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Microbial messaging to the marrow

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In this issue of Blood, Boettcher et al demonstrate that endothelial cell–intrinsic MyD88 signaling is critical for synthesis of the cytokine granulocyte colony-stimulating factor (G-CSF) and induction of emergency granulopoiesis during gram-negative bacterial infection. This work answers long-standing questions centered on which cell types transform systemic pathogen signals into the molecular cues needed to elicit neutrophil generation in demand situations.

Neutrophils are vital for host defense. A severe reduction in numbers predisposes to life-threatening infection and, consistently, numerous mechanisms regulate neutrophil homeostasis. Emergency granulopoiesis describes a rapid physiological response in which circulating neutrophil amounts become significantly elevated. This occurs during bacterial or fungal infection and is important for effective microbial clearance. Steps include a swift elevation in serum G-CSF amounts, release of mature neutrophils from the bone marrow reserve to the circulation, and enhanced synthesis of new neutrophils from bone marrow–localized hematopoietic progenitors. Emergency granulopoiesis can be mimicked in animals by recombinant G-CSF administration and may be activated when this agent is used to treat human congenital or therapy–induced neutropenia. Although G-CSF has indisputable roles in regulating neutrophils in demand and homeostatic conditions, how invading pathogen signals are transmitted to the bone marrow to elicit emergency granulopoiesis has been far less clear.

Using a variety of genetically engineered mouse models and bone marrow chimeras, Boettcher et al elegantly reveal the key role for endothelial cells in emergency granulopoiesis. This builds on earlier work by the same group, which ruled out cells of hematopoietic origin as principal mediators of emergency granulopoiesis after stimulation via Toll-like receptor 4 (TLR4), a signaling pathway activated upon infection with gram-negative bacteria, as well as studies that implicated nonhematopoietic sources of G-CSF. Here, the authors demonstrate that endothelial cells from diverse tissues express significant amounts of TLR4 and its crucial signaling adaptor MyD88. Surprisingly, this expression surpassed, at the messenger RNA level, TLR4 and MyD88 expression in splenic dendritic cells, suggesting endothelial cells might act as sensors of circulating bacterial pathogens. Evidence from mice with restricted MyD88 expression indicates that TLR4 signaling is required for efficient induction of emergency granulopoiesis upon LPS challenge, it remains possible that systemic LPS delivery and bacterial infection induce production of additional granulopoietic cytokines (eg, interleukin–6, granulocyte–macrophage colony–stimulating factor), which contribute to the emergency response. In fact, emergency granulopoiesis was not affected as severely in mice lacking endothelial MyD88 after Escherichia coli infection compared with systemic LPS treatment, suggesting E. coli stimulates multiple danger recognition pathways and additional downstream mediators that ensure induction of a robust hematopoietic response.

Granulopoiesis appears to be controlled by distinct mechanisms in homeostatic and demand conditions, including reliance on G-CSF and transcriptional regulators in the CCAAT enhancer–binding family. Steady-state neutrophil production is largely but not entirely dependent on G-CSF, whereas demand-driven granulopoiesis can proceed in at least some conditions without this cytokine. Endothelial MyD88 deficiency does not suppress basal neutrophil amounts, indicating that other cellular sources and mechanisms control steady-state G-CSF amounts and neutrophil development. Although G-CSF administration can compensate for endothelial MyD88 deficiency by eliciting emergency granulopoiesis upon LPS challenge, it remains possible that systemic LPS delivery and bacterial infection induce production of additional granulopoietic cytokines (eg, interleukin–6, granulocyte–macrophage colony–stimulating factor), which contribute to the emergency response. In fact, emergency granulopoiesis was not affected as severely in mice lacking endothelial MyD88 after E. coli infection compared with systemic LPS treatment, suggesting E. coli stimulates multiple danger recognition pathways and additional downstream mediators that ensure induction of a robust hematopoietic response.

The discovery that endothelial cells are fundamental intermediates in emergency granulopoiesis might seem logical in hindsight because cells lining the vasculature would be a first point of encounter between a host and systemically introduced bacterial pathogens. Indeed, mice with TLR4 expression restricted to endothelial cells recruit neutrophils and clear features of granulopoiesis under demand conditions. The importance of this pathway was further highlighted by the observation that endothelial cell–intrinsic MyD88 signaling is required for efficient induction of emergency granulopoiesis during systemic Escherichia coli infection. A fundamental intermediate in emergency granulopoiesis might seem logical in hindsight because cells lining the vasculature would be a first point of encounter between a host and systemically introduced bacterial pathogens. Indeed, mice with TLR4 expression restricted to endothelial cells recruit neutrophils and clear features of granulopoiesis under demand conditions. The importance of this pathway was further highlighted by the observation that endothelial cell–intrinsic MyD88 signaling is required for efficient induction of emergency granulopoiesis during systemic Escherichia coli infection.

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systemic gram-negative bacterial infection. These observations, however, were far from predictable because cells of hematopoietic origin (eg, macrophages, dendritic cells) are typically considered front-line sensors of extracellular pathogens and are potent mediators of TLR signals, with numerous critical roles in regulating the interplay between innate and adaptive immune responses. Moreover, hematopoietic progenitors express TLRs, which suggested a potential direct mechanism of pathogen sensing to activate emergency hematopoiesis. Evidence herein argues strongly in favor of the importance of indirect sensing via the endothelium for response to systemic bacterial challenge.

By contrast with systemic presentation, a common route of bacterial infection is via localized responses such as skin exposure or inhalation. Because white blood cell counts can increase in these conditions, localized infection may trigger G-CSF and/or other granulopoietic cytokine production by endothelial and/or hematopoietic populations to induce emergency granulopoiesis. One hopes the results here will prompt further investigation into cell populations and mechanisms that regulate physiologically relevant production of hematopoietic growth factors in response to local and systemic stressors (eg, infection, chemotherapy) as well as the primary sources of these molecules in steady state. Regardless, the fundamental idea that the vasculature can sense and respond to systemically delivered TLR agonists will no doubt shape our view of how other potential danger signals, such as extracellular nucleic acids or cell damage molecules, might affect hematopoiesis. Because these mediators are released in numerous disease states, including inflammation and cancer, and emergency hematopoietic responses might in turn influence disease outcomes (eg, production of inflammatory or immunosuppressive populations), understanding roles for endothelial cell–derived hematopoietic factors in different disease or pathogen-elicited scenarios is of critical importance. Both the vasculature and the marrow are dispersed throughout the body, potentially receiving not only systemically delivered messages, but also local tweets that could reveal important changes in environmental conditions warranting emergency hematopoietic responses. Our ability to recognize and detect such signals is clearly evident in healthy individuals in vivo. This capacity needs to be correspondingly enhanced in the laboratory to understand the full spectrum of microbial messaging to the marrow.

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

**REFERENCES**


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**Comment on Moorman et al, page 1434**

**The clinical path to integrated genomics in ALL**

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In this issue of *Blood*, Moorman et al show that most good-risk patients can now be classified robustly by integrating the information from prevalent copy-number alterations (CNAs) in relevant combinations and classical cytogenetic risk factors in acute lymphoblastic leukemia (ALL).1

ALL is a complex genetic disease that results from the combination of lesions in genes involved in the regulation of hematopoiesis, lymphoid differentiation, cell cycle, and proliferation. High-resolution genetic profiling of ALL reveals an increasing number of underlying mutations that modify the function of transcription factors, epigenetic regulators, or components of signaling pathways, among others. For decades, cytogeneticists have been aware of specific chromosomal translocations, some of which have been established as reliable risk factors (see figure). However, these genetic risk factors identify only some of the patients at risk. Most study groups therefore also include the assessment of minimal residual disease (MRD) during induction chemotherapy for risk stratification, a powerful approach that was pioneered by European study groups. The challenge is now to devise new approaches to translate the rapidly expanding knowledge of ALL genomics to the clinic in order to define better prognostic markers and specific druggable targets already at diagnosis. The most common submicroscopic genetic lesions include CNAs or sequence mutations of hematopoietic transcription factors, including *PAX5, IKZF1*, and *EBF1*; gene rearrangements leading to overexpression of the cytokine receptor component *CRLF2*, mutations in *JAK1* and *JAK2* kinases; and deletions of the *CDKN2A/B* loci encoding the *1NK4/ARF* tumor suppressor genes, to name representative examples. Several of these lesions have been associated with less favorable outcome (such as *CDKN2A/CDKN2B* and *IKFZ1* deletions and...
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