intact. Another important characteristic of the LOX-1/L5-based blocking approach is that hemorrhaging in the brain was not observed. This is an important point showing a certain potential of this strategy for future prevention of ischemic stroke or acceleration of patients recovery after the event.

One of the connections between stroke pathology and platelet function is uptake and secretion from platelets of amyloid β peptide. Interestingly, platelets serve as one of the main sources of αβ in the circulation. Several studies demonstrated that αβ peptide accumulates in the cerebral vasculature with aging and contributes to endothelial damage, which in turn might serve as another pathologic player in a series of events leading to stroke.

Secretion of αβ amyloid was shown to be inducible by well-known platelet agonists such as thrombin and collagen. Shen et al show that αβ is secreted from platelets activated by L5 via LOX-1 and involves a signaling pathway including IκB kinase 2 (IKK2) and nuclear factor-κB (NF-κB) activation (see figure). This creates an additional stimulatory "autocrine" loop during platelet aggregation. There is an apparent synergism between L5 and the αβ peptide; both are able to augment platelet responses that are induced by their respective "partners in crime." The clear evidence of NF-κB involvement in platelet responses came from the use of an IKK2 inhibitor in an in vivo hemostasis model. In the presence of both L5 and the αβ amyloid peptide, the IKK2 inhibitor attenuated hemostasis returning bleeding times to control levels. Altogether, this study establishes an important pathway of platelet activation with an apparent relevance to stroke pathobiology.

One of the most remarkable findings of this study is that L5 levels are almost 40-fold higher in stroke patients compared with 10-fold the cost and twofold the hospitalizations and mortality rate of noninhibitor patients. Thus, preventing or reducing inhibitors is a major goal of hemophilia management. Unfortunately, this is an exciting time in hemophilia treatment with the promise of novel therapeutics that potentially bypass existing inhibitor anti-FVIII response, ie, small

rather complex mixture. Overall, it remains unknown what exact chemical moieties on L5 lipoproteins are recognized by LOX-1 and stimulate platelet activation.

Taken together with a series of previous reports, it appears that prothrombogenic activities of LDLs are mediated by several pattern recognition receptors expressed on platelets. At present, these include TLR9, a receptor for final oxidized products of phospholipids, and 2 distinct scavenger receptors: CD36, a receptor for intermediate products of phospholipid oxidation, and LOX-1, a receptor for the negatively charged L5 fraction of LDL. The involvement of pattern recognition/scavenger receptors is understandable because platelets seem to respond to the rather wide spectrum of compounds. Many of these compounds remain to be identified and fully characterized.

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Comment on Chen et al, page 1346

Platelet VIII pack evades immune detection

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In this issue of Blood, Chen et al demonstrate that platelets expressing factor VIII (FVIII) shield FVIII from immune detection. In the naive FVIIInull hemophilia A (HA) mouse, platelet-derived VIII prevents both a primary and memory anti-FVIII immune response, and together with total body irradiation, suppresses anti-FVIII immune response.1

Among the most serious complications of hemophilia treatment is the formation of inhibitor antibodies, which occurs in 25% to 30% of those with severe HA, usually within the first 20 to 30 exposures.2 Inhibitor formation is a T-cell-dependent, B-cell-mediated immune response to foreign infused FVIII,3 which renders standard FVIII replacement therapy ineffective. Treatment is difficult, as bypass agents such as FVIIa or factor-eight inhibitor bypass activity, are less effective than FVIII in noninhibitor patients, and result in poorly controlled bleeding, with 10-fold the cost and twofold the hospitalizations and mortality rate of noninhibitor patients.3 Thus, preventing or reducing inhibitors is a major goal of hemophilia management.

Fortunately, this is an exciting time in hemophilia treatment with the promise of novel therapeutics that potentially bypass existing inhibitor anti-FVIII response, ie, small

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interfering RNA-antithrombin 3 and bispecific FVIII monoclonal antibody, or suppress new anti-FVIII inhibitor response, ie, rFVIIIFc. In this study, Chen et al take advantage of their lentiviral-mediated platelet-specific FVIII gene delivery model in which FVIII is protected from protease degradation by storage in platelet α-granules, to explore the immune response to platelet FVIII (see figure). In their model, hematopoietic stem cells (HSCs) from the FVIIInull HA mouse are transduced with 2bF8 LV. Platelets were isolated from HSCs in the presence of cytokines, and 2bF8 LV was detected in platelet α-granules. Following conditioning by a lethal dose of 1100 cGy total body irradiation, 6- to 8-week FVIIInull mice received 2bF8-transduced platelets by retro-orbital or tail-vein injection. Platelet FVIII expression was measured by chromogenic assay and anti-FVIII by Bethesda assay. FVIII function was assessed by survival after tail clip. 

Platelet-derived FVIII. The 2bF8 lentiviral vector (LV) was constructed by transfection of lentivirus in 293T cells by plasmids containing hBD-FVIII complementary DNA (cDNA). HSCs collected from the bone marrow of tibia and femurs of FVIIImm mice were transduced with 2bF8 LV. Platelets were isolated from HSCs in the presence of cytokines, and 2bF8 LV was detected in platelet α-granules. Following conditioning by a lethal dose of 1100 cGy total body irradiation, 6- to 8-week FVIIImm mice received 2bF8-transduced platelets by retro-orbital or tail-vein injection. Platelet FVIII expression was measured by chromogenic assay and anti-FVIII by Bethesda assay. FVIII function was assessed by survival after tail clip. 

In their current elegant studies, Chen et al showed that infusion of 2bF8 genetically manipulated platelets, ie, platelet–derived FVIII, into the FVIIImm HA mouse not only restores normal hemostasis after tail clip and corrects FVIII levels, but also prevents de novo inhibitor formation. Further, they demonstrated that platelet–derived FVIII prevents a memory immune response to FVIII in FVIII–null mice with inhibitors. Moreover, platelet–derived FVIII suppressed anti-FVIII response even when preconditioning included a nonmyeloablative regimen, thus confirming that platelet–derived FVIII reduces immunogenicity and suppresses anti-FVIII response, independent of the effects of a myeloablative conditioning regimen.

So, how does platelet–derived FVIII escape the immune system? First, these studies indicate that infusion of platelet–derived FVIII is effective against bleeds, and, further, does not trigger an immune response in the FVIIImm HA mouse. The mechanism by which platelet–derived FVIII escapes immune detection is not fully understood. Platelets sequester FVIII in α-granules, and thus FVIII exposure to the blood stream and immune system is very limited. Even in the setting of bleeds, such as during retro-orbital infusion, or endothelial injury when FVIII is released from activated platelets to promote clot formation, or when aged platelets are phagocytosed by macrophages, FVIII would be expected at the site of bleed or injury or phagocytosis in sufficient amounts to trigger the immune system, but no immune response has been detected. The reduced immunogenicity afforded by platelet–derived FVIII appears to be long-lived, as additional experiments have shown anti–FVIII-inhibitor response is suppressed in the HA FVIII–null mouse even weeks after these studies were completed, and after subsequent exposure to standard recombinant FVIII.

Whether platelet–derived FVIII suppresses FVIII–specific T–regulatory cell response is not known, but plausible, as platelets contain many biologically active proteins that are increasingly recognized to play a role in the regulation of immune response in inflammation and infection.

In conclusion, the transfusion of platelets containing FVIII appears to provide a less immunogenic factor replacement for HA mice. Whether this is also true for patients with HA will require the conduct of prospective clinical trials. If confirmed, this platelet VIII approach may provide a much needed therapeutic alternative to current therapies for hemophilia patients with inhibitors.

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Comment on Jacoby et al, page 1361

**Allogeneic CAR19 cells clear ALL**

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Chimeric antigen receptor T cells redirected to CD19 (chimeric antigen receptor [CAR19]) show great promise in the clinic to treat refractory CD19⁺ acute lymphoblastic leukemia (ALL). However, production of autologous CAR19 cells from these patients can be difficult as patients frequently have T-cell dysfunction, due to disease and/or treatment-related effects. In this issue of *Blood*, Jacoby et al¹ addressed this by exploring whether allogeneic donor CAR19 cells could be used to treat ALL-bearing mice using a minor mismatch bone marrow transplant model.

In this murine model, allogeneic CAR19 cells were very effective in eradicating ALL; they also induced graft-versus-host disease (GVHD) in host mice. The GVHD only occurred when the recipient mice were leukemia-bearing and was caused by CD4⁺ CAR19 cells. This GVHD response occurred in the context of a proinflammatory microenvironment, as CAR19 cells were given 2 days after bone marrow transplantation (BMT) conditioning therapy. In contrast, when an alternate BMT model with delayed allogeneic CAR19 infusion was used, GVHD was ameliorated. Furthermore, GVHD was abrogated when CAR19 cells were given to leukemia-bearing hosts reconstituted with interleukin (IL)-6⁻/⁻ donor BM. It is not yet clear which donor BM cells are the critical source of IL-6 in this model (see figure).

These data at first appear to contradict the clinical data reported by the authors²; however, the patients who received allogeneic CAR19 received treatment of persistent disease after transplant and donor lymphocyte infusion (DLI) infusion with no or very mild GVHD. The CAR19 cells were given with no chemotherapy or conditioning regimens. Jacoby et al show that the timing of the CAR19 therapy and conditioning therapy is critical for determining clinical outcome. In addition, their model system provides key information regarding the importance of IL-6 biology in clinical response to CAR T-cell therapy. The IL-6R blocking antibody (tocilizumab) has now been used successfully to treat patients with cytokine release syndrome (CRS) following CAR19 therapy³,⁴ or GVHD.⁵ The study by Jacoby et al indicates that tocilizumab could be used for autologous CAR19 therapy, as there was no apparent difference in antitumor efficacy, and toxicity may be reduced. In addition, tocilizumab could be used for allogeneic donor-derived CAR19 DLI therapy after BMT.

The CAR T-cell field is going through rapid change, and new CARs are being designed, tested in preclinical models, and rapidly transitioned into the clinic. In parallel with the study by Jacoby et al, alternate strategies include donor-derived CAR T cells where endogenous TCR expression has been silenced.⁶ This CAR19 product induced a favorable antitumor response, with no GVHD, in 1 patient with refractory ALL. We wait for ongoing clinical data to establish whether the...
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