vein endothelial cell (HUVEC) tube formation under both normoxic conditions (20% O₂, remote effect) and hypoxic conditions (1% O₂, local effect). Thus, hypoxic MM cells may modulate their microenvironment to enhance angiogenic potential by secretion of exosomes (see figure).

Recently, miRNAs have been implicated as critical factors in exosomes because they largely decide exosome functional consequences in recipient cells. To dissect how hypoxic exosomes released from MM cells promote angiogenesis, Umezu et al profiled miRNA expression in 3 lines of HR-MM cells and their released exosomes. They observed that the levels of miR-210 and miR-135b were consistently upregulated in both acute and chronic hypoxia-treated MM, and also were highly encased in exosomes released from these cells. However, the authors further identified that high levels of miR-210 were only maintained in hypoxic culture and gradually disappeared in the normoxic condition. By contrast, upregulation of miR-135b could be maintained in the normoxic condition. On the basis of these findings, Umezu et al suggest that miR-210 is a universal hypoxia-responsive miRNA with transient effect, whereas miR-135b is an HR-MM-cell–specific miRNA with chronic effect. Given that exosomes are able to shuttle their contents between cells, the authors also provided convincing evidence showing that exosomal miR-135b could be effectively delivered to HUVECs and functionally targeted to the 3′-UTR of the factor-inhibiting hypoxia-inducible factor-1 (HIF-1) gene, leading to reduced expression of FIH-1 (see figure). FIH-1 is an asparaginyl hydroxylase enzyme that inhibits the transcriptional activity of HIF-1. Accordingly, the HIF-1 activity was dramatically increased in hypoxic exosome–treated HUVECs. Therefore, this work by Umezu et al suggests that hypoxia-driven, accelerated angiogenesis is ascribed to exosomal miR-135b shed from HR-MM cells by targeting the FIH-1/HIF-1 signaling pathway (see figure).

It should be commented that Umezu et al provide compelling evidence in vivo and in vitro evidence showing that knockdown of miR-135b in HR-MM exosomes dampened their proangiogenic effects. However, a note of caution should be added: exosomes derived from chronic hypoxia MM cells might encapsulate many specific proteins (ie, receptors and kinases), miRNAs, and mRNAs. In fact, the authors have presented in this study that chronic hypoxia MM-exosomes encase tens of other miRNAs in addition to miR-135b. It is therefore possible, if not likely, that other exosomal contents may additionally contribute to the enhanced angiogenesis in MM.

Finally, the authors nicely made an effort to characterize exosomes from the primary myeloma cells of 2 MM patients. Although their findings indicate that miR-135b levels were elevated in both exosomes and parental myeloma cells from one of the MM patients, the authors could not define any association between upregulation of exosomal miR-135b and therapy-resistant MM patients with this limited number of MM patients. Another puzzling issue is that, even when MM cells exhibit high levels of miR-135b, its levels in plasma are very low. The plausible interpretation Umezu et al provided in this study is that exosomal miR-135b might play a role in local niche rather than circulation. Thus, future studies will need to clarify the clinical significance of exosomal miR-135b in a large number of MM patients. Clinically, it will be very interesting to explore in the future whether blockade of exosome biogenesis/release in MM cells can improve patients’ survival.

Overall, this work by Umezu et al is exciting because they report for the first time that under chronic hypoxia conditions, MM cells enhance angiogenesis through the exosomal transfer of miR-135b to endothelial cells, resulting in a reduced expression of FIH-1 and increased activity of HIF-1 (see figure).

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PLATELETS & THROMBOPOIESIS

Comment on de Stoppelaar et al, page 3781

Platelets: balancing the septic triad

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In this issue of Blood, de Stoppelaar et al further unravel the relevance of platelets in a mouse model of pneumonia-derived sepsis by illustrating how platelets dynamically modulate infection and the inflammatory response.1

A major challenge in sepsis research is understanding the complex interplay of thrombotic and inflammatory processes that lead to a widespread vascular collapse and death. Although strong individual paradigms exist for describing clotting and inflammation, when and how these processes influence each other is less clear. Thrombocytopenia is a common finding in severe sepsis and is increasingly recognized as more than just
a simple marker of consumptive coagulopathy.\(^2\) Indeed, together with other studies, the work of de Stoppelaar et al suggests that circulating platelets promote inflammation during the early stages of infection, but later inhibit cytokine release when the inflammatory response becomes high.

Beyond the mechanistic complexities of sepsis are the limitations imposed by various animal models and the ability to translate findings to the human condition. Several options exist, and de Stoppelaar et al have used a mouse model of Klebsiella infection, a common respiratory pathogen and causative agent for sepsis in pneumonia. The investigators chose to deplete platelets in animals to varying degrees by using antiplatelet antibodies and then evaluate in vivo parameters such as survival, bacterial growth, hemorrhage, distant organ damage, and proinflammatory cytokine release.

To fully appreciate the findings of de Stoppelaar et al requires a detailed consideration for the temporal sequence of events that lead to the septic condition. One of the simplest conclusions of their study is that after platelet depletion and inoculation with Klebsiella, serum markers of inflammation such as tumor necrosis factor (TNF)-\(\alpha\) and interleukin-6 (IL-6) are elevated, and animal survival is decreased. This data alone would suggest that normal platelet levels are important for survival and for impairing cytokine release. However, during the earliest stages of infection, platelet depletion increases the bacterial load in the bloodstream, which suggests a platelet-dependent mechanism in the innate immune response. This indicates that platelets participate in a protective manner in early sepsis by lowering the bacterial load, and then in the later stages of sepsis, they modulate cytokine levels during the “cytokine storm.” Thus, the platelet has a central role in modulating the balance between antimicrobial response, inflammatory response, and hemostasis/thrombosis (see figure).

The molecular basis for how platelets participate in the pathophysiology of sepsis is still to be defined. An initial, perhaps overly simplistic, thought is to attribute the platelet-dependent consequences during sepsis to circulating and intact platelets. However, platelet activation and the release of platelet microparticles may be more relevant mediators of the pathophysiology associated with sepsis.\(^3\) Work by Xiang et al\(^4\) supports the relevance of platelets in a lipopolysaccharide (LPS)-induced endotoxemia and a bacterial infusion mouse sepsis model while implicating activation of a platelet COX1 pathway in regulating the inflammatory response. The platelet adhesion receptor glycoprotein Ib-IX (GPIb-IX) has also been implicated in the regulation of the immune response in models of both LPS and polymicrobial peritonitis.\(^5,6\) GPIb-IX–dependent platelet interactions with neutrophils and monocytes were also documented to influence neutrophil and monocyte phenotype.\(^6\) Going forward, a more comprehensive view of the molecular interactions between platelets and immune cells is needed. This will require establishing models that allow dissecting the platelet’s role in hemostasis, thrombosis, and inflammation.

There have been major criticisms for how closely any murine models of sepsis mimic the human septic situation. Indeed, every model has its limitations, and the human septic situation is a heterogeneous clinical situation that results from differences in the primary sites of infection, the pathogens involved, and the overall health status of the patient. Some have even suggested that studies of inflammation in murine models have no merit because of observed differences in global gene expression patterns during infection or inflammation when comparing mice and humans.\(^7\) Recently however, others have argued the opposite, suggesting that mouse models are an important tool for modeling inflammatory diseases.\(^8\) Independently, both hemostasis/thrombosis and inflammation have been modeled for decades by using rodent models with significant impact on and relevance to our understanding of human pathophysiology. Thus, mouse models are likely to continue as a major experimental approach to the topic of sepsis for generating new hypotheses that can be further evaluated for the clinically challenging topic of human sepsis.

To the practicing physician, and even more so the practicing hematologist, defining the relevance of platelets in the inflammatory events occurring in the progression of sepsis is likely to be important for guiding patients in their use of antiplatelet medications. Important questions need to be addressed. How relevant are the major antiplatelet medications, such as aspirin, in the progression of sepsis? Are the molecular events associated with sepsis simply too robust for any antiplatelet medication to impact the course of the disease? Is targeting platelet-leukocyte interactions a potential therapeutic approach in sepsis? Although these questions are far beyond the implications of the work by de Stoppelaar et al in this issue of Blood, they do remind us of the platelet’s exclusive presence in mammals. How much relevance to inflammation has been retained from its counterpart in lower vertebrates—the thrombocyte—in lower vertebrates? Although the platelet has been the cornerstone of research in thrombosis, adapting platelet paradigms to bridge hemostasis, thrombosis, and inflammation marks a major challenge in platelet research going forward. More studies, such as those reported in this issue of Blood, are needed and are likely to have major impacts in our understanding and therapeutic approaches to address unmet clinical needs.
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Comment on Kanakry et al, page 3817

GVHD prophylaxis made safe, easy, and inexpensive

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In this issue of Blood, Kanakry and coworkers report on the use of posttransplant cyclophosphamide (Cy) as sole immunoprophylaxis against graft-versus-host disease (GVHD) in HLA-matched related or unrelated allogeneic stem cell transplant (HSCT).1

This pilot study included 209 consecutive adult patients with acute myeloid leukemia (n = 138), acute lymphoblastic leukemia (n = 43), or myelodysplastic syndrome (n = 28). All patients received T-cell–replete allografts. The cumulative incidence of acute GVHD of grades II-IV at 100 days was 45%; that of acute GVHD of grades III-IV was 11%. In line with experience using posttransplant Cy in haploidentical HSCT, the probability of chronic GVHD was strikingly low (13%). Three-year probability of relapse was 36%. Thirty percent of the patients were not in morphologically complete remission at the time of transplant. At 3 years, disease-free survival was 46% and overall survival was 58%. Transplant-related mortality (TRM) at 3 years was 15% (95% confidence interval: 10-20%) for the entire cohort.

Although the probability of severe acute GVHD was comparable to that using a calcineurin inhibitor combined with a short arm of methotrexate, TRM was low, which may suggest a low risk of death from infections and toxicity—demonstrating the safety of this protocol.

Advantages of using posttransplant Cy are that it selectively depletes proliferating alloreactive T cells while preserving resting memory T cells, which are essential for immunological recovery after HSCT. This approach is certainly much simpler and less expensive than alternative approaches to improve immune reconstitution after HSCT, such as T-cell depletion and add-back of suicide gene–manipulated T cells, in vitro depletion of alloreactive donor T cells, or the development of cytotoxic donor T cells against cytomegalovirus, adenovirus, Epstein-Barr virus, and fungi, which may cause severe infections after HSCT.2

The team from the Johns Hopkins University pioneered the use of posttransplant Cy to control posttransplant alloreactivity using T-cell–replete grafts and HLA-haploidentical donor transplantation.3 Posttransplant Cy was based on a mouse study in which Luznik and coworkers demonstrated that posttransplant Cy administered on day +3 allowed stable engraftment across major histocompatibility complex barriers using a nonmyeloablative regimen.4 This concept has revolutionized haploidentical transplants, with an increasing number of such transplants being performed worldwide.5 The concept of posttransplant Cy in haplo-HSCT has challenged double cord blood transplants6 and unrelated donor transplants with similar survivals (Mary Eapen et al, Center for International Blood and Marrow Transplantation Research, unpublished data).

Now the concept of posttransplant Cy has been extended to HLA-matched HSCT.

The low TRM rates with posttransplant Cy may be due to the low rate of posttransplant infections, and there have been no posttransplant lymphoproliferative disorders reported so far with this immunosuppression.1,3,5 Using myeloablative conditioning and high-dose busulfan especially, there may be an increased risk of hemorrhagic cystitis using Cy, as found in a prospective, randomized study.7 The low risk of chronic GVHD is certainly welcome because it reduces suffering and improves the patients’ quality of life. However, chronic GVHD has a strong graft-versus-leukemia effect and reduces the probability of leukemic relapse after HSCT.8 It is possible that the low risk of chronic GVHD using posttransplant Cy may be associated with an increased risk of leukemic relapse. The depletion of alloreactive T cells may also deplete leukemia-reactive T cells. Future randomized studies will determine whether this is the case.

Since the Seattle team introduced cyclosporine and a short arm of methotrexate as GVHD prophylaxis, this has been the gold standard for immunosuppressive therapy after HSCT worldwide.9 After several decades of immunosuppressive dominance using a calcineurin inhibitor and a short arm of methotrexate post-HSCT, this prophylaxis was challenged by Cutler and coworkers, who introduced a combination of tacrolimus and sirolimus as an alternative.10 In a randomized study comparing tacrolimus/sirolimus with tacrolimus/methotrexate as GVHD prophylaxis, it was found that the probability of acute and chronic GVHD, relapse, relapse-free survival, and overall survival was similar between the 2 groups. However, there was more rapid engraftment and less oropharyngeal mucositis in the patients treated with sirolimus

3672 BLOOD, 11 DECEMBER 2014 • VOLUME 124, NUMBER 25
Platelets: balancing the septic triad

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