heparin, and, in contrast, less efficient in binding to the platelet-receptor glycoprotein Ibα. Because the protein backbone is similar between plasma and platelet VWF, it seems conceivable that variations in the glycosylation pattern may contribute to such functional differences. Indeed, initial analysis revealed that platelet VWF contains less sialic acids and lacks the blood group A and B glycan structures that are found on plasma VWF.5,6

In the present study, McGrath et al confirm the observation that sialylation of N-linked glycans (but not O-linked glycans) is reduced by more than 50% for platelet VWF compared with plasma VWF (see figure). As expected, blood group A- and B-glycan structures were absent but surprisingly enough, blood group O structures were normally present. Why specifically the A- and B-glycan structures are missing remains unclear, because other platelet proteins do contain these structures, suggesting that the necessary machinery is available during VWF protein synthesis in megakaryocytes.

Previously, it has been reported that ABO blood group structures modulate the efficiency by which VWF is degraded by its cleaving protease ADAMTS13, with O-structures rendering VWF more susceptible to cleavage than A or B structures.7 Because platelet VWF selectively carries the blood group O-structures, one would expect that it is degraded efficiently by ADAMTS13. However, McGrath et al observed the opposite: platelet VWF is much more resistant against ADAMTS13-mediated proteolysis than plasma VWF. Apparently, the explanation behind this paradoxical finding lies in the reduced quantity of sialic acids that are attached to the N-linked glycans of platelet VWF. Indeed, previously, the same group reported that α2,6-linked sialic acids promote proteolysis by ADAMTS13; a reduction of >50% of these sialic acids could explain why platelet VWF is proteolysed less efficiently by ADAMTS13. It further indicates that the role of sialic acids is more dominant than the role of the blood group determinants in regulating ADAMTS13-mediated proteolysis. This would be in agreement with sialic acids being much more abundant (>90% of the glycans being sialylated in plasma VWF) than the blood group ABO structures (1-2 per monomer).8

Altogether, this article reveals new insights into platelet VWF with the observation that its glycome is markedly different from plasma VWF, providing it with a higher resistance to ADAMTS13 proteolysis. However, this report also highlights how much we have yet to learn about this particular VWF that, despite being highly multimerized and ADAMTS13-resistant, is less efficient than plasma VWF in binding to its platelet receptor glycoprotein Ibα. Additional studies focusing on platelet VWF-specific functions are necessary to further unveil its mysteries.

Conflict-of-interest: The authors declare no competing financial interests.

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T cells then become functionally redirected to the specific tumor antigen, which is recognized by the immunoglobulin portion of the molecule and can proliferate and mediate non–major histocompatibility complex restricted cytotoxicity against cells that express the tumor antigen target. Advances in vector technology, specifically the advent of lentiviral vectors that can efficiently target both dividing and nondividing lymphocytes, have facilitated the translation of genetically engineered T cells to the clinic.4

The most advanced clinical trials have focused on CD19, which is restricted in its expression to normal and malignant B cells. One of the major impediments to the clinical development of CAR technology has been the limited in vivo persistence and expansion of CAR-modified T cells.5 In a pilot clinical trial, autologous T cells, which were genetically engineered to express an anti-CD19 CAR, were used to treat 3 patients with refractory chronic lymphocytic leukemia (CLL), 2 of whom achieved durable complete responses (CR; ≥2 years) and 1 a stable partial response (PR).6 Two patients with refractory pediatric acute lymphoblastic leukemia (ALL) were successfully treated by a similar approach with 1 remission ongoing at nearly 1 year, although this patient experienced a severe cytokine release syndrome that required treatment with cytokine blockade.7 Five patients with refractory adult ALL, including 2 patients with 63% and 70% marrow blasts at enrollment, achieved a molecular remission after CD19-CAR T-cell therapy, allowing 4 of 5 patients to proceed to allogeneic stem cell transplantation.8 Other groups have reported successful treatment of progressive CD195 B-cell malignancies, including follicular lymphoma, using CD19-CAR T cells.9

In this issue, Kochenderfer and colleagues report for the first time the results of a pilot study of 10 patients who received CD19-CAR-engineered and donor-derived allogeneic T cells for treatment of persistent or relapsed CD19-positive B-cell malignancies after allogeneic stem cell transplantation and at least 1 standard DLI infusion (see figure).1 This 2-arm study included 6 patients with relapses after matched sibling transplants and 4 patients with relapses after matched URD transplants. Peripheral blood mononuclear cells were obtained from the healthy donors, transduced with a gammaretroviral vector encoding the CD19-CAR construct (which includes a portion of the CD28 costimulatory molecular and the signaling domain of CD3ζ), and infused on day 8 of culture. None of the patients received any pre-CAR T-cell lymphodepletion treatment. Even so, 3 URD patients, including 2 with CLL and 1 with mantle cell lymphoma, exhibited complete or partial regression of disease after CD19-CAR therapy; 1 of the CLL patients has an ongoing CR, and the patient with mantle cell lymphoma has an ongoing PR. Importantly, although 6 of 10 study patients had GVHD earlier in their transplant courses, none of the patients exhibited GVHD after CAR-modified T-cell transfers. However, all of the responders had transient cytokine-mediated toxicities, including hypotension and fever, and 1 patient had depressed cardiac function that lasted for 4 months. Although these early observations are encouraging, enthusiasm for the specific approach taken in this study is tempered by the fact that the majority of patients (7/10) did not exhibit an objective response. The authors provide some clues to explain the low response rate: (1) After peaking at ~10 days after infusion, the CD19-CAR T cells disappeared from the blood in all the patients before 1 month. (2) Expression of the inhibitory receptor PD-1 increased rapidly (by day +10) on the CAR-modified T cells. Although the objective responses occurred early after T-cell transfers, it is also unclear how well this approach would work for patients with more proliferative B-cell malignancies such as B-cell ALL. Conceivably, the response frequency and duration could be augmented by using cytoreductive and/or lymphodepleting therapies prior to CAR T-cell transfers, serial T-cell infusions, and/or the incorporation of anti-PD1 or anti-CTLA4 antibodies to minimize T-cell exhaustion. In addition, further studies will be needed to establish the best CAR “model” because the inclusion of the CD137 (4-1BB) cytoplasmic signaling domain to the CD3ζ chain may enhance T-cell persistence, proliferation, and antitumor activity compared with CARs that carry the CD3ζ chain alone.10 Nonetheless, the work by Kochenderfer and colleagues...
represents a bold and elegant step forward for CARs and hopefully for their drivers.

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Shockingly: the loss of Lyn leads to leakage

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In this issue of Blood, Han et al demonstrate that endotoxin-induced mortality in a murine model of acute lung injury (ALI) was associated with increased vascular permeability attributable to loss of the Src family kinase (SFK) Lyn.1

Endothelial barrier stability required Lyn’s phosphorylation of another tyrosine kinase, focal adhesion kinase (FAK). Could this mechanism also explain the pleural effusions and pulmonary edema observed infrequently and transiently to the SFK inhibitor dasatinib? Could promoting the Lyn–FAK pathway lead to decreased vascular leakage and decreased mortality in acute respiratory distress syndrome (ARDS)?

The vascular-endothelial (VE) barrier is critical for tissue homeostasis, regulating normal fluid balance, inflammatory cell infiltration, vascular tone, angiogenesis, and thrombogenic responses. Injury to the endothelial barrier results in tissue edema, inappropriate inflammatory cell infiltration, coagulopathy, and exudation of serum proteins, which can lead to shock and death. Catastrophic damage to the pulmonary capillary barrier function, as occurs in ALI and ARDS, can be induced by lung infection or sepsis. ARDS is frequently associated with the failure of other organ systems, where their endothelial barriers may also be compromised. ALI and ARDS have been postulated to have an important immune cell component, and a variety of inflammatory cells have been implicated for the production of inflammatory mediators that affect subsequent vascular leakage. However, it remains unclear which inflammatory cells and intracellular signaling pathways are critical for inflammation-related endothelial dysfunction.2

Efforts to identify the essential cellular activities underlying vascular leakage have focused on altered signaling in the endothelial cells that comprise the vascular barrier. The endothelial barrier function is maintained by cell–cell contacts and by cellular adhesion to the extracellular matrix and the basement membrane. These attachments can be affected by alterations in cytoskeleton, gap junctions, tight junctions, and adherens junctions, as well as changes in the extracellular matrix.2 Interestingly, alteration of the endothelial barrier function involves cross talk between multiple signaling pathways and a balance between opposing signals. For instance, the interplay between the second messengers cyclic guanosine monophosphate and cyclic adenosine monophosphate (cAMP) regulates cell adhesions through the activation/inhibition of the serine/threonine protein kinase A. The subcellular compartmentalization of cAMP generation further complicates these signals because different locations of protein kinase A activation have opposing effects on barrier function. Similar complexity may be found in tissue homeostasis and their regulation of the endothelium. Early studies of SFKs suggested that activation of c-Src and Yes induced endothelial barrier permeability. However, evidence from recent clinical trials suggested that this may be a simplified picture of tyrosine kinases in barrier function. Fluid retention and pleural effusions, involving exudates with lymphocytic accumulations, were demonstrated to be an adverse effect of dasatinib, which inhibits SFKs, BCR-ABL, platelet-derived growth factor receptor β, and c-Kit. A study of patients receiving dasatinib for the treatment of chronic myeloid leukemia showed that 9 out of 40 patients developed fluid retention and lung abnormalities.3 Interestingly, this side effect dissipates with time, suggesting tolerance or emergence of counterregulatory mechanisms.

Mice or humans express 8 closely related SFKs: Blk, Fgr, Fyn, Hck, Lck, Lyn, c-Src, and Yes.4 The Src kinases are potent, phosphorylating a diverse range of substrates, many of which are involved in cytoskeletal assembly or reorganization. They differ in their tissue expression patterns, such as epithelial or neural, lymphoid or myeloid. They also differ in the amino acid sequences at their N terminus, a region that has not been solved structurally or characterized functionally other than serving as an acceptor site for acyl groups that localize the kinases to the plasma membrane. Subtle differences likely exist in their Src homology 3 and Src homology 2 domains, which interact with
Donating used CARs

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