to platelets, erythrocytes, inflammation, or genes without any so far known biological link with thrombosis (see figure). The results will be important not only for future genetic and clinical studies but also for directing future function-structure studies of the molecular cause of VTE.

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TRANSPLANTATION

Comment on Koehn et al, page 1670

We didn't start the fire, MDSC inflammasome signaling in GVHD

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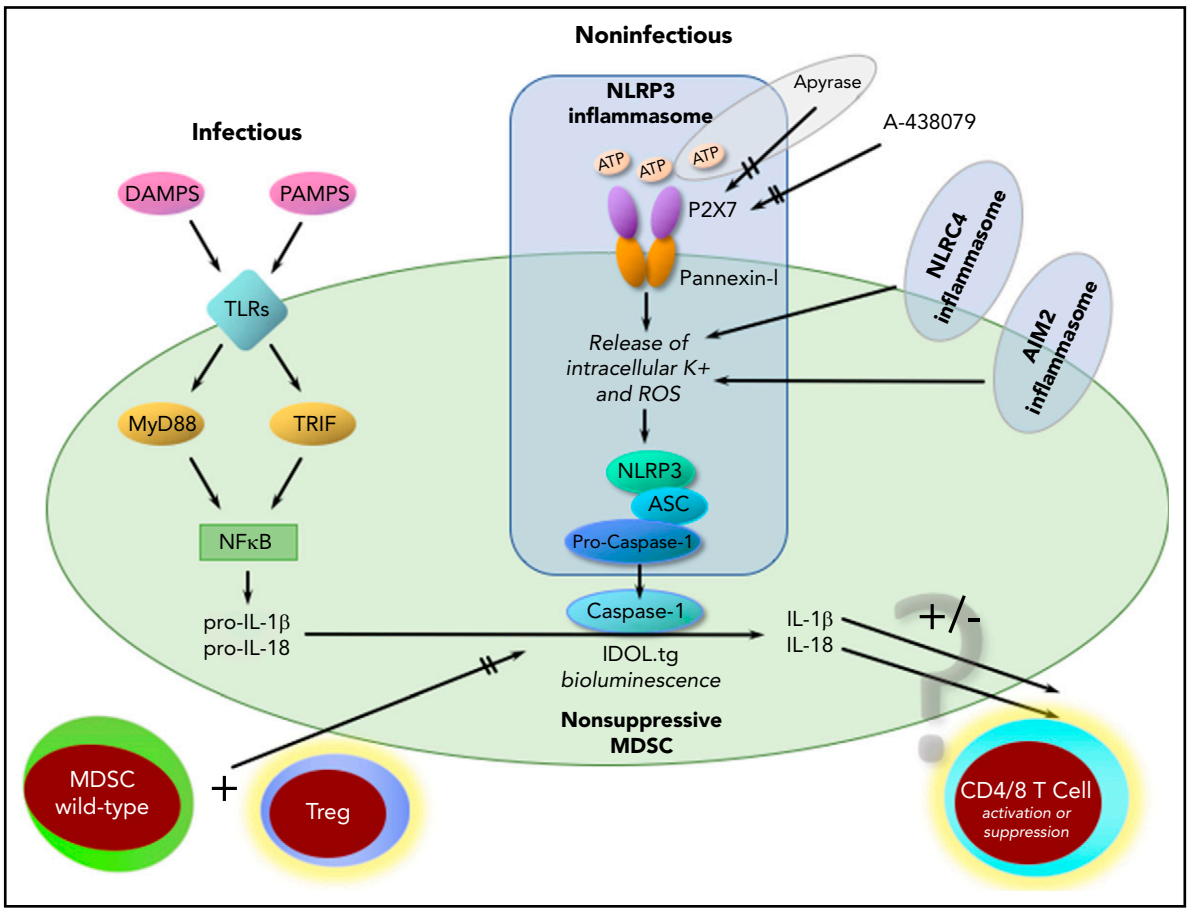
In this issue of *Blood*, Koehn et al investigate how activation of the nucleotide-binding domain and leucine-rich repeat (NLR) pyrin family domain 3 (NLRP3) inflammasome pathway influences the function of myeloid-derived suppressor cells (MDSCs) in the setting of acute graft-versus-host disease (aGVHD).[1]

The authors use a wide range of genetic and pharmacologic tools to dissect the contributions of the inflammasome signaling pathway components to loss of suppressive function in MDSCs. Although T cells are the main drivers of GVHD, MDSC suppression of alloreactivity is a promising strategy for inhibiting GVHD because MDSCs have the potential to suppress $CD4^+$ and $CD8^+$ T cells, as well as natural killer and natural killer T cells.[2] Efficacy of MDSCs generated ex vivo in aGVHD was previously explored, but efficacy was limited because MDSCs lost their suppressive ability when they were placed into a highly inflammatory milieu within the hematopoietic stem cell transplant (HSCT) recipient. MDSC function or its loss is highly dependent on environmental cues. To delineate the environmental and intrinsic mechanisms of MDSC activation, Koehn et al explored the role of the NLRP3 inflammasome pathway in an aGVHD major histocompatibility mismatch mouse model.

MDSCs are a diverse population of immature myeloid cells produced during chronic inflammatory states that have the ability to suppress anti-tumor immunity as well as inflammation in a variety of clinical settings.[2,3] Dampening MDSC suppressor function is an attractive strategy for optimizing cancer immunotherapy because MDSCs are implicated in tumor progression and metastasis. Conversely, enhancing or sustaining MDSC suppressive function would be beneficial in inhibiting inflammation associated with GVHD. MDSCs can be targeted through a variety of pharmacologic approaches. However, to successfully maintain MDSCs in the desired suppressive state in vivo, it is essential to understand the pathways that direct MDSC behavior in the context of the specific biological setting.

Previous investigations in aGVHD identified the NLRP3 inflammasome as a key pathway for MDSC alloimmune activation (see figure).[4] MDSCs can be generated from bone marrow stem cells in an interleukin-13 (IL-13) culture system, and they are able to ameliorate aGVHD after being infused into the host.[5,6] The effect on aGVHD is modest, however, and repeated infusions can further improve outcomes but do not fully abrogate aGVHD. MDSC loss of suppressive function after HSCT occurs when the conditioning regimen induces the release of adenosine triphosphate (ATP) into the extracellular compartment (see figure). The release of ATP results from cell damage induced by pretransplant conditioning, particularly irradiation. ATP binding to the P2x7 receptor (P2x7R) on MDSCs results in downstream NLPR3 inflammasome activation. The canonical inflammasome activation then initiates a cascade of proinflammatory effects that fuel aGVHD. Koehn et al show that P2x7 knockout (KO) or inhibition of ATP binding to the receptor reduced inflammasome activation. This was demonstrated with extracellular ATP depletion via apyrase and pharmacologically via administration of A-438079, a highly selective P2x7R inhibitor. Both approaches resulted in improved post-HSCT survival in the aGVHD model, with important clinical implications.

The downstream result of NLRP3 inflammasome activation is caspase-1–mediated



NLPR3 inflammasome activation results in loss of suppressive function in the MDSCs. Blockade of ATP signaling via intraperitoneal injection of apyrase or administration of P2x7 inhibitor A-438079 can abrogate NLRP3 inflammasome activation and restore MDSC suppressive function. IL-1β release can be visualized in vivo through the use of an IDOL transgenic mouse strain (IDOL.tg), in which IL-1β activation results in luciferase production that can be detected with bioluminescent imaging. When MDSCs were co-infused with Tregs, overall survival was improved and inflammasome activation was abrogated as assessed by bioluminescent imaging. The effects of IL-1β and IL-18 released from MDSCs have been associated with T-cell suppression and T-cell activation: blockade of IL-1β resulted in amelioration of GVHD,[8] IL-1β KO donor MDSCs were inferior to wild-type MDSCs in suppressing GVHD (study by Koehn et al), addition of IL-1β enhanced MDSC suppressive function (study by Koehn et al), and administration of IL-18 can improve survival and decrease GVHD severity.[7] Transfer of myeloid differentiation primary response 88 (MyD88) KO or transfer of double-KO MyD88/TIFF MDSCs did not result in improved survival over infusion of wild-type MDSCs. NLR family CARD domain-containing 4 (NLRC4) KO and administration of absent in melanoma 2 (AIM2) KO MDSCs resulted in improved aGVHD but offered no additional protection compared with wild-type MDSCs, whereas infusion of NRLP3 KO MDSCs resulted in a significant survival advantage. ASC, adaptor protein apoptosis-associated speck-like protein–containing caspase activation and recruitment domain (CARD); DAMPS, damage-associated molecular patterns; NFκB, nuclear factor κ light-chain enhancer of activated B cells; P2x7R, B6.129P2-P2rx7tm1Gab/J [mouse cell line]; PAMPS, pathogen-associated molecular patterns; ROS, reactive oxygen species; TLRs, Toll-like receptors; TRIF, TIR-domain-containing adapter-inducing interferon-β.

cleavage of pro-IL-1β and pro-IL-18 to the active forms, IL-1β and IL-18, respectively. Interestingly, these cytokines can either suppress or stimulate $CD4^+$ and $CD8^+$ T cells. Although both cytokines have been dubbed proinflammatory, their role in aGVHD is not yet fully elucidated. Reddy et al[7] previously demonstrated that IL-18 can ameliorate aGVHD and prolong survival after allo-HSCT by suppressing effector T cells in the presence of interferon-γ. Although IL-1 inhibition can ameliorate GVHD,[8] Koehn et al show that IL-1β KO did not improve aGVHD, but adding exogenous IL-1β enhanced the suppressive function of MDSCs. Thus, the downstream effects of IL-1β and IL-18 on alloreactive T cells have yet to be deciphered.

A novel tool was used to noninvasively detect inflammasome activation in vivo through the use of MDSCs from an IDOL.tg (IL-1β–based dual-operating luciferase transgenic) bioluminescent mouse strain. In this system, inflammasome activation results in quenching of the bioluminescence (see figure). This model can be used to noninvasively screen drugs for inflammasome modulation. The authors used this transgenic mouse model to show that regulatory T-cell (Treg) co-infusion with MDSCs results in amelioration of aGVHD (ie, Tregs cooperate with MDSCs to prevent inflammasome activation). Both Tregs and MDSCs have been previously shown to suppress GVHD without detriment to graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effects, and understanding the mechanisms of this cooperation may illuminate future therapeutic approaches for GVHD.

In summary, in the article by Koehn et al, the required components for inflammasome activation and the resultant subversion of MDSC suppressive function indicate that triggers upstream of the NLPR3 inflammasome can be successfully targeted to maintain MDSC immunosuppressive phenotype in GVHD. Future work will need to delineate downstream effects of inflammasome signaling (ie, the effects of IL-1β and IL-18 on $CD4^+$ and $CD8^+$ T-cell function in GVHD). The synergy between Tregs and MDSCs is another

promising direction that warrants further study to ameliorate GVHD without negative impact on GVL or GVT. Finally, improved understanding of the role of microRNAs (miRs) in MDSC and T-cell function can be explored for enhancing T-cell activity and reduced MDSC suppression in the setting of cancer and enhanced MDSC suppression in the setting of auto- and alloimmunity, respectively. In particular, miR-155 has been implicated in NLRP3 inflammasome activation and $CD8^+$ T-cell fitness.[9,10] With their elegant series of experiments, Koehn et al have made an important contribution to the field of GVHD and the expanding field of MDSCs.

Conflict-of-interest disclosure: N.P.B. is an inventor for "Stable water isotope labeling and magnetic resonance imaging for visualization of rapidly dividing cells," patent pending (PCT/US2017/058886). ■

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