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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Dinauer et al, page 2855

NOX2: is the best defense a good offense?

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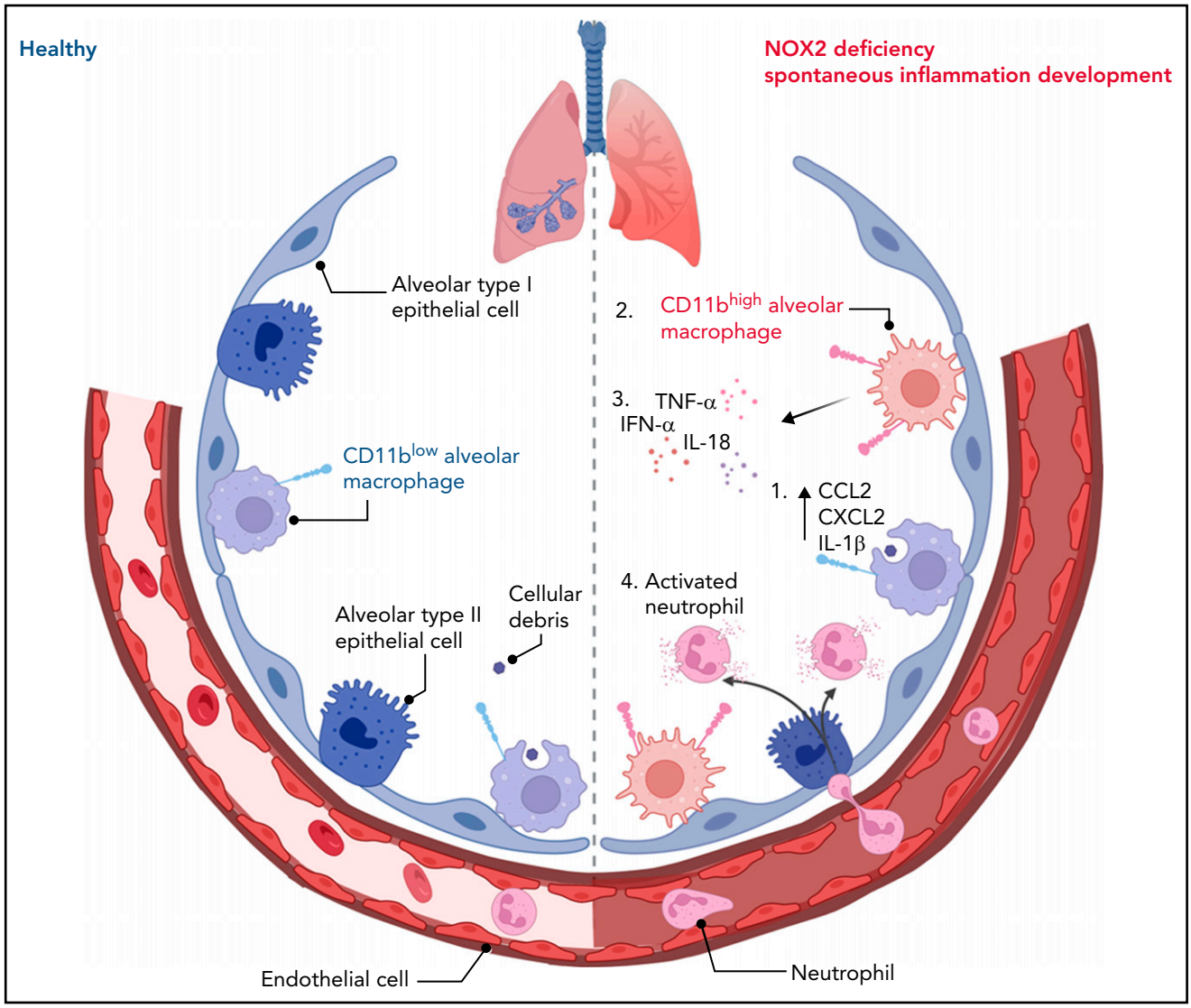
In this issue of *Blood*, Dinauer et al used NADPH oxidase 2 (NOX2)-deficient mice to study the underlying mechanisms of the noninfectious inflammatory conditions that arise in patients with chronic granulomatous disease (CGD).[1] They identified the development of a new population of alveolar macrophages (AMs) with significant inflammatory properties.

After fetal liver monocytes populate each of the organs during development, they receive important instructions from the local environment to develop into noninflammatory, tolerant macrophages that regulate homeostasis. For example, in the liver, the monocyte reaches out and touches hepatocytes, stellate cells, and sinusoidal endothelium and, through key molecules that activate specific transcription factors, acquires its Kupffer cell identity.[2] In the liver, these Kupffer cells sustain their number through replication. In other organs, such as the lung, the AMs are slowly replaced by monocyte-derived macrophages that are rapidly trained to take on the identity of original tissue-resident macrophages. Because only 30% to 40% of alveoli contain AMs at any one time, the cells migrate from alveolus to alveolus, ensuring that there is full coverage of all alveoli within the lung so that debris and any inhaled pathogens are removed.[3] Despite the fact that we breath in 10 000 L of nonsterilized air each day, AMs are so efficient, that neutrophils are generally not needed. The small increase in bacterial burden is handled by the AMs through protective mechanisms, such as phagocytosis and the release of reactive oxygen species. Patients with CGD lack NOX. They develop infections and have increased inflammation, especially in the lungs. Clearly, an efficient offense is the best defense against inhaled foreign particles, and a perturbation in this well-oiled immune machine, such as loss of the oxidant-generating enzyme NOX2, causes a shift away from homeostasis.

Dinauer and colleagues unveil what appears to be an anti-inflammatory role for the bacteria-killing enzyme NOX2 in regulating sentinel AMs to ensure measured responses to disturbances, thereby maintaining pulmonary homeostasis. In NOX2 deficiency, neutrophil recruitment was noted in the absence of infection (see figure). Neutrophil recruitment into lungs is almost always associated with excessive inflammation that can damage bystander tissues and cells. However, the healthy AM is trained to remove even 1 million bacteria instilled directly into mouse lungs without a need for neutrophils. It is only when an overwhelming infection occurs that blood-borne immune cells are recruited.[4] Because NOX2-deficient mice have lost their capacity to maintain homeostasis, neutrophils are inadvertently recruited, even at steady state. NOX2-deficient neutrophils may also contribute to the increased inflammation. Indeed, the global NOX2-knockout mice have greater inflammation than the macrophage-selective NOX2-knockout mice, suggesting that other cells, such as the neutrophils, also contribute to the inflammation and perhaps are even more inflammatory.

Dinauer et al have also uncovered a new population of CD11b^{high} AMs within the CGD-affected mouse lung. Whether it is the loss of NADPH oxidase per se or the increased inflammation that derives this population is unclear. It has been reported that inflammation in the lung, triggered by external infectious pathogens also drives an increase in CD11b^{high} AMs that is transcriptionally and epigenetically distinct from the resident CD11b^{low} AMs.[5] Comparably, using RNA sequencing and ATAC sequencing, Dinauer et al observed the greatest expression of proinflammatory genes in CD11b^{high} AMs compared with wild-type AMs, suggesting a contribution of CD11b^{high} AMs to the lung proinflammatory landscape. They did not actively examine the source of these cells; both monocyte-derived and tissue-resident macrophages could be contributors to this population. It is nevertheless intriguing that NOX2-deficient macrophages increased CCL2 production 250-fold in bronchoalveolar lavage fluid in response to lipopolysaccharide, a chemokine absolutely critical for monocyte mobilization from the bone marrow and recruitment into tissues.[6,7] A monocyte-derived, cell-specific effect has also been shown in the peritoneum, where NOX2 absence delayed the maturation of monocyte-derived macrophages after zymosan instillation, resulting in a persistent inflammatory phenotype.[8] Although Dinauer et al noted no increased apoptosis or decreased replication in the resident AMs, monocyte-derived macrophages could simply be added to the AM pool. Although they have been careful not to overinterpret the origin of these CD11b^{high} AMs, a future monocyte lineage-tracing experiment using Ms4a3-RFP mice would clarify the extent of monocyte-derived macrophages on a NOX background.[9] It would unveil whether the resident macrophages or monocyte-derived macrophages are being maladaptively trained in the absence of NOX2.

Although there is always criticism levied against inferring human phenotypes from



A healthy and NOX2-deficient alveolus. A healthy alveolus populated with sentinel resident CD11b^{low} AMs that can capture cellular debris to maintain homeostasis (left). NOX2 deficiency results in (1) phagocytic CD11b^{low} AMs that can capture cellular debris but also release production of proinflammatory cytokines and chemokines, followed by the emergence of (2) a CD11b^{high} AM population, derived from either resident AMs or recruited monocytes, and by overall lung inflammation in mice and humans, characterized by (3) an increase in proinflammatory cytokines and (4) recruitment and activation of neutrophils (right). IFN-α, interferon-α; IL-18, interleukin-18; TNF-α, tumor necrosis factor-α.

mouse experiments, in this case the mouse largely corresponds to the human clinical condition. There are some notable differences; for example, whereas humans with CGD develop opportunistic infections such as *Aspergillus fumigatus*,[10] mice remain reasonably devoid of such infections. However, this is almost certainly not a mouse model problem but an environmental problem. Mice kept in pathogen-free conditions are often devoid of many phenotypes found in humans living in the outside pathogen-laden world. Indeed, it would be fascinating to examine the CGD mouse in the wild to determine the full extent of the inflammatory phenotype in this deficiency. Although the germ-free environment did not unveil any difference in specific-pathogen–free mice, we predict that placing the mice in their natural environment and exposing them to mouse fungal, bacterial, and viral pathogens would further exacerbate the phenotype seen in this study. Indeed, exposing the NOX2-deficient mice to zymosan and Toll-like receptor ligands elicited more potent inflammatory responses than in wild-type mice. So, an ongoing challenge with pathogens and microbial products may further replicate the human condition.

The vulnerability to opportunistic infections and an increased inclination toward a proinflammatory landscape illustrates an extremely dangerous lung profile in patients with CGD. Dinauer et al inform us of the NOX2 absence–induced, altered-AM phenotype and its association with the spontaneous development of noninfectious inflammation in the lung. This study is a notable contribution to our understanding of the CGD lung and reminds us that the best immune defense is a very effective offense.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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TRANSPLANTATION

Comment on Zaiken et al, page 2983

GVHD … it is all about the microenvironment!

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In this issue of *Blood*, Zaiken et al describe the impact on chronic graft-versus-host disease (cGVHD) affecting the formation of germinal centers (GCs) in secondary lymphoid organs (SLOs).[1] Using 2 different small molecules targeting polycomb repressive complex 2 (PRC2) or bromodomain and extraterminal (BET)-bromodomain inhibitor, they demonstrate both can improve cGVHD in a murine model of bronchiolitis obliterans (BO) and one also improved scleroderma in another model.

GVHD remains the major complication following allogeneic hematopoietic cell transplantation (HCT). We have come a long way in understanding the pathobiology of this process with novel approaches and drugs that are effective in prevention and therapy. Even with these gains, there is still so much more that is needed. This need is especially pressing for cGVHD because we are transplanting older patients with more comorbidities. Improvements in early treatment-related mortality have led to more patients surviving and at risk for developing cGVHD.

One major insight into the pathology of cGVHD is the role of B cells. B-cell tolerance and signaling are aberrant in cGVHD.[2] B cells contribute to cGVHD pathology but are also required for the formation of the lymphoid follicle. In a healthy individual, GCs are structures in the follicles where mature B cells are activated, proliferate, and mature and where somatic hypermutation occurs in response to an antigen. In addition, the follicle is a critical tolerogenic site in SLOs.[3] These SLOs are a tightly regulated site of T- and B-cell tolerance induction via exquisite control of a reticular network comprising nonhematopoietic stromal cells, including fibroblastic reticular cells (FRCs).[4,5] FRCs play a known pathological role in cGVHD as they fail in their immune tolerogenic role, and they promote aberrant B-cell activation by providing notch ligand and producing B-cell activating factor.[6,7] In addition, there are data to suggest that extrafollicular CD4 T- and B-cell interactions are sufficient for inducing cGVHD.[8]

The authors had previously developed a murine model of multiorgan cGVHD with BO, which is dependent on the formation of GCs. These observations suggest that modulation or inhibition of the GC reaction may be a new therapeutic target. Thus, the investigators set out to test 2 pathways known to be required for GC formation. The first target was enhancer of zeste homolog 2 (EZH2), a histone-lysine *N*-methyltransferase enzyme that participates in histone methylation and, ultimately, transcriptional repression. EZH2 is the functional enzymatic component responsible for the methylation activity of the PRC2, which is responsible for health through the epigenetic maintenance of genes responsible for regulating development and differentiation. Specifically, PRC2 is a critical regulator in GC formation. A small molecule called JQ5 was used to target EZH2. The second target was directed to BET-bromodomain enzymes, specifically BRD4. These "readers" of the genome recognize acetylated lysine residues and are responsible in transducing the signal and translating it into various normal or abnormal phenotypes. The investigators used another molecule, JQ1, to target this area. In vitro, JQ1 hinders T-cell interleukin-21 expression required for T-follicular helper cell (TFH) function, and in vivo, JQ1 impaired GC B-cell formation via BCL6 repression.

These new data demonstrated that both JQ1 and JQ5 improved pulmonary function, including improvement of lung histology, and, as predicted, these drugs impaired the GC reaction, which was required for the cGVHD, pathological, and clinical changes (see figure). They also demonstrated that JQ5 was on target by demonstrating reduced EZH2-mediated methylation in donor T cells. Using a second model of sclerodermatous cGVHD, JQ5 also reduced the severity of skin changes, but surprisingly JQ1 did not. To determine the mechanistic targets of these 2 drugs, the investigators performed RNA-seq of GC B cells from spleens of cGVHD mice with BO (through all the difficulties during COVID-19). Each drug led to different changes. Moving to gene set enrichment analysis comparing