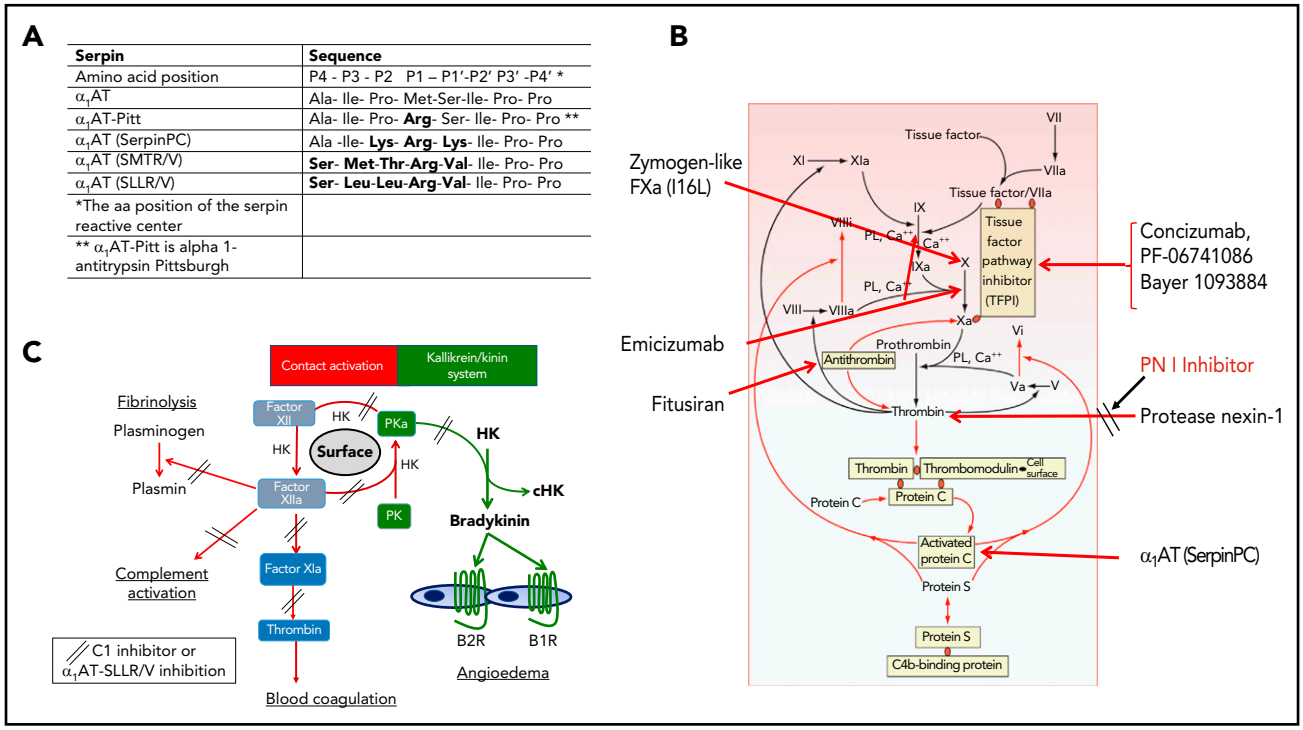
In this issue of *Blood*, the papers of Aymonnier et al[1] on the use of protease nexin 1 and de Maat et al[2] on the use of altered α-1-antitrypsin propose therapeutic uses of these serpins for the management of coagulation and contact system disorders, respectively. Serpins are a family of serine protease inhibitors that regulate proteases of plasma, mostly enzymes of blood coagulation, complement, and inflammatory systems.

Interest in serpins as possible therapeutic tools began in 1978, when Lewis et al published a paper about a 10-year-old boy who had an unknown life-long hemorrhagic disorder.[3] After his death, an abnormal plasma α-1-antitrypsin (α-1-antitrypsin Pittsburgh [α_1AT-Pitt]) was characterized that has a reactive center Met358Arg polymorphism, converting this elastase inhibitor to a thrombin inhibitor (see figure panel A).[4] α_1AT-Pitt is a tragic event of nature, but therapeutic serpin modification, this commentary's focus, as in the papers of Aymonnier et al and de Maat et al, has clinical importance. α_1AT-Pitt also is a potent inhibitor of plasma kallikrein (PKa), αFXIIa, and βFXIIa.[5,6] It anticoagulates simulated cardiopulmonary bypass circuits, but its infusion into septic baboons did not prevent the associated coagulopathy due to in vivo inactivation.

Much recent effort has been made to derive novel treatments for hemophilia by rebalancing the reduced coagulation in hemophilia patients with designer immunoglobulin made to function like the missing factors or immunoglobulin or other inhibitors to regulate hemostasis (see figure panel B). Emicizumab is a monoclonal antibody that binds FIXa/IX and FX/Xa to function like FVIII/FVIIIa in patients with hemophilia A or inhibitors to FVIII.[7] Monoclonal antibodies to TFPI (eg, concizumab, PF-06741086, Bayer 1093884) rebalance hemostasis by allowing more factor VIIa–tissue factor complex to activate factor X directly.[8] In addition, forms of factor X less susceptible to antithrombin inhibition (zymogen-like FXa [I16L]) have been produced to bypass a hemophilia block or coagulation protein inhibitor.[9]

Lessons learned from α_1AT-Pitt also have been applied to hemophilia rebalancing. If one has FVIII or FIX deficiency, lowering antithrombin with a siRNA (fitusiran) rebalances the hemostatic defect (see figure panel B).[10] Altering α_1AT by substituting a Lys and Arg in the P2 and P1 positions, respectively, and a Lys in the P1′ spot (see figure panel A) changes α_1AT into an improved activated protein C inhibitor that reduces thrombin inhibition and, presumably, improves hemostasis.[11] Like α_1AT-Pitt, the Arg in the P1 position makes it a serine protease target, but the Lys in the P2 and P1′ position prevents the reactive center from thrombin interference.

In this issue of *Blood*, a novel approach to hemophilia rebalancing via a serpin is presented. Protease nexin-1 (PN-1) has specificity toward thrombin. Very early PN-1 was recognized on the activated platelet surface in complex with thrombin.[12] Aymonnier and colleagues observed that platelet PN-1 deficiency in activated *f8*$^{-/-}$ platelets or PN-1 inhibition in platelet-rich plasma from severe hemophilia patients significantly improved thrombin generation. In murine models, combined *f8*$^{-/-}$/*Serpine2*$^{-/-}$ (PN-1 knockouts) mice have reduced blood loss and bleeding times compared with *f8*$^{-/-}$ mice alone.[1] Neutralizing antibody to PN-1 enhances clot stability and lengthens clot lysis time on thromboelastometry in blood from *f8*$^{-/-}$/*Serpine2*$^{-/-}$ mice and hemophilia A patients. These data indicate that removal of PN-1 from plasma or platelet-rich plasma produces increased thrombin generation. Because PN-1's target is thrombin, an end point in the hemostatic pathway, its inhibition additionally may be applicable to correct hemostasis in hemophilia B and factor XI deficiency (hemophilia C). Development of a single form of rebalancing therapy for hemophilias A



(A) Reactive center amino acid sequence of α_1AT and its biologically active mutants. Three letter abbreviations are used for amino acids (aa). (B) Agents and targets to rebalance hemostasis. The schematic is a drawing of tissue factor and factor VIIa–induced hemostasis (TF-VIIa) with FIX (IX) and FX (X) activation leading to FXa (Xa) (tenase) with subsequent thrombin formation (prothrombinase). Overlying this hemostatic pathway are the 3 major anticoagulant systems in vivo: antithrombin, activated protein C being formed by thrombin when both are bound to thrombomodulin, and tissue factor pathway inhibitor (TFPI). A listing of hemostatic rebalancing agents that are approved for clinical use or are in clinical development and their hemostatic targets are shown. A zymogen-like FXa containing an I16L polymorphism is more resistant to constitutive levels of antithrombin and may overcome a hemostatic block of tenase. Emicizumab is a monoclonal antibody that binds factors X/Xa and IX/IXa to overcome a factor VIII deficiency or inhibitor. Fitusiran is a small interfering RNA (siRNA) that lowers levels to antithrombin to rebalance hemostasis in patients with factor VIII or factor IX deficiency. Concizumab, PF-06741086, and Bayer 1093884 are monoclonal antibodies that block TFPI and increase thrombin generation due to more direct FVIIa-TF activation of FX. Protease nexin I is a serpin thrombin inhibitor. Its inhibition increases thrombin generation in hemophilic plasma (see text). Activated protein C–specific serpin (α_1AT [SerpinPC]) made from strategic amino acid replacement in α_1-antitrypsin (see text) leads to increased thrombin formation that may rebalance hemostasis in patients with hemophilias. (C) Targets of C1 inhibitor and α_1AT-SLLR/V in contact activation and hereditary angioedema (HAE). This figure shows the contact activation and kallikrein/kinin systems. The contact activation system consists of zymogen FXII (XII) autoactivating on an artificial or biologic negatively charged surface to FXIIa. This pathway is accelerated by the presence of H-kininogen (HK). Formed FXIIa activates zymogen prekallikrein (PK) to PKa. PKa activates more FXII to FXIIa, leading to reciprocal activation and amplification of activation of the system. Formed FXIIa activates plasminogen to plasmin, the classic complement pathway, and factor XI to FXIa to initiate blood coagulation. PKa also cleaves HK to liberate bradykinin (BK) from HK that activates its vascular receptors, the bradykinin B2 (B2R) and B1 (B1R). cHK is cleaved BK-free HK that is a marker for contact and kallikrein/kinin system activation. Formed BK is thought to be the major agent inducing angioedema in the majority of patients with HAE. Importantly, C1 inhibitor or the iterated forms of α_1AT (eg, α_1AT-SLLR/V) (see text) block all forms of FXIIa, PKa, and FXIa to inhibit all their activities.

to C is attractive because it makes patient management less complex.

This issue of *Blood* also has an investigation by de Maat et al to iterate a more potent C1 inhibitor (C1INH) analog.[2,5] C1INH accounts for ~92% of the plasma inhibitory activity against FXIIa and related proteases' inhibition and ~48% of the inhibitory activity against PKa (see figure panel C). C1INH is not a potent PKa inhibitor. Fourfold molar excess of plasma C1INH to formed PKa is unable to eliminate the enzyme's activity. The disorder of acute attacks of HAE occurs in patients with 40% to 60% normal C1INH. In vivo, C1INH probably has a role to balance physiologic BK production. C1INH regulates the constitutive levels of BK, but does not abolish its production. In the recent clinical studies with lanadelumab, a monoclonal antibody to plasma PK, only a 50% decrease in PK levels was sufficient to reduce the incidence of acute attacks of HAE.[13]

de Maat et al developed a C1INH analog also using α_1AT-Pitt as a template. These investigators kept the Arg358 for a reactive center to coagulation proteases. Two forms of α_1AT-Pitt were iterated: Ser-Met-Thr-Arg-Ser and Ser-Leu-Leu-Arg-Ser for the P4, P3, P2, P1, and P1′ positions, respectively (see figure panel A).[2] Both forms are better PKa and FXIIa inhibitors than C1INH. However, they have reduced but still present specificity to thrombin (IIa), FXa, plasmin, activated protein C, and FXIa. When the P1′ Ser is mutated to Val, the target specificity to IIa, FXa, activated protein C, and plasmin is lost (see figure panel A).[2] α_1AT-SMTR/V is a slightly better FXIIa inhibitor, and α_1AT-SLLR/V is a better PKa inhibitor. Both retain their ability to also inhibit FXIa. These investigators show that α_1AT-SMTR/V and α_1AT-SLLR/V are more potent inhibitors of contact activation, PKa cleavage of H-kininogen, and BK formation than C1INH (see figure panel C). Furthermore, in a series of murine models that examine the roles of FXIIa, PKa, and BK formation in contact activation, α_1AT-SMTR/V and α_1AT-SLLR/V block murine models of ferric chloride–induced carotid artery thrombosis, rodent paw swelling, and dextran-induced colitis.

Developing potent inhibitors to contact activation to control BK formation and factor XI activation is important for better management of HAE and may prove

useful to inhibit thrombosis on artificial medical surfaces. Is too much inhibition of contact activation and BK formation potentially deleterious? *Kgn1*$^{-/-}$ mice exist without BK and obvious consequence. The clinical use of α_1AT-SMTR/V and α_1AT-SLLR/V inhibitors may induce the mild medical hemophilia C (FXIa inhibition), a mild bleeding disorder. This activity may be useful for contact activation-induced thrombosis prevention.

The 2 articles highlighted here show the novelty and diversity of therapeutic development in the areas of hemostasis and contact activation. It is exciting to observe how simple amino acid substitutions make a serpin elastase inhibitor (α_1AT) into a potent antithrombin, activated protein C inhibitor, or anti-PKa/FXIIa inhibitor.[2,4,11] It is important to learn that downregulation of PN-1 alone improves thrombin generation.

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THROMBOSIS AND HEMOSTASIS

Comment on Lindström et al, page 1645

Genetics of venous thromboembolism revised

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In this issue of *Blood*, Lindström and colleagues[1] report genomic and transcriptomic association of 16 novel susceptibility loci for venous thromboembolism (VTE). Moreover, Mendelian randomization causally linked blood traits to thrombosis.

Familial aggregation of VTE was recognized in 1905 by Briggs,[2] but it was not until 1965 that Egeberg identified the first genetic risk factor for VTE (ie, inherited deficiency of antithrombin [SERPINC1]) (see figure).[3] In 1981 and 1984, inherited deficiencies of protein C (PROC) and protein S (PROS1) were recognized as risk factors for VTE. However, it was not until the discovery of resistance to activated protein C (APC resistance) by Dahlbäck et al in 1993 that it became evident that genetic factors are common risk factors of VTE.[4] APC resistance was linked to a mutation in the factor V gene (F5), factor V Leiden (rs6025).[5] In 1996, Poort et al reported a common genetic variation (rs1799963) in the 3′-untranslated region of the prothrombin gene (F2) is associated with elevated plasma prothrombin levels and VTE risk.[6] These 5 inherited defects are called major thrombophilias, and all involve coagulation or anticoagulant genes. No new major thrombophilia has been discovered since 1996. Instead, genome-wide association studies (GWASs) have discovered a number of VTE-associated loci, reviewed by Trégouët and Morange.[7] The GWAS-discovered risk variants are weak risk factors for VTE but are prevalent in the population. Before the present study, all genes linked to VTE were directly or indirectly linked to the coagulation system (FGG, F2, F5, F8, F11, KNG1, VWF, ABO), anticoagulation pathways (SERPINC1, PROC, PROS1, PROCR, THBD), or platelets (VWF, GP6, ABO, STXBP5, ZFPM2), although some loci (SLC44A2, TSPAN15, LRP4) had no known biological link to VTE.

The present study by Lindström et al is the largest meta-analysis of GWAS data for VTE, including 18 studies with 30 234 VTE cases and 172 122 controls. The study is the first *trans*-ancestry GWAS of VTE. It is also the first transcriptome-wide association study (TWAS). The GWAS identified 11 newly associated genetic loci (C1orf198, PLEK, OSMR-AS1, NUGGC/SCARA5, GRK5, MPHOSPH9, ARID4A, PLCG2, SMG6, EIF5A, and STX10). The TWAS identified 5 additional genetic loci using imputed gene expression in whole blood (SH2B3, SPSB1, RP11-747H7.3, RP4-737E23.2) and in liver (ERAP1). Some previous associations could not be confirmed in the present GWAS, such as the THBD loci.[7] The present study gives important contributions to the functions of genes involved in the pathogenesis of VTE (see figure). In the figure, the genes are grouped after potential links to VTE. The strongest genetic risk factors for VTE (ie, classical thrombophilia) are all related to coagulation (F2 and F5) or the anticoagulation