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Immunosuppressive lung neutrophils

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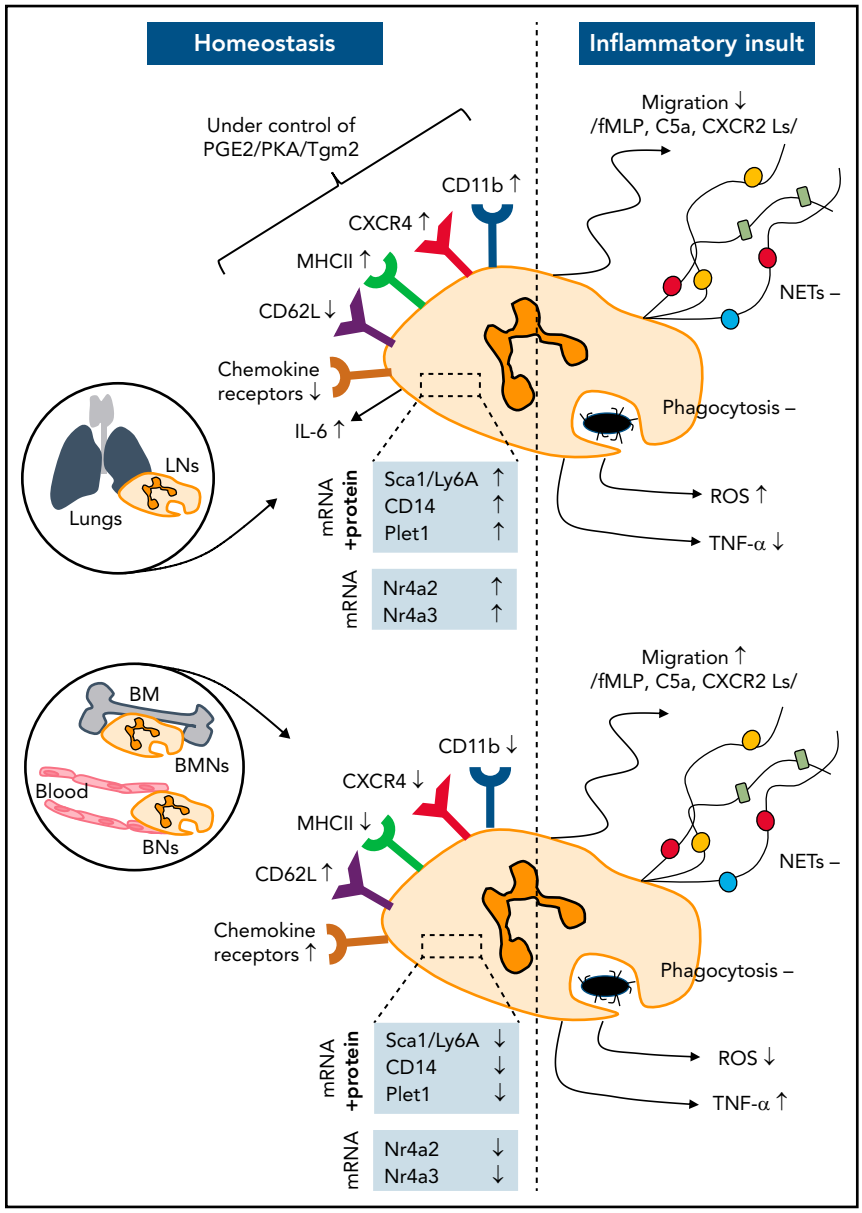
In this issue of *Blood*, Bae et al[1] provide evidence that lung neutrophils form a unique population with primarily immunosuppressive (in homeostasis), and more anti-inflammatory than proinflammatory (upon inflammatory insult), phenotypes that protect the lungs against the damage that may result from an overexaggerated response to injury, infection, or dysfunctional/disordered homeostasis within the lung.

Neutrophils originate from hemopoietic stem cells differentiating into common myeloid progenitors, and as mature cells, they migrate from bone marrow (BM) into the blood, where they circulate unless activated and recruited to sites of inflammation.[2,3] Some neutrophils are marginated into pools in various loci (liver, spleen, and BM itself), but probably the largest intravascular reservoir of these cells is harbored in the pulmonary circulation.[2,4] These marginated neutrophils are thought to combat infection locally or to be released into the circulation when needed. In all these locations, resident neutrophils must be tightly regulated to avoid damage to tissues such as the fragile alveolar structures of the lungs. To identify mechanism(s) controlling the quiescent phenotype of alveolar neutrophils, Bae et al compared lung neutrophils (LNs) to the much better characterized BM neutrophils (BMNs) and blood neutrophils (BNs), and showed that LNs have a unique immunosuppressive phenotype, both during hemostasis and inflammatory activation. First, multiple genes and surface markers were shown to be altered in LNs (all are listed in the figure), and among them, the expression of CXCR4 was upregulated, whereas that of CD62L was decreased. In line with this finding, CXCR4 has been shown to sequester LNs to the lung endothelium,[5] whereas adhesion molecule CD62L (also known as L-selectin) facilitates neutrophil trafficking to the inflammatory site.[2] Interestingly, this phenotype is also characteristic of ageing neutrophils which, during conditions of homeostasis, are cleared in the BM, but during inflammation they are the first to arrive at the inflamed tissue where they efficiently fight infection.[6] Importantly, LNs are also CD11b^{hi}, which parallels the immunosuppressive neutrophils identified in other tissues in conditions such as cancer, pregnancy, or sepsis.[7] The number of LNs is maintained by high levels of interleukin-6 (IL-6) in steady-state conditions, and prostaglandin E2 (PGE2) is critical for induction of their phenotype, acting through protein kinase A (PKA) and transglutaminase 2 (Tgm2). Although mostly regarded as proinflammatory, IL-6 also has regenerative, metabolic, and anti-inflammatory functions. In the lungs, IL-6 is essential for neutrophil survival and for promoting pathogen clearance.[8] Bae et al further show that neutrophils polarize locally within the lung environment, as BMNs cultured in the presence of bronchoalveolar lavage fluid or PGE2 become LNs. PGE2 also increases the lifespan of LNs by delaying their apoptosis. Concentration of PGE2 has been shown to be 50- to 80-fold greater in the lower respiratory tract than in concurrent blood samples, which justifies occurrence of this phenomenon in the lungs. The exact source of PGE2 is still unknown, but interdependence between this lipid mediator and IL-6 has been shown (eg, PGE2 can induce release of IL-6 by airway epithelial cells[9]).

Another noteworthy feature of LNs is upon stimulation with lipopolysaccharide (LPS) or other proinflammatory stimuli. LNs demonstrate efficient phagocytosis and form neutrophil extracellular traps (NETs) in response to ionomycin, similar to BMNs. LNs are less prone to migration toward *N*-formyl-methionyl-leucyl-phenylalanine and C5a after recognition by their respective receptors. In light of this, it is of importance that in vivo lung neutrophils respond rapidly to LPS or *Escherichia coli* by crawling from venules to capillaries (pulmonary intravital microscopy [pIVM]), but this process depends on CD11b, which is highly expressed in LNs.[4] In addition, LNs release less tumor necrosis factor-α (TNF-α) in response to LPS, but they generate somewhat higher levels of reactive oxygen species (ROS) upon phorbol 12-myristate 13-acetate stimulation. This reveals a mixed proinflammatory or anti-inflammatory response. ROS may be necessary for NET generation, yet no more NETs were produced by LNs and, importantly, no NET formation was observed in the mouse lungs (examined by pIVM) upon bacterial stimulation.[4] On the other hand, TNF-α is a very potent proinflammatory cytokine that induces a cascade of inflammatory mediators (eg, it is upstream of IL-1β). Therefore, its lower level of release may weaken a subsequent cytokine storm. Indeed, neutrophils from *Tgm2*$^{-/-}$ mice release higher levels of TNF-α, IL-1β and other inflammatory cytokines in response to LPS compared with their wild-type counterparts.

Notably, numerous immature band neutrophils are rapidly recruited from BM to inflamed organs during inflammation, including the lungs. One can hypothesize that in such conditions, time is limited for the pulmonary microenvironment to induce rephenotyping of band neutrophils into LNs. Newly recruited BMNs present in the lung display a strong correlation with disease severity. Thus, the resident anti-inflammatory LNs alone cannot prevent collateral damage during severe lung inflammation, but certainly they can minimize it.

Bae et al have also significantly contributed to the ongoing discussions on neutrophil heterogeneity. Changes in neutrophil quality, rather than mere changes in cell counts, are now recognized to occur during multiple disorders.[3] Whereas neutrophil commitment in BM is a possible source of neutrophil heterogeneity, reprogramming of mature neutrophils in tissues is equally, if not more probable. Indeed, neutrophils have been shown to change at the transcriptome level when they infiltrate various tissues[3] as Bae et al further confirm. The first reports on heterogenous



Differences in phenotypes of LNs vs BMNs and BNs during homeostasis and inflammatory stimulation. In homeostasis (left), LNs display altered expression of multiple surface receptors and their gene expression (mRNA levels) is changed. This phenotype is induced by PGE2, which acts through PKA and Tgm2. Moreover, LNs produce high levels of IL-6 that maintains their count. Activated LNs release NETs (ionomycin) and phagocytose in manner similar to BMNs and BNs but show impaired migratory activity toward various chemoattractants. In response to LPS they produce less TNF-α but more reactive oxygen species. In homeostasis their phenotype is immunosuppressive, whereas in inflammatory conditions (right), it is mixed with dominance of the anti-inflammatory response. C5a, complement component 5a; CD11b, integrin αM subunit; CD14, cluster of differentiation 14; CD62L, L-selectin; CXCR2 Ls, ligands of the C-X-C chemokine receptor type 2; CXCR4, C-X-C chemokine receptor type 4; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine; MHCII, major histocompatibility complex II; Nr4a2, nuclear receptor 4A2; Nr4a3, nuclear receptor 4A3; Plet1, placenta-expressed transcription 1; Sca1/Ly6A, stem cell antigen-1; — unaltered levels, ↑ increased levels, ↓ decreased levels.

neutrophil populations came from cancer studies identifying anti-tumor N1 and pro-tumor N2 cells which reprogramming depends on factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and transforming growth factor β.[3,10] Downstream action of GM-CSF induces PGE2 generation which is responsible for the immunosuppressive phenotype of N2. Although PGE2 has the same immunosuppressive effect on LNs, GM-CSF has only minor impact on the LN phenotype.

In a recent consensus paper, experts suggested using the term "states" to refer to phenotypically distinct neutrophil populations.[10] Along this line, Bae et al characterized a pathway leading to the generation and maintenance of an immunosuppressive state of lung neutrophils that offers new therapeutic opportunities for inflammatory lung disorders.

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

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