Comment on Zhang et al, page 2889

Fragmenting the platelet to reduce metastasis

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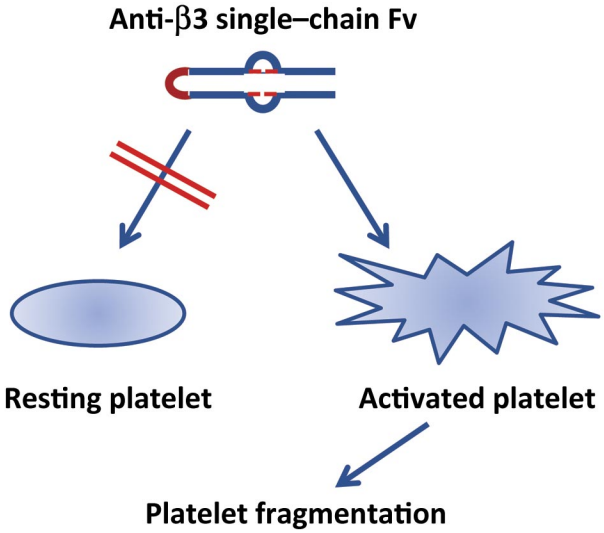
In this issue of *Blood*, Zhang et al describe an antiplatelet reagent attacking metastasis, the most deadly aspect of cancer.[1] Their experiments outline an early-stage preclinical approach providing proof-of-principle for antiplatelet strategies that may ultimately lead to an improved prognosis for the cancer patient.



A single-chain variable fragment (scFv) recognizes the activated conformation of the b3 subunit of the αIIbβ3 platelet receptor. Upon binding to platelets the scFv induces an oxidative platelet fragmentation that reduces tumor metastasis.

The platelet's relevance beyond normal hemostasis and thrombosis continues to grow, due in part to a wealth of reagents and animals models.[2] The supportive role of platelets in various aspects of cancer provides the background for studies targeting the platelet.[3] Specifically, Zhang et al target the β3 subunit of the platelet's fibrinogen receptor, αIIbβ3, with a humanized single-chain antibody (scFv). The generation and antiplatelet properties of the scFv have been previously reported but these investigators have expanded their characterization to include antimetastatic properties.

Rather than a simple inhibition of αIIbβ3 function, the intriguing property of this scFv is its ability to induce oxidative platelet fragmentation preferentially for an activated platelet (see figure). The scFv recognizes a linear sequence within the extracellular portion of both the mouse and human β3 subunits. The success of the reagent in reducing metastasis is probably based on platelet–tumor cell interactions where the platelet shields the tumor cell in the circulation from the immune system and/or provides a fibrin-rich mesh to support extravasation from the bloodstream.[4,5] One of the more interesting results for the scFv is its minimal side effects in causing thrombocytopenia or increasing the bleeding risk in animal models. Supportive experiments confirm the scFv effects are restricted to antiplatelet properties and not via anti-β3 effects on other cell types, such as endothelial cells.

The possibility for this type of antiplatelet targeting is very intriguing both in the realm of cancer therapy and for all platelet influenced pathologies. The results suggest the scFv is only eliciting its antiplatelet activity when activated platelets are bound to tumor cells. Balancing the normally needed platelet properties in hemostasis and inhibiting platelet function in other pathologic settings has remained one of the biggest challenges in the development of antiplatelet therapies. While measurement of mouse bleeding is less sophisticated than assessing human bleeding, the possibility of maintaining normal hemostasis while targeting the activated platelet would be met with great anticipation.

Somewhat unexpected, the scFv appears to have no effect on angiogenesis. While platelets are known to store and release both pro- and anti-angiogenic proteins the inability of the scFv to influence angiogenesis is most likely related to the state of the platelet targeted by the scFv. Indeed, the fragmentation elicited by the scFv may still release stored proteins that support the platelet's relevance in angiogenic pathways, but more work on this topic is needed to completely understand.

If these results are to translate to clinical trials the in vivo mouse models used by Zhang et al must reflect the human cancer situation. The models used are common approaches for studying metastasis but neither may exactly mimic the situation occurring in human disease. In experimental metastasis a large bolus of tumor cells is injected into the venous circulation and the majority of cells are quickly lodged during transport to their first capillary bed in the pulmonary circulation. Thus, experimental metastasis produces a dramatic number of tumor nodules in the lung. Secondly, the spontaneous model represents a large primary subcutaneous tumor where some cells do leave the site to also find a capillary bed for extravasation in lung tissue. How predictive these models are to the complex events surrounding human metastatic disease is still an open question.

Moving forward there still exist challenges. First is the relatively short window within which the anti-β3 scFv is effective. The injection of the scFv 4 hours before, or after, the addition of tumor cells to the vasculature was effective at reducing metastasis. Administration of the scFv 12 hours before, or after, tumor cell addition was not effective. Are there situations where a short time frame of administration could still be advantageous? Purely speculative at this point, but perhaps during surgical removal of a primary tumor the scFv could be a temporary second line of defense to reduce the likelihood of viable tumor cells

traveling through the bloodstream. There may be methods to improve the half-life of the anti-β3 scFv in the bloodstream and this would be obvious improvement now that the exciting basic proof-of-principle data exist.

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Comment on Brennan et al, page 2899

α-1 antitrysin DAMPens GVHD

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In this issue of *Blood*, Brennan et al report that a noninfectious damage-associated molecular pattern (DAMP), heparan sulfate (HS),[1] aggravates graft-versus-host disease (GVHD) and that this enhanced severity can be dampened by administration of serine protease inhibitor α-1 antitrysin (AAT).[2]

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy against many hematologic diseases. However, despite the curative potential its application has been limited by its many complications, most notably GVHD. Detection of tissue damage by innate immune cells using pattern-recognition receptors (PRRs) that sense noninfectious molecular signatures called DAMPs is known to augment adaptive immunity.[3] After conditioning for allo-HSCT, both infectious and damage-associated noninfectious signals (DAMPs) enhance severity of GVHD.[4] However, the nature and type of DAMPs implicated in augmenting GVHD remain poorly understood. Brennan and colleagues provide an important piece of the puzzle by demonstrating that HS, an extracellular matrix component, enhanced host antigen-presenting cell function in a TLR4-MyD88–dependent manner and accentuated alloreactive donor T cell–mediated GVHD.[2]

Excessive inflammation underlies pathologic immunologic processes, including GVHD.[5] Although the GVHD-enhancing aspects proinflammatory cytokines is well appreciated, much remains unknown about the specific instigators that induce, perpetuate, and accentuate inflammation after allogeneic HSCT.[4] The classic instigators of inflammation are the microbial-derived pathogen-associated molecular pattern molecules (PAMPs) and the nonmicrobial tissue damage-associated DAMPs that are recognized by PRR-bearing immune cells.[3] Several endogenous molecules such as high mobility group1, adenosine-5′-triphosphate, heat shock protein (HSP) 70, fibronectin, HS, hyaluronic acid, and uric acid can function as DAMPs. Many PRRs, including the TLR family, recognize both PAMP and DAMP motifs.[3] Activation of innate immunity by these motifs shapes adaptive immunity.[3] Experimental studies have only recently begun to uncover the role of specific DAMPs (likely generated in the allo-HSCT hosts from either disease or conditioning or GVHD-related tissue damage) in modulating GVHD severity.[6]

Brennan et al analyzed in vitro impact of several DAMPs in enhancing alloreactive T-cell proliferation.[2] They found that only HS and HSP70 significantly promoted alloreactive T-cell expansion. This in vitro enhancement of alloreactive T-cell proliferation by HS was dependent on the expression of TLR4 and MyD88 in the stimulators (dendritic cells [DCs]) and not in the responders (allo-T cells). HS also promoted in vitro maturation and secretion of proinflammatory cytokines from DCs in a predominantly TLR4-MyD88–dependent manner. Using transfection studies of epithelial cell lines they found that HS caused NF-κB translocation in a TLR4-dependent manner. They explored the relevance of HS-mediated inflammation, in vivo, by measuring HS levels after MHC matched, minor antigen mismatched experimental allogeneic bone marrow transplantation (BMT). HS levels increased a week after BMT and returned to baseline by 3 weeks after BMT in the allogeneic recipients. The kinetics suggest that the increase in this extracellular matrix moiety, HS, might be more a consequence of GVH-induced damage than perhaps from conditioning-related tissue damage. Using 4C-TCR-trangenic T cells in a creative experimental model system that employed an MCH mismatched BMT model, they suggest persistence of host class II–expressing antigen presenting cells (APCs) at the time of peak levels of HS. However, it is unclear whether the kinetics of HS levels in sera are the same in MHC matched and mismatched BMT systems. Furthermore, the data do not directly show whether the class II–expressing cells are indeed radio-resistant host hematopoietic APCs (as suggested) and/or if they are also nonhematopoietic APCs. It is also formally possible that PAMPs like lipopolysaccharides that are recognized by TLR4 may also simultaneously contribute in an additive or synergistic manner to the role of HS in aggravating GVHD. Nonetheless, administration of an HS mimetic further accelerated GVHD, indicating an important role for HS in aggravating GVHD. In addition, HS levels were elevated in the sera of patients from a relatively small and mixed cohort of allogeneic BMT recipients and correlated temporally with onset of GVHD in these patients. In light of the levels of HS being higher in patients with Hunter syndrome (mucopolysaccharidosis Type II), it would be worth exploring whether these patients suffer from higher-than-anticipated rates of GVHD after allogeneic BMT. Importantly and consistent with previous observations, in multiple experimental models when allogeneic recipients were treated with AAT, a serine protease inhibitor that is clinically available and known to inhibit elastase, GVHD was significantly attenuated.[7-9] AAT-induced reduction in GVHD correlated with reduced HS levels and was observed only in wild-type but not in TLR4-deficient mice. Brennan and colleagues posit that AAT reduced GVHD