types of mechanical and chemical vessel injuries in arterial and venous beds, although the animals have a normal hemostatic capacity. These mouse models show that FXI-mediated procoagulant activity is crucial for pathologic thrombosis. Interestingly, reconstitution of FXI-null mice with human FXI restores thrombus formation in the carotid artery in the ferric chloride injury model, suggesting that the protease operates similarly among the species. Indeed, the critical function of FXI for pathologic thrombosis has been convincingly demonstrated in other species. Targeting FXI in rabbits, rats, and baboons with inhibitory antibodies and peptidomimetic inhibitors potently reduced thrombus formation.

How is FXI activated in pathologic thrombosis? Initially, based on the normal hemostatic capacity of FXII-deficient humans, thrombin-mediated feedback activation was considered to be the major pathway for activating FXI in vivo. However, the discovery that vessel-occlusive thrombus formation both in FXII- and FXI-deficient mice was similarly defective suggests that FXIIa activates FXI in thrombosis. Indeed, we recently identified platelet-released polyphosphates, inorganic polymers of 80–120 orthophosphates, as the initiator of FXII-driven fibrin production in vivo; mice with combined deficiency in the intrinsic pathway proteases FXII and FXI (FXII−/−FXI−/−) were protected from lethal polyphosphate–driven thrombosis to the same extent as the single gene-deficient mice, FXII or FXI nulls. Consistently, anti-FXI antibodies that specifically block FXIIa-mediated activation of FXI interfere with thrombus formation in vivo. Cumulatively, the mouse models reveal a crucial role of FXI for arterial and venous thrombus formation and show that the protease is activated in a FXII-dependent manner—consistent with the classical reaction sequence of the intrinsic pathway.

Zhang and colleagues use antisense technology based on base-pair hybridization of antisense oligonucleotides (ASOs) with mRNA to inhibit FXI in mice (see figure). FXI-specific synthetic ASOs are internalized, translocated to the nucleus, and specifically hybridize with the complementary sequence of the FXI mRNA. The mRNA-ASO duplex is specifically degraded by nuclease RNaseH and consecutive expression of the gene of interest is reduced. Intravenous infusion of FXI-specific ASOs dose-dependently reduced FXI plasma levels. When FXI was <30% of normal, ASO-treated mice were largely protected from arterial and venous thrombus formation in various models such as ferric chloride–induced thrombosis in the aorta, mesenteric veins, and the vena cava, as well as stasis–triggered vena cava thrombosis. Notably, thrombus formation in FXII and FXI heterozygous mice having 50% of normal plasma levels is indistinguishable from normal wild-type mice. Therefore, drugs that target expression of intrinsic pathway proteases need to substantially reduce FXII and/or FXI plasma levels to efficiently block thrombosis. Despite the striking thromboprotective effects, FXI-ASO treatment did not interfere with the hemostatic capacity of injected mice. Co-administration of FXI-ASO with the antiplatelet drug clopidogrel or heparin amplified the anticoagulant potency of these drugs but did not increase bleeding. Application of ASO for targeting FXI is a smart strategy. ASOs specifically interfere with FXI expression and do not affect levels of other coagulation factors.

Antisense nucleotide treatment reversibly reduces FXI levels and the effect lasts for several days (plasma half-life of FXI is ~2 days). A FXI concentrate is available for substitution therapy that could serve as antidote in case of unexpected bleeding or injury.

Is FXI-ASO therapy a potential strategy to interfere with thrombosis in humans? The answer is probably yes. Retrospective clinical studies analyzing thromboembolic disease in a population with severe FXI deficiency indicate that these individuals are protected from ischemic stroke and deep vein thrombosis but not from myocardial infarction. Antisense oligonucleotides are used in clinics and given that FXI-ASOs are functional in humans, the drug offers an exciting opportunity for a novel, effective, and safe antithrombotic therapy with minimal therapy-associated bleeding risk.

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Comment on Melenhorst et al, page 4700

T time for transplants

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In allogeneic stem cell transplantation, unsorted donor T cells are dangerous things: too many and the recipient runs the risk of death from severe graft-versus-host disease (GVHD); not enough and the graft may fail, the disease recur, and opportunistic infection arise. Smarter, more specific donor T cells might be better, but how specific does specific need to be to avoid trouble? In this issue of Blood, Melenhorst and colleagues allay anxieties about the use of virus-specific donor-derived T cells to prevent or treat viral reactivation and infection occurring after allogeneic transplantation. Their analysis of 153 transplant recipients given virus-specific donor-derived cells showed no de novo GVHD secondary to adoptive transfer and a rate of GVHD reactivation of only 6.5% with no reactivation greater than grade II in severity.
This is a striking result for several reasons. First, the cohort included 73 patients receiving cells from HLA-mismatched donors, including 28 receiving haploidentical virus-specific cells in which one might reasonably expect that the risk of GVHD would be higher. In fact, there was no difference in the rate of GVHD in patients receiving virus-specific cells from matched and unmatched donors (both were low) despite the fact that the cells were lines rather than clones and were administered for the most part in the absence of immunosuppressive therapy. Concerns had been heightened earlier this year when Amir and colleagues demonstrated that 80% of virus-specific cell lines (including Epstein-Barr, cytomegalovirus [CMV], varicella zoster, and influenza-specific T cells) cross-reacted with allogeneic HLA molecules in vitro. Melenhorst and colleagues looked for this phenomenon in their own cell lines to be certain that the absence of GVHD that they observed clinically was not simply a fortunate association of nonreactive donor T cells. Posttransplant immune suppression required to control GVHD promotes opportunistic infection and disease relapse. When the virus-specific cells were given reacted to a variety of allogeneic HLA molecules, including some of their own? The findings of Melenhorst and colleagues do not extend to providing the explanation, although some possibilities might include the relatively young age of the recipients (there was a weighting toward pediatric patients), the nature of their peritransplant T-cell depletion (many had received grafts depleted of T cells in vitro), the relatively small T-cell doses administered, and the memory cell as opposed to naive characteristics of the virus-specific cells. Whatever the case, the implication of their observation is clear. Virus-specific T cells, at least as prepared and administered by the groups in Washington and Houston, do not cause severe GVHD. Results from other groups using virus-specific T cells are also relevant. In an extension of a previously reported study, 10 of 33 adults receiving bivirus-specific T cells after allogeneic transplantation in Sydney developed GVHD, de novo in 9 patients. Three patients developed grade III or IV GVHD. The group from London reported similar results with 11 of 30 patients developing acute GVHD after CMV-specific T-cell infusions with grade III disease in 3 of the patients. In both these series, GVHD rates were not different from those in similar cohorts not receiving virus-specific T cells. Randomized studies of both prophylactic and preemptive CMV-specific T cells currently running in the United Kingdom will provide further information on the risk of GVHD related to virus-specific T cells in patients receiving uniform conditioning.

In the meantime, the results of Melenhorst et al raise the prospect of developing an approach to allogeneic transplantation that combines reduction of GVHD using CD34+ or other purified hemopoietic stem cell grafts with rapid reconstitution of the immune system posttransplantation using specific T cells recognizing a broad range of infectious and malignant antigens (see figure). Such a transplant could theoretically be performed with little or no posttransplant immune suppression if the GVHD risk from T-cell infusions specific for other pathogens and malignant targets is as low as it appears to be for virus-specific T cells. Aspergillus-specific T cells have already been used clinically in the setting of haploidentical transplantation and several groups are studying the in vitro generation of T cells specific for other clinically relevant opportunistic pathogens including BK virus, varicella zoster virus, respiratory viruses, and others.

In addition to infections, cell therapy in the setting of T-cell depletion will need to deal with the issue of disease relapse. T-cell clones targeting minor histocompatibility antigens have been administered for relapsed leukemia in Seattle; although GVHD was observed, it did not appear to be worse than what existed before cell administration. Other cellular approaches to targeting malignant antigens include the production of cells bearing single-chain chimeric antigen receptors or artificial T-cell receptors recognizing relevant tumor antigens such as CD19 or WT-1. When used in the allogeneic transplant setting, every product will need to be assessed for both efficacy and GVHD risk. Each product targeting a specific infectious or malignant antigen that demonstrates efficacy with little or no GVHD risk will build one more brick in the wall of an immune system demolished by allogeneic stem cell transplantation. In coming years, we should welcome reports of new products that safely increase the spectrum of activity of cell therapy against specific antigens in the setting of allogeneic transplantation. More T anyone?
Conflict-of-interest disclosure: The author declares no competing financial interests.

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Comment on Langer et al, page 4395

Complement halts angiogenesis gone wild

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In this issue of Blood, Langer and colleagues demonstrate the inhibitory role of the complement system during neovascularization, which is mediated by complement-stimulated macrophages in both a mouse model of retinopathy of prematurity and in a Matrigel culture model.

Complement is important for both innate and adaptive immunity, providing the first line of defense against invading microorganisms. Complement is also involved in the phagocytosis of apoptotic cells and cellular debris in response to injury, the regulated removal of neurons during brain development, neuroprotection, neuronal migration, organ regeneration, and homing of hematopoietic stem cells. As coagulation, complement activation involves proteolytic activation of multiple factors under the tight regulation of inhibitors, and, not surprisingly, regulatory disturbances can result in disease. During acute inflammation, complement anaphylatoxins mediate changes in vascular flow, permeability, leukocyte extravasation, and migration, sometimes contributing to tissue damage after ischemia and reperfusion. Autoanti-

The complement system involves more than 40 soluble and surface-bound proteins, with membrane-associated receptors and regulators interacting with effectors to target microbes or damaged cells for elimination. Complement response is initiated via the classical, lectin, and alternative pathways, which converge on the proteolytic activation of C3 (see figure) to produce C3a and C3b. C3b contributes to the formation of the C5 convertase, which cleaves C5 to produce C5a and C5b. The effectors C3a and C5a (anaphylatoxins) bind to their respective receptors C3aR and C5aR (C5a also binds to C5a receptor-like 2, C5L2), found on neutrophils, eosinophils, mast cells, monocytes/macrophages, dendritic cells, endothelial cells, astrocytes, and microglia, as well as other tissues. C5b recruits C6, C7, C8, and C9 to form the terminal membrane attack complex, leading to pore formation and cell destruction.

The study by Langer and colleagues in this issue reveals the importance of complement in regulating neovascularization in a mouse model of retinopathy of prematurity (ROP). Their ROP model used newborn mice exposed to high oxygen concentration (75%) in an incubator followed by exposure to room air, leading to retinal hypoxia causing a hypoxia-driven proangiogenic response with increased pathologic neovascularization. Retinal angiogenesis was quantified by enumerating stained epiretinal vascular cell nuclei. They found unexpectedly that neovascularization was increased in C3-deficient (C3-/-) mice compared with controls. The inhibitory role of the C5a receptor during angiogenesis was shown by increased neovascularization in C5aR-/- mice compared with control mice. Antibody-mediated blockade of C5 and terminal complement activation in wild-type mice also resulted in increased neovascularization. Similarly, wild-type mice injected with C5aR antagonist peptide showed increased neovascularization, whereas treatment with C5a agonist peptide reduced retina neovascularization, confirming the inhibitory role of C5a and C5aR in this process. Furthermore, treatment of C3-

Although no direct effect on angiogenesis was observed with C3a or C5a in endothelial cells, C5a-stimulated macrophages caused an inhibitory response. Specifically, C5a-treated macrophages had an antiangiogenic signature (increased IL-6, TNF-α, soluble VEGF receptor 1 [sVEGFR1], and decreased IL-10 mRNA). Secreted sVEGFR1 protein was increased in the supernatant of C5a-stimulated mouse macrophages and human monocytes, and the latter supernatant also inhibited angiogenesis in an in vitro Matrigel tube formation assay. Immunodepletion of sVEGFR1 from the supernatant reversed the inhibitory effect, confirming the key role of sVEGFR1 in monocyte/macrophage-mediated down-regulation of angiogenesis (see figure).
T time for transplants

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