the target may be more relevant based on the biology of an IgM-secreting cell. Indeed, previous studies using the same assay did not demonstrate a difference in proteasome levels in CD138$^+$ cells from normal donors and myeloma patients.[6] More importantly, the data demonstrate inhibition of both proteasomes. Because the immunoproteasome is only expressed constitutively in hematopoietic cells, this may provide an explanation as to why B-cell malignancies are exquisitely sensitive to this class of therapeutics.

The constitutive proteasome and immunoproteasome are nearly identical, with both possessing 3 catalytic subunits in their 20s barrel.[7] These subunits have chymotryptic-like, tryptic-like, and caspase-like activity, with the chymotryptic-like subunit being the target of ONX0912 as well as the primary site of action of bortezomib. However, the subunits that contain these activities are different in the constitutive proteasome (β5, β2, and β1, respectively) and the immunoproteasome (LMP7, MECL1, and LMP2, respectively). These changes result in differences in the roles that each proteasome plays. In addition to degradation of the unneeded, damaged, or misfolded proteins from the cytoplasm and endoplasmic reticulum, the constitutive proteasome regulates signal transduction, cell-cycle progression, and apoptosis. While initially it was believed that the reason for the different subunits in the immunoproteasome was to optimize peptide generation during antigen processing for MHC class I presentation, recent evidence suggests that additional roles exist for the immunoproteasome including cytokine production.[8] Amazingly, the use of proteasome-specific inhibitors demonstrates that the cellular processes controlled by each proteasome are in part distinct as selective inhibition of the constitutive proteasome does not affect IL-23 production in monocytes.[8] However, these selective inhibitors do not induce apoptosis when used individually,[6] thus overlapping functions are likely to exist as well. Therefore, normal and transformed B cells may be dependent on both proteasomes and explain why these cells are so sensitive to proteasome inhibition. It will be important to determine which processes are uniquely controlled versus those that are regulated by both proteasomes to further understand the biology of diseases like WM (see figure).

These findings may also explain why it is hard to alter the effect of proteasome inhibitors by blocking the individual effects of proteasome inhibition. For example, the authors demonstrate activation of the unfolded protein response yet pharmacologic inhibition of this response provides minimal, albeit statistically significant protection from ONX0912-induced death.[1] This is similar to previous studies that demonstrated that myeloma cells are significantly more sensitive to bortezomib than NF-κB inhibition.[9] In contrast, JNK is activated and its inhibition has a significant effect on ONX0912-induced death. This indicates that JNK activation is downstream of both proteasomes and may provide clues to how proteasome inhibition results in cell death. More intriguing may be the effects of ONX0912 on the bone marrow stromal cells that can support WM cell growth and survival through the production of cytokines. ONX0912 does not kill these cells but inhibits cytokine production. It remains to be determined which proteasomes are present in these cells. However, it adds to the promise of this class of agents for the treatment of B-cell malignancies such as WM, and now this double-barreled inhibition can be achieved with an orally available drug.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● THROMBOSIS & HEMOSTASIS

Comment on Schulz et al, page 4102

Proteomics unravels platelet function

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In this issue of *Blood*, Schulz and colleagues report promising findings using differential proteomics as a discovery tool to identify functionally important proteins in platelet activation. The authors identify several proteins that change in abundance upon platelet activation. These findings implicate several novel pathomechanisms relevant to platelet activation and identify novel potential therapeutic targets for platelet inhibition.[1]

Platelets have a pivotal role in thrombosis, vascular repair, and inflammatory reactions. They lack nuclei and genomic DNA and are thus not amenable to most of the classical cell, molecular biology, and genomic techniques. For the identification of proteins and novel protein functions, proteomic studies are therefore the method of choice. Proteomic approaches are further favored by easy access to large amounts of platelet proteins via blood donations. Platelets possess a pre-mRNA splicing machinery and also have remnants of megakaryocyte-derived mRNA, which both can result in rapid translation upon platelet activation.[2,3] Thus, proteomic comparisons between resting and activating platelets are expected to reveal differences in abundance of proteins. Technical advances in proteomic technologies and recent achievements in generating protein data repositories hold great promise for important discoveries in platelet research.[4,5]

The proteomic comparison of 2 functional states of platelets by Schulz et al is based on the elegant use of the 2-dimensional difference gel electrophoresis (2D-DIGE).[1,6] Classical 2D gel electrophoresis is hampered by inter-gel variability as well as difficulties in protein quantification. In 2D-DIGE, samples are labeled with different fluorescent dyes (typically Cy3 and Cy5, providing a wide dynamic range of fluorescence quantification) and run on the same 2D gel. Gels are scanned with the excitation wavelength of each dye. This allows a direct comparison to each other and to an additional sample (eg, labeled with Cy2), which is used as an internal control allowing a quantitative comparison between gels. These dyes are designed to bind to lysine-residues, to have the same molecular masses, and to preserve the charge of the labeled proteins. Thus, this provides a true reflection of the protein abundance throughout the investigated proteome.[6] Together with advances in the rapidly advancing fields of mass spectrometry and protein database content and management, 2D-DIGE is an ideal discovery tool for platelet research.

Mechanistic research on platelet activation is very much driven by the prospect of identifying novel targets for antiplatelet therapy. Although current antiplatelet drugs are among the most successful drugs ever developed (in regard to both direct mortality/morbidity benefits and commercial success), there appears to be a link between higher efficacy and higher rates of bleeding complications. Proteomic discovery approaches that aim to identify novel players in platelet activation are therefore also inherently drug discovery programs in the search for better therapeutic targets.

Tucker et al recently reported such a discovery approach focusing on the membrane-associated proteome of thrombin-activated platelets.[7] Using biotinylation of the platelet surface, the authors isolated and identified proteins that were abundantly associated with the platelet membrane upon platelet stimulation with thrombin. One of the proteins identified, HIP-55, which is an SH3-binding adaptor protein, becomes associated with Syk and the integrin subunit β_3 upon platelet activation. The authors also demonstrated the functional relevance of this protein in $\alpha_{IIb}\beta_3$ (GPIIb/IIIa) activation.

Schulz et al chose to study glycoprotein VI (GPVI)–induced platelet activation. GPVI mediates platelet binding to collagen, which itself induces cross-linking (ligation) of the receptor causing outside-in signaling and platelet activation.[1] Genetic deficiency and ex vivo as well as in vivo receptor blocking data suggest a central role of GPVI in platelet adhesion and aggregation as well as in thrombus formation. This makes the receptor as well as the associated signaling pathways attractive as therapeutic targets.[8] Indeed, one of the 9 proteins found in higher abundance upon GPVI ligation, aldose reductase, is the kind of protein one would like to "discover" with a differential proteomic approach. Functional investigations revealed that inhibition of aldose reductase impaired GPVI-induced platelet aggregation. This property warrants further investigation on its use as a therapeutic target for antiplatelet therapy. Another protein "discovered" on 2D-DIGE upon GPVI ligation was ERp57, a disulfide isomerase, which Schulz et al could show to be released after platelet activation and which converts tissue factor from an inactive to an active form.[1] This finding describes an interesting link between GPVI-induced platelet activation and tissue factor–induced blood coagulation.

The report of Schulz et al is a good example of how modern proteomic technologies can become a driving force to better our understanding of platelet (patho)physiology and to pursue discovery of novel targets for antiplatelet therapy.[1] Using 2D-DIGE with a systematic evaluation of the stimulation of other platelet receptors such as P_2Y_{12}, GP1bα, and protease-activated receptors seems a most promising approach for further discoveries.

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● ● ● TRANSPLANTATION

Comment on Brown et al, page 4111

The TREC to less CMV after UCBT

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In this issue of *Blood*, Brown and colleagues demonstrate the importance of thymic recovery and T-cell reconstitution in CMV-specific immunity after allogeneic UCB transplantation.

Umbilical cord blood (UCB) is increasingly being used as an alternative donor source for hematopoietic cell transplantation (HCT). In addition, double UCB transplantation has overcome the obstacle of cell dose and opened the use of UCB as a stem cell source for larger adults. T-cell reconstitution after HCT depends on both the homeostatic expansion of the adoptively transferred T cells from the UCB graft, as well as de novo stem cell–derived T-cell production. In this latter situation, the thymus is required for both positive and negative T-cell selection and durable immunity. The T cells transferred in UCB transplantation are immunologically naive and do not provide passive immunity to the transplant recipient. As a result, there has been concern for prolonged reconstitution of antigen-specific immunity and increased risk for viral infections after UCB transplantation.[1,2] In fact, some studies show fewer CMV-specific $CD4^+$ and $CD8^+$ T cells and a higher incidence of viral infections after UCB transplantation.[3-5]