The notion that mucosal delivery of antigen can lead to unresponsiveness came from seminal experiments a century ago by Wells and Osborne. These workers demonstrated that prior oral feeding of antigens could prevent the immune response to a number of proteins in guinea pigs. This was followed up by Sulzberger and Chase, who pointed out its immunologic specificity, and was pioneered later by Weiner’s group in applying oral tolerance to treat autoimmune diseases. Indeed, there is evidence for oral tolerance in humans based on anecdotal epidemiologic data in that the incidence of peanut allergy is 10-fold lower in Israel, where peanut snacks are widely given to infants, than in the United Kingdom. A formal clinical study is now being done in the United Kingdom (http://www.leapstudy.co.uk/).

This use of mucosal administration of antigen to induce tolerance for inhibitor formation to clotting factors was first described by Alpan et al at the American Society of Hematology (ASH), who persuaded mice to drink milk loaded with factor IX (FIX). Rawle et al in Lillicrap’s group tested the effect of oral and nasal delivery of the FVIII C2 domain in FVIII−/− mice with hemophilia A. They found that such mucosal administration of the FVIII C2 domain, a major target of inhibitors, not only blocked the response to C2 but also led to partial reduction of total anti-FVIII antibodies. However, relatively large amounts of protein were necessary for efficacy so that this approach would not seem to be feasible due to the high cost of therapeutic FVIII.

In this issue, Sherman et al, in a collaboration between the Herzog and Daniell groups, report that oral gavage of plant cell extracts expressing FVIII antigens (heavy chain = A1, A2, and B domains; C2 domain) led to significant unresponsiveness to FVIII challenge in terms of both total antibody and inhibitor titers in 2 different hemophilia mouse strains. In this study, transplastomic (using chloroplasts) lines were created in tobacco to express these FVIII domains fused to the cholera toxin B subunit in order to promote transmucosal delivery. The expressed proteins assembled into pentameric forms, to facilitate binding to GMI ganglioside receptors, and were shown to be protected and delivered intact to the mucosal immune system. This extends the results of these collaborators who used oral delivery of transgenic plant extracts for tolerance to FIX (presented at the 2012 ASH annual meeting, Atlanta, GA, December 8–11, 2012). Oral tolerance to plant-expressed FVIII led to an increase in immunomodulatory cytokines, transforming growth factor-β/latency associate protein, and interleukin-10; in addition, regulatory T cells were induced that could transfer tolerance to naive murine recipients. Tolerance did require multiple injections over an 8-week period, with FVIII challenge commencing after 4 weeks.

Importantly, oral delivery into previously immunized recipients, as a model for subjects with preexisting inhibitors, also led to a reduction in antibody and inhibitor titers (see figure). This approach is an important advance because it does not require large amounts of FVIII. It is not known, however, how long the tolerogenic effect will last. Additional administration may just be necessary to maintain tolerance. Thus, transgenic plants may offer less costly alternative therapies for tolerance, not only for hemophilia inhibitor formation, but also for autoimmune diseases. Indeed, there is precedent for the latter in a diabetes model. Further development of edible plants, such as lettuce, and efforts for scaled-up low-cost production will clearly be needed to move this therapy into the clinic. Then, there will be good reason to say, “Eat your veggies and become tolerant!”

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REFERENCES


Go with the flow: *S. aureus* and vascular infection

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In this issue of Blood, Claes et al elegantly illustrate the importance of the *Staphylococcus aureus* von Willebrand factor binding protein (vWbp) in the initiation of vascular lesions under flow. There has been considerable discussion in the field with respect to the hierarchy of importance of different virulence factors of *S. aureus* that contribute to the pathogenesis of endovascular infections such as infective endocarditis, an infection of the heart valve. Do the interactions of *S. aureus* with serum proteins, platelets, or endothelial cells and exposed extracellular matrix proteins make the most significant contribution? This paper shows for the first time that vWbp is critical in establishing lesions on the surface of the in vivo vasculature under physiological flow via its von Willebrand factor (VWF) binding and procoagulant activity.

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Comment on Claes et al, page 1669
Previous research by Pappelbaum et al. found that VWF is the host mediator of *S. aureus* vascular endothelial cell binding. This study augments those findings by clearly identifying that the *S. aureus* bacterial virulence factor, vWbp, is responsible for this interaction. The VWF binding protein of *S. aureus* (vWbp) was first described in 2002. However, its role in endovascular infection by *S. aureus* remained unclear until this study. Protein A, another *S. aureus* surface protein, was originally identified as a VWF binding protein. However, under conditions of high shear stress, Protein A does not promote the adherence of *S. aureus* to VWF. Claes et al. show that a combination of its adhesive and procoagulant activity contributes to the ability of vWbp to promote endovascular infection, an effect that becomes apparent under physiologically relevant shear stress conditions.

This paper also shows that exogenously produced vWbp associates with *S. aureus* cells to promote the observed VWF adherence under physiological shear stress. Therefore, although the Pappelbaum et al. study indicated that teichoic acids are involved in the interaction of *S. aureus* with VWF, it is tempting to speculate that the reduction in adherence to vascular endothelial cells under flow that was observed in a *S. aureus* strain deficient in wall teichoic acid is, in part, a result of a reduced association of vWbp with *S. aureus* due to the lack of wall teichoic acids on its cell surface. Further studies may demonstrate whether this is, in fact, the case.

It will be interesting to follow-up on the observations in this study by studying the VWF binding capacity of other clinically relevant bloodstream pathogens under physiologically relevant shear stress. This will be particularly interesting in the case of bacteria that frequently cause bacteremia and bloodstream infections and that have been shown to cause thrombocytopenia but are not frequent causative agents of infective endocarditis, for example, *Escherichia coli*. It remains possible that bacterial interactions with VWF under flow are the critical discriminating factor between these different types of cardiovascular infections. This is especially relevant seeing that inflammation-activated endothelial cells appear to be a critical source reservoir of VWF that serves to capture platelet bacteria thrombi on the vessel surface.

The outstanding contribution in this paper is the real-time intravascular imaging of bacterial platelet thrombi in vivo using bacterial and mouse isogenic mutations to illustrate the conclusions drawn from the in vitro data. Encouragingly, this study shows that dabigatran and related compounds offer the possibility of adjunctive therapy to antibiotics in *S. aureus* sepsis by slowing down or halting the coagulation of bacteria and platelets by inhibiting the action of staphylothrombin, a combination of prothrombin, *S. aureus* coagulase (Coa), and vWbp. This vWbp- and Coa-mediated coagulation significantly contributes to the lethal outcomes of sepsis in vivo as observed by McAdow et al. However, the complications of anticoagulation therapy in sepsis represent a significant drawback in terms of bleeding risks in the clinic. Perhaps the approach taken in a 2013 study that identifies vWbp and Coa as effective vaccine targets represents a more sustainable strategy and an indication that the function of vWbp and Coa can be inhibited to successfully treat *S. aureus* bloodstream infections. The data presented in this paper are a significant step in our understanding of *S. aureus* bloodstream and vascular infections and offer a real opportunity to develop a therapeutic treatment of these infections that may have an important clinical impact and one that does not rely solely on antibiotic therapies.

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### REFERENCES

Comment on Pai et al, page 1677

Proteasome: target for acute and chronic GVHD?

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In this issue of Blood, Pai et al provide exciting murine and clinical data for proteasome inhibition in the treatment of chronic graft-versus-host disease (cGVHD).1

With increased utilization of unrelated donors after allogeneic hematopoietic stem cell transplantation (HSCT), cGVHD is now the leading cause of late morbidity and mortality. Most patients (≥50%) develop cGVHD requiring corticosteroids, and about half of those will require additional therapies, a major risk factor for poor survival. Several immunosuppressant agents, largely empirically tried, have shown little benefit as second-line therapy. Thus, an urgent need exists for novel approaches with improved activity and tolerability in patients that develop cGVHD that is not responsive to steroids.

Newly established National Institutes of Health criteria now provide a comprehensive classification that encompasses the diverse clinical manifestations of cGVHD; however, its underlying biology has largely remained obscure. Emerging experimental data have implicated dysregulation of humoral immunity with germinal B-cell aberrations and generation of pathologic allo- and autoantibodies as potentially key mechanisms.2,3 Consistent with these murine data, correlative studies from patients have implicated antibodies against HY antigens in female-to-male HSCT,4 antibodies against platelet-derived growth factor receptor-α,5 and increases in B-cell activation factor (BAFF)6 in clinical cGVHD. These observations, together with the finding that rituxan may prevent clinical cGVHD, further support a putative role of B cells in its pathogenesis.

Bortezomib, which is Food and Drug Administration approved for 2 malignant B-cell neoplasms (multiple myeloma and mantle cell lymphoma), possesses broad immunomodulatory properties. In previous murine models and in a clinical trial, bortezomib prevented acute GVHD (aGVHD).7,8 Whether bortezomib would have a similar effect on cGVHD was previously unknown. In a murine model of sclerodermatous cGVHD, Pai et al show that administration of bortezomib attenuates clinical and pathologic skin lesions after the onset of cGVHD. Animals treated with bortezomib showed reduced germinal center B cells and decreased expression of nuclear factor-κB and BAFF in cGVHD lesions. Because thymic epithelial damage is believed to contribute to immune dysregulation, the findings of increased thymic cellularity and regulatory T-cell/conventional T-cell ratios further suggested proteasome inhibition had broad salutary effects on the immunological aberrations of cGVHD. Bortezomib also did not impair antitumor immunity in animals. Furthermore, variations in the timing of administration had an impact on the rates of response of cGVHD but did not accelerate or increase cGVHD severity. These experiments were necessary because in previous studies, timing critically altered the response and toxicity of aGVHD. The most exciting aspect of this work by Pai et al is that they performed a pilot proof of concept clinical trial in 10 patients with steroid refractory cGVHD. Informed by their murine data, suggesting skin necrosis might occur at higher bortezomib doses, a careful approach to intrapatient dose escalation was undertaken. Preliminarily, bortezomib had good tolerability and potential for inducing some striking clinical responses. These data are also supported by another recent clinical study.9 Thus, the study by Pai et al is exciting because, for the first time, they implicate proteasome inhibition as a pathogenic pathway for targeting and ameliorating cGVHD.

The authors should be commended for melding preclinical and human studies in such a thoughtful manner; however, several questions remain. First, much remains to be understood about the specific dysfunction of B cells and the potential complex interactions with other immune cells in cGVHD. The role played by the immune dysfunction in causing fibrosis and the impact on reversing fibrosis remain poorly understood. In the current study, although the authors demonstrate clinical responses, the specific and causative cellular and molecular mechanisms responsible for the effects of proteasome inhibition on fibrogenesis were not explored. Some of these questions have, in the past, been hampered in part due to the absence of murine models that completely mimic all aspects of clinical cGVHD (kinetics and manifestations). As such addressing these questions will require considerable and continuous efforts. Second, the nature and composition of the specific ubiquitinated proteins targeted by the proteasome that are critical for cGVHD remain unknown. It remains a possibility that targeting a limited number of the ubiquitinated proteins may allow for improved tolerability and less toxicity than global proteasome inhibition.10 Third, cGVHD has protean manifestations, and whether all or only some patients will respond, and if so, under what circumstances, has been challenging to study in a rigorous manner. Thus, the clinical observations by Pai et al, while exciting, are obviously limited by the small patient numbers. Furthermore, the authors suggest established fibrosis may be difficult to treat, and even in responders, recurrences of cGVHD were observed after...
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