Comment on Gibbings et al, page 1707

Environment tames CGD macrophages

Juhi Bagaitkar | University of Louisville

In this issue of *Blood*, Gibbings et al[1] show that the inflammatory milieu rather than the cell-intrinsic Nox2 deficiency skews monocyte-derived macrophages (MoMacs) into a state of inflammatory hyperresponsiveness and prevents their immunophenotypic maturation in chronic granulomatous disease (CGD).

CGD is a rare primary immunodeficiency of phagocyte function characterized by life-threatening infections, granulomas, and dysregulated inflammatory responses toward a wide array of sterile and nonsterile stimuli/agonists. CGD is genetically heterogenous and caused by null mutations in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (*phox*) subunit genes. These null mutations cause the failure of neutrophils and monocytic cells to undergo respiratory burst and generate superoxide (O_2^-). The membrane-restricted flavocytochrome b_{558}, a heterodimer of gp91^{phox} (Nox2) and p22^{phox} subunits, is the main catalytic subunit of the NADPH oxidase complex. Approximately two-thirds of CGD cases are X-linked and are due to null mutations in the gene encoding gp91^{phox} (Nox2). The remaining cases are caused by autosomal recessive defects in the cytosolic p47^{phox}, p67^{phox}, and p40^{phox} subunits.[2] NADPH oxidase-derived O_2^- and downstream reactive oxygen species (ROS) are essential antimicrobial agents. However, they are increasingly recognized to play important modulatory roles in host immune responses, in part by regulating immune effector functions of phagocytes and inflammatory signaling downstream of pattern recognition receptors.[2,3]

CGD patients often present with inflammatory complications that are unrelated to infections such as sterile granulomas in hollow organs, colitis, discoid lupus-like lesions, and other autoimmune complications.[2,4] Thus far, studies to understand the molecular and cellular basis of inflammatory complications and in CGD have focused on elucidating specific Nox2/ROS regulated pathways that alter neutrophil or macrophage effector functions in a cell-intrinsic manner. For instance, failure to optimally activate NADPH oxidase delays apoptosis, compromises NETosis, dysregulates ionic fluxes, and leads to excessive degranulation of Nox2 null of CGD neutrophils.[3] CGD macrophages also have significant defects in autophagy pathways, have reduced efferocytic capacity, and exhibit delays in phagosomal maturation, consistent with delayed proteolysis of apoptotic cell-derived antigens.[5-8] Besides dysregulation of multiple phagocyte-associated effector responses, CGD neutrophils and macrophages are also hyperactive or hyperresponsive to inflammatory stimuli, often producing more cytokines and chemokines on a per-cell basis compared with cells derived from healthy individuals or wild-type mice. Mechanistically exaggerated responses to pathogen-associated ligands of endogenous ligands released during sterile injury were linked to dysregulated or prolonged activation of redox-sensitive transcription factors (NF-kB, Nrf2) as well as MAP kinases.[9] Based on these data, it was largely assumed that dysregulated cell-intrinsic inflammatory pathways and immune processes continue to sustain CGD inflammation in vivo, possibly by amplification of inflammatory feedforward loops. However, new findings by Gibbings et al adds another layer of complexity to CGD inflammation by showing that the hyperinflammatory nature of CGD MoMacs can be reversed by the manipulation of their microenvironment, thus showing that extrinsic factors such as the inflammatory milieu foster the dysregulated behavior of CGD macrophages.[1]

Gibbings et al investigated the maturation programs of newly recruited inflammatory monocytes into mature macrophages using a murine model of zymosan-induced peritonitis. Macrophages are highly plastic cells that dynamically alter their transcriptomes, epigenetic landscapes, and phenotypic profiles in response to environmental signals and local tissue milieu. Their studies show that wild-type MoMacs rapidly transition from an inflammatory state into mature macrophages that exhibit expansion in cell size, downregulation of proinflammatory markers, and upregulation of transcripts involved with resolution of inflammation. In striking contrast, CGD MoMacs were continuously recruited to the inflamed peritoneal cavity and failed to undergo phenotypic maturation and transcriptional reprogramming consistent with the acquisition of resolution properties. They show that CGD MoMacs remained in a migratory state, migrating to the diaphragm where they were found in fibrinogen clots surrounding neutrophil clusters in nascent pyogranulomata. Using mixed chimeras and adoptive transfer experiments, Gibbings et al show that the inflammatory milieu primarily regulated this dysregulated behavior of CGD MoMacs in CGD rather than intrinsic loss of Nox2 activity within the MoMacs themselves. Another important finding of these studies is that even though CGD macrophages have been known to produce more inflammatory cytokines on a per-cell basis, they can be reprogrammed into less inflammatory states by modulating their microenvironment. Overall, these studies show a previously underappreciated role of the inflammatory milieu in sustaining CGD inflammation and hyperresponsiveness of CGD macrophages.

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

REFERENCES

1. Gibbings SL, Haist K, Nick HJ, et al. Heightened turnover and failed maturation of monocyte-derived macrophages in murine chronic granulomatous disease. *Blood*. 2022;139(11):1707-1721.
2. Dinauer MC. Inflammatory consequences of inherited disorders affecting neutrophil function. *Blood*. 2019;133(20):2130-2139.
3. Zeng MY, Miralda I, Armstrong CL, Uriarte SM, Bagaitkar J. The roles of NADPH oxidase in modulating neutrophil effector responses. *Mol Oral Microbiol*. 2019;34(2):27-38.

4. Schäppi MG, Jaquet V, Belli DC, Krause KH. Hyperinflammation in chronic granulomatous disease and anti-inflammatory role of the phagocyte NADPH oxidase. *Semin Immunopathol.* 2008;30(3):255-271.

5. Bagaitkar J, Huang J, Zeng MY, et al. NADPH oxidase activation regulates apoptotic neutrophil clearance by murine macrophages. *Blood.* 2018;131(21):2367-2378.

6. Fernandez-Boyanapalli RF, Frasch SC, McPhillips K, et al. Impaired apoptotic cell clearance in CGD due to altered macrophage programming is reversed by phosphatidylserine-dependent production of IL-4. *Blood.* 2009;113(9):2047-2055.

7. de Luca A, Smeekens SP, Casagrande A, et al. IL-1 receptor blockade restores autophagy and reduces inflammation in chronic granulomatous disease in mice and in humans. *Proc Natl Acad Sci USA.* 2014; 111(9):3526-3531.

8. Huang J, Canadien V, Lam GY, et al. Activation of antibacterial autophagy by NADPH oxidases. *Proc Natl Acad Sci USA.* 2009;106(15):6226-6231.

9. Segal BH, Grimm MJ, Khan AN, Han W, Blackwell TS. Regulation of innate immunity by NADPH oxidase. *Free Radic Biol Med.* 2012;53(1):72-80.

DOI 10.1182/blood.2021014417

THROMBOSIS AND HEMOSTASIS

Comment on Cebo et al, page 1722

Chewing the fat on platelet CXCR7

Hannah Stevens[1-3] and James D. McFadyen[1-4] | [1]Alfred Hospital; [2]Monash University; [3]Baker Heart and Diabetes Institute; and [4]The University of Melbourne

In this issue of *Blood*, Cebo et al[1] demonstrate that the platelet chemokine (C-X-C motif) receptor 7 (CXCR7) plays a novel role in regulating the platelet lipidome, and through the generation of "antithrombotic" lipid species, CXCR7 ligation can regulate thromboinflammatory platelet functional responses.

The quest for novel therapeutic strategies for the treatment of cardiovascular disease (CVD) represents an ongoing unmet clinical need because of the significant global burden of CVD. One of the fundamental mechanisms underlying the shortcomings of current therapies for CVD is the fact that thromboinflammation plays a key role in driving the thrombotic response; however, this is not abrogated by current antithrombotic therapies. Thromboinflammation reflects the bidirectional crosstalk between thrombosis and inflammation and is implicated in a broad range of clinical conditions, including CVD, sepsis, ischemia reperfusion injury, and coronavirus disease 2019 (COVID-19).[2,3] Platelets are well recognized as central players mediating thromboinflammation owing to their expression of a large repertoire of receptors essential for adhesion and immune function, in conjunction with their ability to produce and release a range of cytokines and chemokines resulting in leukocyte recruitment and activation.[2]

In addition to releasing a number of important inflammatory mediators, such as chemokine (C-X-C motif) ligand 4 (CXCL4)/platelet factor 4, chemokine (C-C motif) receptor 5, CXCL12, migration inhibitory factor (MIF), interleukin-1β (IL-1β), and transforming growth factor-β, platelets also express the chemokine receptors, CXCR7 and CXCR4.[4,5] As such, significant attention has recently been focused on how these chemokine receptors modulate the thrombotic and inflammatory function of platelets. Previous work has demonstrated that the interaction between CXCR7 with its 2 ligands, CXCL12 and MIF, promotes platelet lifespan and mediates an antithrombotic effect by inhibiting phosphatidylserine (PS) exposure.[4] Moreover, platelet CXCR7 expression levels increase after myocardial infarction and correlate with an improved prognosis.[6] However, the precise mechanisms governing how increased platelet CXCR7 expression may confer benefit in the setting of myocardial infarction and how this may be exploited therapeutically have remained elusive.

In this context, the study by Cebo and colleagues now links these critical observations and defines how platelet CXCR7 can regulate the thromboinflammatory function of platelets (see figure). Utilizing platelets from a large cohort of patients with CVD, the authors highlight the protective effect of increased platelet CXCR7 expression by demonstrating that increased platelet CXCR7 expression correlates with a reduction in platelet aggregation in response to a range of platelet agonists. In order to demonstrate that platelet CXCR7 plays a direct role in modulating platelet function, the authors use a CXCR7-specific agonist (VUF11207) to show that CXCR7 signaling significantly attenuates platelet thrombus formation under shear and inhibits soluble agonist activation ex vivo. These findings were recapitulated utilizing in vivo mouse models of myocardial infarction and arterial thrombosis and highlight that, similar to humans, mouse platelets increase CXCR7 expression after myocardial infarction. Furthermore, CXCR7 agonism results in a reduction in infarct size, reduced platelet activation, and reduced platelet-leukocyte aggregate formation in vivo. In accordance with the hypothesis that CXCR7 modulates the thromboinflammatory response, CXCR7 agonist treatment significantly alters the expression of proinflammatory mediators after myocardial infarction and inhibits the release of proinflammatory cytokines from thrombin-activated platelets. Indeed, treatment of mice with a CXCR7 agonist was found to be associated with a marked reduction in the levels of plasma cytokines, including IL-1α, interferon-γ, tumor necrosis factor-α, monocyte chemoattractant protein 1, IL-1β, and IL-6 after myocardial infarction. Moreover, although the treatment of mice with the CXCR7 agonist afforded protection from occlusive thrombus formation, no bleeding phenotype was observed by measuring tail bleeding time or basic coagulation parameters. This finding will require further investigation, as the tail bleeding time described in the current study does not correlate with the bleeding risk of antithrombotics.[7] Indeed, it is noteworthy that CXCR7 agonist treatment inhibits platelet PS exposure, which is significant because the rare platelet function defect (Scott syndrome) associated with defective platelet PS externalization is associated with reduced thrombin generation and increased bleeding.[8]