The VWF-GPIb interaction is independent of platelet activation. Rather, VWF senses mechanical force responding with elongation of multimers that expose A1 domain interaction sites initiating adhesion. Once formed, the VWF-GPIb bond is mechanically stabilized; the association of a rapid rate of bond formation with resistance to tensile stress supports the initial capturing of platelets that transition from rapid flow to low-velocity translocation on reactive surfaces.[4] During this stop/go motion, GPIb-IX-V transduces tensile stress on the VWF bond into intracellular signals[5] linked to platelet activation.[6] Platelet adhesion at sites of vascular injury typically involves VWF immobilized onto adhesive substrates, such as collagen, which are capable of activating platelets on their own. Thus, discerning how signals induced by VWF-A1–GPIb binding contribute to thrombogenesis requires separating adhesion from transduction effects, which has not been possible to date.

On this background, the article by Zhang and colleagues is a decisive step toward understanding how GPIbα senses force.[1] Noting that the mucin-like macroglycopeptide interposed between the ligand-binding and juxtamembrane regions of GPIbα argued against long-distance allosteric effects following VWF ligation, they devised an approach to document force-induced structural changes at the single-molecule level. Thus, they identified a mechanosensitive domain (MSD) that is structured but unstable and unfolds by pulling on VWF-A1 bound at the opposite end of the GPIbα extracytoplasmic portion (glycocalicin). They also documented that altering the MSD in GPIbα mutants abolished force-induced unfolding of the protein, indicating that no other region of the molecule can serve as an alternative mechanotransducer.

With the findings presented in this issue,[1] new directions emerge for future studies. Developing models that can differentiate between GPIbα-mediated adhesive and signaling functions will illustrate how stimulation by force transmitted through the WFA1 bond is integrated with signals from other platelet receptors to promote thrombus growth and stability. This may lead to identifying new targets for antithrombotic intervention. Of note, the GPIbα cytoplasmic domain is linked to filamin-A,[7] contributing to membrane stability. In a mouse model, mutated GPIbα with normal VWF binding (but lacking anchorage to filamin-A) causes membrane disruption when platelets translocate on VWF,[8] signifying that force is transmitted to the cytoskeleton through the VWF bond under normal conditions. This could be one reason for the hitherto unexplained influence that gain-of-function type 2B VWF mutants with enhanced GPIbα binding have on thrombocytopoiesis, causing increased platelet size and altered structure as seen when GPIb-IX expression is defective.[9] Therefore, lack of GPIbα produces effects on thrombocytopoiesis similar to its enhanced stimulation. This is apparently paradoxical, but it may indicate, in agreement with the proven role of shear stress in megakaryocyte biology,[10] that fine-tuning of the response to mechanical stimuli is key for normal platelet production. Determining the possible role of GPIbα-mediated mechanotransduction in thrombocytopoiesis will be facilitated by the identification of the MSD in this receptor.

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REFERENCES

1. Zhang W, Deng W, Zhou L, et al. Identification of a juxtamembrane mechanosensitive domain in the platelet mechanosensor glycoprotein Ib-IX complex. *Blood*. 2015; 125(3):562-569.

2. Hernandez-Hernandez V, Rueda D, Caballero L, Alvarez-Buylla ER, Benitez M. Mechanical forces as information: an integrated approach to plant and animal development. *Front Plant Sci*. 2014;5:265.

3. Breslin JW. Mechanical forces and lymphatic transport [published online ahead of print August 5, 2014]. *Microvasc Res*. doi:10.1016/j.mvr.2014.07.013.

4. Savage B, Saldívar E, Ruggeri ZM. Initiation of platelet adhesion by arrest onto fibrinogen or translocation on von Willebrand factor. *Cell*. 1996; 84(2):289-297.

5. Mazzucato M, Pradella P, Cozzi MR, De Marco L, Ruggeri ZM. Sequential cytoplasmic calcium signals in a 2-stage platelet activation process induced by the glycoprotein Ibalpha mechanoreceptor. *Blood*. 2002;100(8): 2793-2800.

6. Kasirer-Friede A, Cozzi MR, Mazzucato M, De Marco L, Ruggeri ZM, Shattil SJ. Signaling through GP Ib-IX-V activates alpha IIb beta 3 independently of other receptors. *Blood*. 2004;103(9):3403-3411.

7. Nakamura F, Stossel TP, Hartwig JH. The filamins: organizers of cell structure and function. *Cell Adhes Migr*. 2011;5(2):160-169.

8. Cranmer SL, Ashworth KJ, Yao Y, et al. High shear-dependent loss of membrane integrity and defective platelet adhesion following disruption of the GPIbα-filamin interaction. *Blood*. 2011;117(9):2718-2727.

9. Nurden P, Gobbi G, Nurden A, et al. Abnormal VWF modifies megakaryocytopoiesis: studies of platelets and megakaryocyte cultures from patients with von Willebrand disease type 2B. *Blood*. 2010;115(13):2649-2656.

10. Jiang J, Woulfe DS, Papoutsakis ET. Shear enhances thrombopoiesis and formation of microparticles that induce megakaryocytic differentiation of stem cells. *Blood*. 2014; 124(13):2094-2103.

● ● ● TRANSPLANTATION

Comment on Hechinger et al, page 570

Anti–common γ-chain antibody: one for all in GVHD

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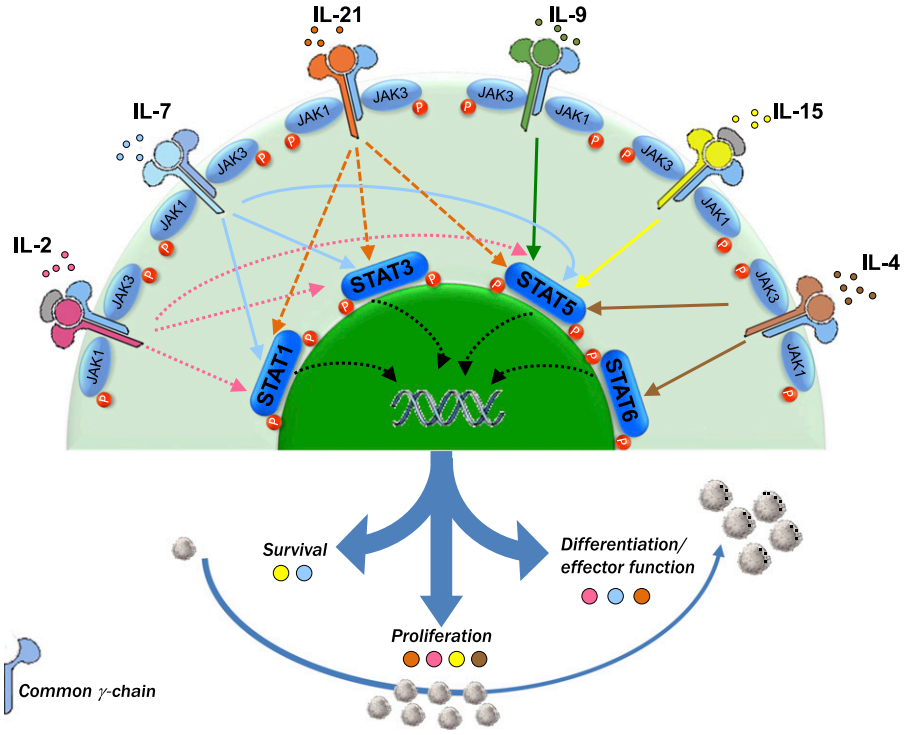
In this issue of *Blood*, Hechinger and colleagues investigate the efficacy of the blockade of the common γ-chain receptor (CD132) as a novel approach in the management of both acute and chronic graft-versus-host disease (GVHD).[1]

The common cytokine receptor γ-chain family consists of interleukin-2 (IL-2), IL-4, IL-7, IL-9, IL-15, and IL-21, and is so called because the receptors for these cytokines share the same γ chain.[2] The gene encoding the γ chain (*IL2RG*) is mutated in humans with X-linked severe combined immunodeficiency (XSCID), and these patients lack T cells and natural killer (NK) cells, which indicates that the γ chain is crucial for the development of these cells.

Acute GVHD has been described as a "cytokine storm" involving a 3-step disease process[3]: (1) conditioning regimen–associated inflammation,



The common cytokine receptor γ-chain family includes IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. This receptor acts mainly through the JAK-STAT pathway. The blockade of these cytokines with an antagonistic antibody directed against CD132 inhibits proliferation and differentiation of T cells and, in turn, might also inhibit GVHD.

(2) donor T-cell priming, and (3) an effector phase mediated by cytokines and cellular effectors. In this regard, numerous studies have correlated the levels of cytokines with the risk of GVHD. More specifically, the plasma levels of IL-7 and IL-15 measured along the first month posttransplant have been identified as highly predictive biomarkers for acute GVHD.[4] All of these data have prompted the development of clinical trials evaluating the efficacy of cytokine inhibitors either for the prophylaxis or for the treatment of GVHD.[5] Although some studies have reported promising results, most have failed to demonstrate a significantly favorable impact on outcome. In contrast to these studies, the blockade of multiple cytokines with an antagonistic antibody directed against CD132 might avoid the redundant effect of most cytokines, which might underlie the poor results reported when a single cytokine is inhibited, and, furthermore, this strategy might be useful both in the acute and in the chronic setting. In this regard, although early studies supported the paradigm of acute GVHD being a T-helper cell 1 (Th1) and chronic GVHD being a Th2 process,[6] recent data suggest a specific cytokine's signature depending on the targeted organ, both in acute and in chronic GVHD. For example, Th2 differentiation, characterized by secretion of IL-4, may be involved in liver and skin GVHD. Similarly, chronic lichenoid GVHD shows a mixed Th1/Th17 signature. Interestingly, treatment of established chronic GVHD using anti-CD132 monoclonal antibody (mAb) reversed liver and lung fibrosis and prevented immunoglobulin deposition. It is worth mentioning that aberrant B-cell function and alloantibody deposition have been shown to play a key role in lung and liver chronic GVHD.[7] Furthermore, from a pathophysiological point of view, chronic GVHD is the result of a highly complex network involving both B cells and T cells, this interaction taking place in the germinal center. By targeting T follicular helper cells that produce IL-21, anti-CD132 mAb might also inhibit germinal center B-cell proliferation.[8] Moreover, IL-21 favors B-cell differentiation into antibody-secreting plasma cells through the Janus kinase–signal transducer and activator of transcription 3 (JAK-STAT3) pathway, which is inhibited by anti-CD132. Consistent with the fact that the γ-chain family cytokines signal through the JAK-STAT pathway, T cells exposed to anti-CD132 mAb showed reduced JAK3 phosphorylation upon activation. This is of particular interest considering the role of JAK3 in lymphocyte activation and, therefore, in GVHD, as shown in several studies demonstrating a decreased risk of GVHD upon exposure to JAK inhibitors, both in preclinical and in clinical models.[9]

Finally, the perforin-granzyme pathway plays a key role in $CD8^+$ T-cell–mediated GVHD. In addition to the effect of anti-CD132 mAb on cytokines, Hechinger and colleagues elegantly show that the blockade of the common γ chain inhibits granzyme B production by CD8 T cells.[1] Remarkably, the cytotoxic molecule granzyme B is required for CD8 T-cell function and, therefore, its reduction could affect antitumor immunity. Nevertheless, recent studies suggest that *GzmB* deficiency significantly enhances the ability of donor $CD8^+$ T cells to control tumor growth in the hosts via other mechanisms.[10] Thus, although further studies will be required to elucidate this issue, this strategy might decrease GVHD while maintaining a graft-versus-leukemia effect.

In summary, the common γ-chain receptor is a key player in survival, proliferation, activation, and differentiation of T cells. Furthermore, it also affects other subpopulations such as dendritic cells, B lymphocytes, and regulatory T cells. Not surprisingly, its blockade dramatically modifies the immune response after transplantation and opens a potential new route for the management of GVHD.

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REFERENCES

1. Hechinger A-K, Smith BAH, Flynn R, et al. Therapeutic activity of multiple common γ-chain cytokine inhibition in acute and chronic GVHD. *Blood.* 2015;125(3):570-580.

2. Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by γ(c) family cytokines. *Nat Rev Immunol.* 2009;9(7):480-490.

3. Ferrara JL, Deeg HJ. Graft-versus-host disease. *N Engl J Med.* 1991;324(10):667-674.

4. Thiant S, Labalette M, Trauet J, et al. Plasma levels of IL-7 and IL-15 after reduced intensity conditioned allo-SCT and relationship to acute GVHD. *Bone Marrow Transplant.* 2011;46(10):1374-1381.

5. Alousi AM, Weisdorf DJ, Logan BR, et al; Blood and Marrow Transplant Clinical Trials Network.

Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. *Blood*. 2009; 114(3):511-517.

6. Coghill JM, Sarantopoulos S, Moran TP, Murphy WJ, Blazar BR, Serody JS. Effector CD4+ T cells, the cytokines they generate, and GVHD: something old and something new. *Blood*. 2011;117(12):3268-3276.

7. Srinivasan M, Flynn R, Price A, et al. Donor B-cell alloantibody deposition and germinal center formation are required for the development of murine chronic GVHD and bronchiolitis obliterans. *Blood*. 2012; 119(6):1570-1580.

8. Flynn R, Du J, Veenstra RG, et al. Increased T follicular helper cells and germinal center B cells are required for cGVHD and bronchiolitis obliterans. *Blood*. 2014;123(25):3988-3998.

9. Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. *Blood*. 2014;123(24):3832-3842.

10. Bian G, Ding X, Leigh ND, et al. Granzyme B-mediated damage of CD8+ T cells impairs graft-versus-tumor effect. *J Immunol*. 2013;190(3):1341-1350.