the alternative pathway of complement was also able to abolish residual lytic activity.

These findings give insight into the mechanism of action of eculizumab and improve our understanding of the eculizumab breakthrough hemolysis that may occur in patients with PNH following infections, surgery, pregnancy, and other conditions that overwhelm complement regulation.

A limitation of this study is that the experiments were performed ex vivo and used rabbit, sheep, or human erythrocytes as a readout for terminal complement activity. Thus, these findings may not be as relevant in vivo and may not directly apply to atypical hemolytic uremic syndrome where terminal complement-mediated attack targets nucleated cells that have the ability to repair their membrane. It is also important to point out that PNH patients do extremely well on eculizumab, and only a minority experience severe breakthrough hemolysis. In some, but not all, instances this can be overcome by increasing the dose of eculizumab. Nevertheless, the work by Harder et al lends valuable insight into our understanding of complement activation and may inform future clinical trials of novel complement inhibitors in human disease.

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Comment on Sebastian et al, page 991

Oxygen bubbles to predict sensitivity to IMiDs

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In this issue of Blood, Sebastian et al provide evidence that the immunomodulatory drugs (IMiDs) inhibit intracellular decomposition of H2O2 resulting in oxidative stress and that the individual cellular antioxidative capacity of multiple myeloma (MM) cells predicts response to this widely used class of drugs.
Alongside proteasome inhibitors (PIs), the thalidomide analogs, lenalidomide and pomalidomide, are key agents in the current treatment of MM and have an increasing role as powerful combination partners in the dawning era of immunotherapy. However, mechanisms of action and resistance are still not fully understood. It has recently been shown that the antitumor effect of all IMiDs requires binding to the ubiquitously expressed E3 ligase, cereblon (CRBN), leading to degradation of the zinc finger proteins IZKF1/Ikaros and IZKF3/Aiolos, mediating abrogation of IRF4 and Myc expression. Additional downstream sequelae of this IMiD/CRBN interaction include the destabilization of the CD147—cereblon (CRBN) complex. As a result, either the destabilization of the CD147—cereblon (CRBN) complex. As a result, either the absence of CRBN or the mutations within, for example, the IMiD-binding domain, lead to IMiD resistance. However, these studies cannot explain how MM cells become resistant to IMiDs despite the continued expression of wild-type CRBN.

Sebastian et al here turn to the long-known observation that thalidomide triggers oxidative stress. They exposed MM cell lines with different degrees of IMiD sensitivity to H₂O₂ and assessed their capacity to decompose this oxidative agent by simply monitoring oxygen bubble formation. The inverse correlation observed between IMiD sensitivity and oxygen production led them to a more sophisticated analysis using flow cytometry, taking advantage of the autofluorescent properties of flavin adenine dinucleotide (FAD) and NADPH (reduced nicotinamide adenine dinucleotide phosphate) to assess cellular antioxidative capacity in a more standardized, quantitative assay. They demonstrated a striking inverse correlation between IMiD sensitivity and antioxidative capacity in both a variety of MM cell lines and primary patient samples (see figure).

Although this potential predictive biomarker will need to be prospectively validated in clinical trials, the authors went on to show that IMiDs increase peroxides in a CRBN-dependent manner by inhibiting the enzyme, thioredoxin reductase. Although the direct mechanism by which this enzyme is inhibited by IMiDs remains elusive, further studies using direct inhibitors of thioredoxin reductase strongly suggest that this enzyme is a promising therapeutic target in MM, even more so as the efficacy of these direct inhibitors appeared to be CRBN independent. Furthermore, the authors found that oxidative stress triggered by IMiDs increased the formation of dimers between immunoglobulin light chains. This excess of misfolded protein overrides the cellular chaperone capacity, thereby inducing ER stress, which finally results in the proapoptotic activation of the BH3-only protein, BIM (see figure). The recognition of this cascade is another important finding of this study as it finally identifies a mechanistic link between exposure to IMiDs and the induction of proteotoxic stress, a well-known “Achilles’ heel” of immunoglobulin-producing MM cells, and might help explain why IMiDs are more effective in plasma cell malignancies than in most other B-cell disorders.

Thinking 1 step ahead, the other major therapeutic strategy in MM, the inhibition of proteasomes, is also well known to strongly increase proteotoxic stress by inhibiting the proteasomal degradation of misfolded proteins. It is therefore no surprise that not only were the authors not able to confirm the synergistic efficacy of combining IMiDs and PIs, as known from clinical trials, but also they found an augmented production of ER stress and proapoptotic sequelae. This observation is of great interest as there has been an ongoing debate regarding the mechanisms underlying this clinically observed synergy even though the main mechanism of action of IMiDs on MM cells, as previously understood, required the ubiquitination and proteasomal degradation of misfolded proteins, a well-known mechanism by which thioredoxin reductase is inhibited by IMiD-bound CRBN, and might help explain why IMiDs are more effective in plasma cell malignancies than in most other B-cell disorders.

As with any research that introduces new concepts, this work by Sebastian et al will stimulate several lines of investigation, which are likely to include elucidation of the mechanism by which thioredoxin reductase is inhibited by IMiD-bound CRBN, validation of antioxidative capacity as a robust predictive biomarker within clinical trials, the identification of clinical grade agents that directly target reactive oxygen decomposition to overcome or prevent IMiD resistance, and, finally, further investigation of this proposed cascade of mechanisms within cells of the (immune-) microenvironment.

Most importantly, it will be essential to clarify the clinical relevance of this proposed mechanism of resistance to IMiDs in the context of those of which we are currently aware, including loss of CRBN expression, the occurrence of CRBN-IZKF1/3 mutations, and differences in IMiD-induced immune cell activation.
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Comment on Noubouossie et al, page 1021

Demystifying the prothrombotic role of NETs

Christian Schulz and Steffen Massberg

In this issue of Blood, Noubouossie and colleagues report surprising findings on the role of neutrophil-derived nuclear material in blood coagulation. The authors provide evidence that, in contrast to DNA and histone proteins, neutrophil extracellular traps (NETs) do not contribute directly to coagulation of human plasma. These findings implicate differential functions of nuclear material in thrombosis and are of importance for the development of antithrombotic therapies targeting NETs.1

Innate immune cells play an important role in blood coagulation by releasing prothrombotic molecular cues. Monocytes and their microvesicles provide tissue factor, which initiates the extrinsic pathway of coagulation. Neutrophils release, among other factors, peroxidase and proteases, which inactivate anticoagulants such as tissue factor pathway inhibitor and thrombomodulin to promote blood coagulation. These prothrombotic pathways culminate in the generation of fibrin, the end product of the coagulation cascade. Fibrin provides an intravascular scaffold to trap and eliminate pathogens.2 Propagation of blood coagulation by innate immune cells therefore contributes to intravascular immunity and is now recognized as an integral part of host defense.3

In addition to soluble molecules, activated neutrophils release nuclear material in form of chromatin lattices, known as NETs.4 Extracellular nucleosomes consist of DNA wound around histone proteins reaching variable diameters dependent on the extent of chromatin decondensation. In addition, NETs are decorated with antimicrobial peptides and proteases derived from neutrophil cytoplasmic granules. They contribute to the elimination of bacteria and fungi, which bind directly to NETs and are exposed to a variety of antimicrobial defenses.4 NETs thereby contribute to establish an intravascular scaffold for the containment and elimination of pathogens. Importantly, NET formation not only contributes to host defense but also has been associated with cardiovascular diseases and other clinical conditions. In fact, NETs are present in arterial thrombi of patients with myocardial infarction and in thrombus specimens retrieved from the venous circulation.5 Inhibition of NET formation or dismantling NETs by DNase treatment reduces thrombosis in mice.6,7 Thus, targeting NETs has evolved as an interesting strategy for the treatment of thrombotic conditions. However, the precise contribution to clot formation of the nuclear elements comprising NETs has been unclear.

Noubouossie et al addressed the differential effects of purified NET components on the coagulation of human plasma in vitro. They report that purified DNA not only activates the intrinsic pathway of coagulation through FXII but also amplifies tissue factor–dependent thrombin generation. Further, single histones induce thrombin generation in a platelet–dependent manner (see figure). In contrast, intact NETs have no procoagulant effect in vitro, and purified DNA loses its procoagulant activity when histones are added.1 This is surprising, since studies in mice provided evidence that NET destruction represents an efficient antithrombotic strategy.2,8 Why should intact NETs be less thrombogenic? The authors speculate that this could be due to neutralization of the negative charge of DNA on the NET surface. Pending further proof under these experimental conditions, this concept is not unlikely, since histones provide a positive net charge, and charge–charge interactions may result in its neutralization.9 This could limit activation of the contact system and thrombin generation. Interestingly, the antimicrobial activity of NETs depends on its negative charge. Neutralizing this property by providing excess cations reduces bacterial killing.10

The report of Noubouossie et al therefore provides an important contribution to our understanding of the role of neutrophil nuclear material in blood coagulation. It suggests that therapeutic strategies targeting NETs should be directed against specific NET structures such as histone proteins, DNA, or NET-bound serine proteases, which give free rein to blood coagulation by proteolysis of the coagulation suppressor tissue factor pathway inhibitor. Nevertheless, future studies will have to assess whether these findings hold in more complex settings.
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Marc S. Raab