

appropriate next step is the development of a multivariate prognostic model that combines relevant prognostic indicators, including primary cause of postpartum hemorrhage and other clinical characteristics. After validation, this model could serve to categorize women according to a low or a high risk for severe postpartum hemorrhage in order to be able to adjust treatment strategies accordingly.

A second potential role for Fibtém in clinical practice would be to improve the timely diagnosis of coagulopathy. However, the clinical actions that follow the diagnosis of coagulopathy as assessed with Fibtém (possibly combined with other tests) have not been established. The results of the ongoing randomized trial studying whether fibrinogen concentrate can improve the outcome of women with moderate postpartum hemorrhage and low Fibtém values (ISRCTN46295339) will need to be awaited. Yet, even if fibrinogen concentrate is proven to be efficacious in women with low Fibtém values, we still don't know whether the Fibtém with-or-without-fibrinogen-concentrate strategy is better (optimal cost-effectiveness) than other strategies.

Besides the strategy of Fibtém measurement possibly followed by fibrinogen concentrate infusion, there is a myriad of other strategies to treat persistent postpartum hemorrhage. The strategies can be roughly categorized as laboratory driven and formula driven. In the laboratory-driven strategies, treatment decisions depend on laboratory parameters and treatment triggers to guide treatment decisions during persistent postpartum hemorrhage. The formula-driven strategies use a prearranged delivery system of (blood) products in various mixtures of products, such as tranexamic acid, red cells, plasma, platelets, and coagulation factors, to stabilize a hemorrhagic patient. These strategies have not been defined for postpartum hemorrhage, but it would be worthwhile to consider development of such a strategy. Coagulopathy and reduction in clotting factors can develop rapidly and soon after having a reassuring test result. With the formula-driven strategy, all women presenting with persistent postpartum hemorrhage can be treated without delay in a standardized way. The disadvantage of such a formula-driven strategy is that

this strategy is not individualized and may therefore lead to both over- and undertreatment. The next sensible step would be to compare a well-designed, evidence-based, formula-driven strategy with a well-designed, evidence-based, point-of-care-test-driven strategy. The findings of Collins et al provide evidence in favor of Fibtém as a test in the laboratory-driven strategy.

Particularly noteworthy in the report by Collins et al is the observation that in the multivariate model, fibrinogen is no longer associated with progression to severe postpartum hemorrhage, whereas Fibtém remains appreciably associated. This suggests that in these early phases of postpartum hemorrhage, other coagulation factors involved in clot formation, not fibrinogen concentration, might be more important for progression to severe postpartum hemorrhage. So an important question is, is fibrinogen at low normal concentrations causally related to progression to severe hemorrhage, or is it but an indicator of the severity and stage of the postpartum hemorrhage?

An additional point that needs to be addressed is laboratory quality control. The TEG-Rotem working group reported large differences in test results among laboratories.⁴

Fibtém is a relatively costly commercial test, widely implemented in the thoracic surgery setting, and commercial forces are increasingly pushing its application in labor wards. Despite the promising results of Collins et al presented in this issue of *Blood*,

we should bear in mind that new tests first need to undergo scrutinized testing for their clinical usefulness. Otherwise, implementation of this test might lead to needlessly high increases in health-care costs with insufficient gain or even loss in patient outcomes.

Postpartum hemorrhage is common and potentially lethal. The study described by Collins et al¹ provides promising findings with regards to the prediction of severe postpartum hemorrhage and early diagnosis of coagulopathy. A number of issues have to be addressed before implementation of Fibtém in the treatment strategy for women with ongoing postpartum hemorrhage. Thus, clinicians interested in Fibtém are encouraged to use the test in the framework of a well-designed scientific study.

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

REFERENCES

- Collins PW, Lilley G, Bruynseels D, et al. Fibrin-based clot formation an early and rapid biomarker for progression of postpartum hemorrhage: a prospective study. *Blood*. 2014;124(11):1727-1736.
- Abdul-Kadir R, McLintock C, Ducloy A-S, et al. Evaluation and management of postpartum hemorrhage: consensus from an international expert panel. *Transfusion*. 2014;54(7):1756-1768.
- Steyerberg EW, Moons KGM, van der Windt DA, et al; PROGRESS Group. Prognosis Research Strategy (PROGRESS) 3: prognostic model research. *PLoS Med*. 2013;10(2):e1001381.
- Chitlur M, Sorensen B, Rivard GE, et al. Standardization of thromboelastography: a report from the TEG-ROTEM working group. *Haemophilia*. 2011; 17(3):532-537.

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● ● ● IMMUNOBIOLOGY

Comment on Newson et al, page 1748

Cellular dynamics of resolving inflammation

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In this issue of *Blood*, Newson et al determine the dynamics of inflammatory cell recruitment and their diversification into functionally distinct, cellular subsets in a previously uncharacterized “postresolution” phase of inflammation.¹ The postresolution tissue retains some of the recruited populations of monocytic and lymphoid cells and remains in a state of “adaptive homeostasis” long after inflammation has resolved.

The host inflammatory response is a complex, well-orchestrated process temporally regulated by the release of various cytokines, chemokines, lipid mediators, and immune cellular subsets. In the acute phase of inflammatory response, following tissue injury, resident macrophages and mast cells release chemical mediators (eicosanoids, chemokines, and proinflammatory cytokines) that initiate rapid influx of neutrophils and monocytes into the tissue. After neutralization of the inciting stimuli, neutrophils undergo apoptosis and are cleared by efferocytosing macrophages, subsequently resulting in resolution and restoration of normal tissue architecture.² Several studies have elegantly demonstrated that resolving inflammation is a complex process comprising multiple regulatory events such as phenotypic transformation of macrophages from inflammatory to antiinflammatory type, catabolism of proinflammatory cytokines, and release of proresolving lipid mediators that synergistically promote wound healing.³⁻⁵ Persistent, unresolving inflammation is often correlated with severity and unfavorable outcomes in various inflammatory diseases such as arthritis, lupus, and periodontitis.

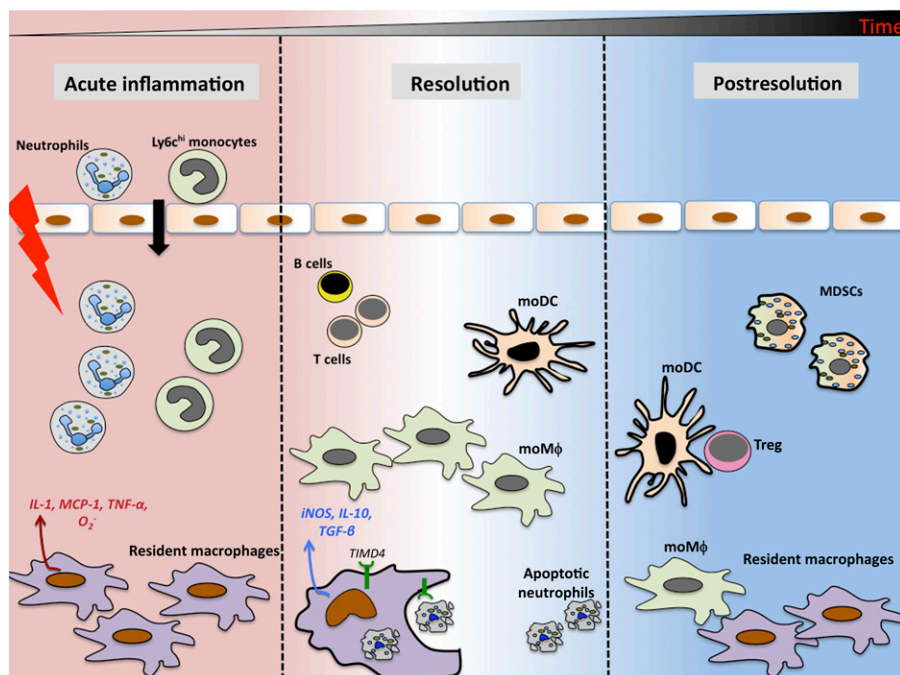
Insight into the cellular players and molecular signals that drive resolution is critical in devising therapeutic interventions for the treatment of chronic inflammatory diseases.

Several functionally specialized immune cell types shut down inflammation. Macrophages are a highly plastic, functionally diverse, myeloid cell population that produces a battery of immune-modulating mediators to regulate both pro- and antiinflammatory arms of immune response. They can undergo phenotypic alterations and reprogram their cell intrinsic effector functions to alter the tissue microenvironment.⁶ Myeloid-derived suppressor cells (MDSCs) are another subset of highly heterogeneous immune regulatory cells that limit T-cell proliferation, produce immunosuppressive inducible nitric oxide synthase (iNOS), and participate in a range of pro- and antiinflammatory pathways.⁷ However, little is known about the dynamics of how these cellular subsets are recruited to the inflammatory site, roles they play in altered tissue microenvironment, and whether their dysregulation perpetuates inflammation.

Newson et al¹ demonstrate a previously uncharacterized “postresolution” phase of inflammation that persists weeks after classical

resolution and is dominated by the presence of resident macrophages, MDSCs, monocyte-derived macrophages, and monocyte-derived dendritic cells (moDCs). They present evidence that each of these monocytic subsets (identified based on lineage and activation makers) play distinct yet overlapping roles in creating a microenvironment conducive for the development of adaptive immune responses. In a model of low-dose zymosan-induced peritoneal injury, there is a rapid influx of neutrophils during the acute phase of inflammatory response (see figure). The neutrophils subsequently become apoptotic. Resident macrophages preferentially efferocytose apoptotic neutrophils and acquire an immunosuppressive phenotype by producing antiinflammatory cytokines TGF- β and interleukin-10. Resident macrophages from the peritoneal cavities of mice challenged with bacteria also exhibit similar properties and behavior. Furthermore, iNOS expression in resident macrophages dampened T-cell proliferation in the peritoneal cavity and promoted lymph node contraction. Although postresolution macrophages induced FoxP3 expression in CD4-positive T cells, moDCs enabled their proliferation, thus enriching the peritoneal cavity with CD4⁺, CD25⁺, or FoxP3 regulatory T cells (Tregs). Tregs are immunosuppressive populations of T cells that modulate the intensity of other immune cells and regulate immunity by maintaining tolerance to self-antigens.⁸ Treg development was hampered in mice injected with higher doses of zymosan, resulting in hyperinflammation and significantly higher inflammatory cell infiltrate. The authors speculate that the blunted development of Tregs might contribute to maladaptive immune responses and may predispose to autoimmune/chronic inflammatory disorders.

To summarize, Newson et al elegantly demonstrate that inflammatory resolution is not just a mere contraction of inflammatory cells, but a complex, well-regulated process comprising multiple proresolution cellular and molecular pathways. The recruited cells persist in the tissue for months after resolution and dictate the magnitude of subsequent inflammatory challenge. Resolving inflammation thus generates a microenvironment conducive for “bridging” innate and adaptive arms of immunity. Whether the phenomenon of “adaptive homeostasis” is restricted to the peritoneal



The acute phase of inflammatory response is accompanied by rapid (within minutes) influx of neutrophils and monocytes in response to soluble mediators released by tissue resident cells. During resolution, resident macrophages clear apoptotic neutrophils and make iNOS and proresolving cytokines such as interleukin-10 (IL-10) and transforming growth factor- β (TGF- β). Monocyte-derived macrophages (moM ϕ) and MoDCs persist in the tissue along with MDSCs and lymphocytes long after classical resolution. MoDCs preferentially drive T-cell proliferation and generation of adaptive immunity in the postresolution phase.

cavity or is a common mechanism of resolution remains to be tested in other tissues and animal models.

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

REFERENCES

1. Newson J, Stables M, Karra E, et al. Resolution of acute inflammation bridges the gap between innate and adaptive immunity. *Blood*. 2014;124(11):1748-1764.
2. Fullerton JN, O'Brien AJ, Gilroy DW. Pathways mediating resolution of inflammation: when enough is too much. *J Pathol*. 2013;231(1):8-20.
3. Novak ML, Koh TJ. Phenotypic transitions of macrophages orchestrate tissue repair. *Am J Pathol*. 2013; 183(5):1352-1363.

4. Buckley CD, Gilroy DW, Serhan CN. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity*. 2014;40(3):315-327.

5. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol*. 2005;6(12): 1191-1197.

6. Locati M, Mantovani A, Sica A. Macrophage activation and polarization as an adaptive component of innate immunity. *Adv Immunol*. 2013;120:163-184.

7. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol*. 2009;9(3):162-174.

8. Corthay A. How do regulatory T cells work? *Scand J Immunol*. 2009;70(4):326-336.

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the responses of donor T cells that drive GVHD. The authors of the current study reasoned that extracorporeal photopheresis (ECP), a modality that exposes cells to methoxypsoralen (8MOP) and UV radiation to induce apoptosis, might be used to prevent GVHD. Indeed, they show that this strategy reduced mortality from GVHD (see figure) and increased the number of regulatory T cells (Tregs), but importantly did not reduce beneficial graft-versus-leukemia effects. Injections of lipopolysaccharide abrogated the protective effect, confirming that modulation of the inflammatory milieu surrounding APCs is key to the success of the strategy.

Others have demonstrated that several infusions of donor ECP-treated cells following BMT can reverse ongoing acute GVHD in mouse models through the induction of donor Tregs.² Clinically, successful treatment of acute GVHD by ECP has also been associated with an increase in Tregs.^{3,4} The principal innovation of this report is the use of host apoptotic cells to modulate the function of host APCs to prevent GVHD. This advance is important because no single APC subset appears to initiate GVHD. Dendritic cells (DCs), the APC subset par excellence, may even regulate GVHD rather than amplify it.⁵ Current data indicate that donor CD8⁺ T cells are activated by hematopoietic APCs^{6,7} but that donor CD4⁺ T cells can be activated by nonhematopoietic APCs in the gastrointestinal tract.⁵ Once activated, donor T cells may be further stimulated by donor APCs and contribute to GVHD.⁸ Given this complex variety of APCs that may activate donor T cells, an approach that modulates APC function is more attractive than a strategy that targets a specific cell type, potentially leaving other APC populations unaffected. It should also be noted that the use of donor apoptotic cells from HLA-identical siblings may effectively prevent GVHD. A small dose-escalation trial tested a single infusion on day -1 of BMT of apoptotic mononuclear cells from HLA-identical sibling donors when added to cyclosporine/methotrexate prophylaxis. The resulting incidence of grade II to IV GVHD was 23% (with 0% for the 2 highest cell doses) and nonrelapse mortality was 8% at day 100.⁹ Incubation of host DCs with these apoptotic cells also significantly decreased the cell surface expression of the activation markers HLA-DR and CD86. Future

● ● ● TRANSPLANTATION

Comment on Florek et al, page 1832

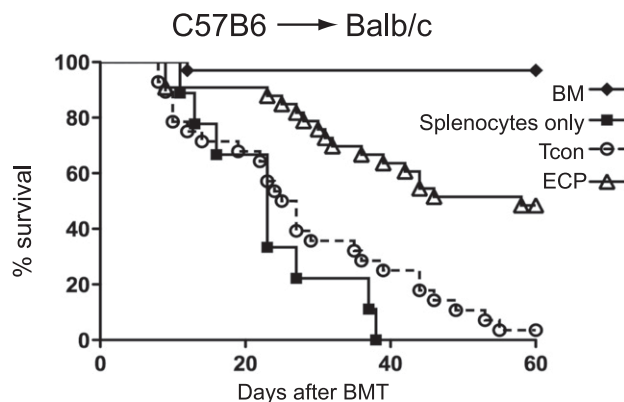
Cellular therapy of the host to prevent GVHD

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In this issue of *Blood*, Florek et al from Stanford describe an interesting new approach to the problem of graft-versus-host disease (GVHD).¹

GVHD remains the scourge of allogeneic bone marrow transplantation (BMT) and limits the use of this important, curative therapy to patients with very-high-risk diseases. The mainstay of GVHD prevention remains broad-spectrum immunosuppression that succeeds in only half of patients, and innovative strategies are desperately needed. The central event of the graft-versus-host reaction is the activation of donor T cells

by host antigen-presenting cells (APCs). High-dose chemoradiotherapy prior to BMT eliminates most, but not all, hematopoietic APCs; the APCs that remain, however, are activated and elicit inflammatory responses from the T cells in the donor graft, eventually resulting in GVHD. Phagocytosis of apoptotic cells by APCs can change their function and make them more regulatory or tolerogenic, thereby limiting



Exposure to apoptotic cells prior to BMT improves survival. BALB/c mice were injected with C57BL/6 BM plus conventional T cells (Tcon) only (circles) or with prior injection of ECP-treated BALB/c-treated cells (triangles) or with prior injection of BALB/c cells treated with 8MOP but no UV light (squares). See Figure 1A in the article by Florek et al that begins on page 1832.