Clearance of apoptotic neutrophils by macrophages (efferocytosis) is an important mechanism regulating inflammation, host responses, and cancer. Clearance of dead cells is important to avoid unwanted inflammatory responses. In this issue, Park and colleagues show that uPAR modulates neutrophil efferocytosis exploiting macrophages and neutrophils isolated from uPAR Ko mice. Indeed, uPAR−/− macrophages show increased engulfing activity of viable (uPAR+/+) neutrophils both in vivo and in vitro. A similar increase in neutrophil uptake is observed when using uPAR−/− neutrophils and uPAR+/+ macrophages, but not when both cell types are uPAR-negative. The data suggest a mechanism different from the “eat me” or “don’t eat me.” Interestingly, administration of exogenous suPAR (a soluble version of uPAR) reverses both phenotypes. Indeed, suPAR inhibits the increased uptake of uPAR+/+ neutrophils by uPAR−/− macrophages as well as the increased uptake of uPAR−/− neutrophils by uPAR+/+ macrophages. The authors’ interpretation is that suPAR modulates the adhesion of neutrophils/macrophages through direct interactions with integrins both in cis and trans. Remarkably, the positive effect of unilateral uPAR deficiency on neutrophil uptake by macrophages seems to require the nonredundant functions of a large number of integrins including αM, αV, β1, β2, and β3 on both the neutrophil and the macrophage, as well as the LDL receptor-related protein, LRP. A direct effect of exogenous suPAR on the activity of Mac1 was previously described.

The complexity of the uPAR/efferocytosis relationship is further underscored by another paper published almost at the same time. D’Mello et al show that uPAR overexpression in nonprofessional phagocytes stimulates efferocytosis of apoptotic cells by cancer cells, specifically. While in this work the cells used are not professional phagocytes, the result is nevertheless opposite that of Park et al. Moreover, in this effect, a direct role of integrins seems to be excluded. It is important to realize that uPAR overexpression is a frequent event in cancer cells and that its presence may induce an environment more favorable for cancer invasion by regulating the infiltration and clearance of inflammatory cells.

Although the precise mechanism underlying uPAR function in efferocytosis still remains to be elucidated, the data from these papers clearly point to an unrecognized role in heterotypic cell–cell adhesion.

The role of the uPAR ligand, uPA, was not addressed in either of the 2 papers. However, contact between apoptotic cells and macrophages induces IL10, which is required for efferocytosis. In turn, IL10 induction requires the activity (and the tyrosine phosphorylation) of a transcription factor, Prep1. Prep1 (at that time called UEF3) was discovered years ago as a transcription factor binding to the enhancer region of the uPA gene in a region that serves to modulate the response to various proliferation (and other) stimuli. Is it possible that there is a connection between Prep1-dependent uPA expression, uPAR, and efferocytosis?

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Comment on Qadura et al, page 871

Plasma-derived and recombinant FVIII

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While plasma-derived and recombinant coagulation FVIII may largely share the same amino acid sequence and restore coagulation equally well, Qadura and colleagues demonstrate in this issue of Blood that these molecules appear quite different to the immune system.
A major complication of treatment of hemophilia A is the formation of inhibitory antibodies against the therapeutic factor VIII (FVIII) protein, which occurs with an overall incidence of 20% to 30% of patients. Using hemophilia A mice and in vitro assays, Qadura et al find that a plasma-derived FVIII product containing von Willebrand factor (VWF) activates dendritic cells (a critical antigen-presenting cell for the initiation of a specific immune response) quite differently than recombinant FVIII. Similarly, when injected into the hemophilia mice, the cytokine profiles of responding T cells differed substantially after administration of the 2 FVIII formulations, suggesting that different subsets of T-helper cells were being stimulated. The response to recombinant FVIII had a bias toward Th1 response but also involved production of the regulatory cytokine IL-10, while plasma-derived protein caused mostly a Th2 response and up-regulation of TGF-β, a cytokine with immune-suppressive properties. In addition, plasma-derived FVIII promoted FoxP3+ Treg responses and caused lower-titer inhibitor and total antibody (IgG) formation compared with recombinant FVIII.

A controversial debate over potential differences in the immunogenicity of FVIII products has been ongoing for years. Plasma-derived products may vary in purity and VWF content. Recombinant products produced in mammalian tissue culture are of high purity, do not contain VWF, and may be full-length or B domain–deleted FVIII. Some studies had claimed a higher propensity of recombinant products to cause inhibitor formation, which, however, may have been influenced by the retrospective nature of such analyses and has not been substantiated by prospective studies.

Questions that fuel the discussion of immunogenicity of factor products include the safety of switching treatment of a patient from one product to another, matching of products to specific patient populations, and the role of VWF in immune responses. Similar discussions are ongoing about which product and dose to use in immune tolerance induction (ITI) protocols. Different hemophilia treatment centers report diverse experiences. For example, a recent report documents a higher success rate using a plasma-derived, VWF-containing product compared with recombinant FVIII in elimination of inhibitors by ITI. It has been hypothesized that VWF could prevent binding of inhibitors by masking epitopes on the C2 domain or otherwise interfere with antibody binding. Qadura et al propose an alternative mechanism, namely antigenic competition for processing by antigen-presenting cells. Interpretation of these animal data are somewhat complicated by murine immune responses to human VWF that may not occur in patients. Nonetheless, this new study provides the hemophilia research community with the exciting perspective of having an animal model available that allows studies that address immunogenic differences between FVIII products, doses, and treatment protocols. In addition, such studies can determine involvement of T regulatory cells in controlling immune responses, as evidence is mounting that active suppression of antibody formation by this subset of T cells is a critical component of maintaining tolerance to coagulation factors.

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Comment on van der Meijden et al, page 881

Thrombosis in flowing blood

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In this issue of Blood, van der Meijden and colleagues report on the mechanisms by which collagen exposure in flow–dependent circulation contributes to thrombus formation.

In their report, van der Meijden et al show that 2 independent summing mechanisms for thrombin generation initiated by fibrillar type I collagen lead to platelet and coagulant thrombus formation. Type I collagen added to citrated human plasma upon recalcification shortens thrombin generation times (TGT). TGT are blocked by the addition of corn trypsin inhibitor (CTI) but not in the presence of inactivated factor VIIa (FVIIa), a tissue factor pathway inhibitor. TGT are also blocked in factor XII— or XI–deficient plasma. Under conditions of whole blood flow, addition of CTI (but not FVIIa treatment), reduced platelet adherence to collagen, and combined CTI and JAQ1 glycoprotein VI (GPVI)–blocking antibody treatment further decreased platelet adhesion and thrombin generation. Platelet activation by GPVI was mediated through LAT and PLCγ2, which are established signaling pathways for GPVI.1 These investigations show that collagen exposure in flowing blood results in at least 2 pathways for thrombus formation, one directly through platelet adhesion and activation, and another indirect mechanism via thrombin generation in response to contact activation.
Plasma-derived and recombinant FVIII

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