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INSIGHTS

T2B or not to B: Calming neutrophils offshore

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In this issue of *JEM*, Podstawka et al. (2021. *J. Exp. Med.* https://doi.org/10.1084/jem.20210409) show that B cells can limit neutrophil responses within the lung microvasculature by marginating and acting on marginated neutrophils. This study provides a new view of B cells and reveals a novel mechanism of cell-mediated intravascular regulation.

The surveillance of blood-borne pathogens is usually considered to be the precinct of the spleen, which has red pulp macrophages and neutrophils to mount innate responses and white pulp lymphocytes to mount adaptive responses, but recent studies have begun to show that the pulmonary microvasculature contains a pool of neutrophils that can neutralize blood-borne pathogens. While immune cells are most often thought of as extravasating from the blood vessels and doing their work in the tissue parenchyma, neutrophils in the lung microvasculature will marginate, or position themselves in the low-flow areas at the vessel walls where they are no longer swept away with the rest of the blood flow and can remain local while intravascular. From there, the neutrophils capture blood-borne pathogens that are immobilized by the endothelial cells to protect the host. With activation, however, the neutrophils can swarm and cluster, causing capillary plugging (Granton et al., 2018). Furthermore, among marginating neutrophils in the lung are aged cells that have entered other tissues and then exited, or reverse transmigrated. These neutrophils are considered to be more proinflammatory (Kim et al., 2018; Wang et al., 2017). The activities of the activated and aged neutrophils together can contribute to their respiratory distress (Granton et al., 2018) observed in a number of infections, including COVID-19. Mechanisms that limit the tissue-damaging activities of these marginating neutrophils, however, are poorly defined. Yipp and colleagues have previously shown that B cells can promote marginating, aged neutrophils to undergo apoptosis. In this issue of *JEM*, the same group expands upon this work by showing that a specific subset of B cells preferentially marginate and can prevent neutrophil clustering in the lung microvasculature (Podstawka et al., 2021).

Using an elegant combination of intravital microscopy and single-cell RNA sequencing, Podstawka et al. (2021) show that B cells marginate within the pulmonary microvasculature even during homeostasis and identify these B cells as transitional type 2 B (T2B) cells. This naive B cell population originates from the bone marrow and accumulates in the spleen, where they develop into mature B cells that subsequently recirculate. Podstawka et al. (2021) observed that the T2B cells respond to CXCL13 expressed by the lung tissue and marginate, remaining endothelial bound within capillaries for up to 10 min, in a similar manner to neutrophils. The prolonged positioning of the T2B cells with neutrophils within the lung microvasculature provides a unique opportunity for these cell types to interact, and the authors show that B cell margination serves to limit intravascular neutrophil clustering and promote their apoptosis in a lipoxin A4 (LXA4)–dependent manner in response to inflammatory insults.

By establishing that B cells can marginate and regulate neutrophil responses in the lung, this paper identifies a previously unknown function of B cells, and specifically of T2B cells in a nonlymphoid tissue. B cell deficiency has previously been shown to increase neutrophil-mediated inflammation and lung-specific bacterial burden (Kozakiewicz et al., 2013), but the mechanisms by which B cells conferred protection to the tissue has been poorly understood. T2B cells have been shown to have an immune-regulatory phenotype, capable of suppressing the cytotoxic T cell response and promoting T reg cell differentiation, but these functions have been previously identified in lymphoid tissues (Zhou et al., 2020). This study now both provides a mechanism for B cell–mediated neutrophil regulation in the lungs and delineates a new role for T2B cells. This opens the door to many additional studies. As T2B cell margination was observed even at homeostasis, it will be interesting to understand whether T2B cells act as constantly patrolling gatekeepers, actively inhibiting aberrant neutrophil activation and inducing death during

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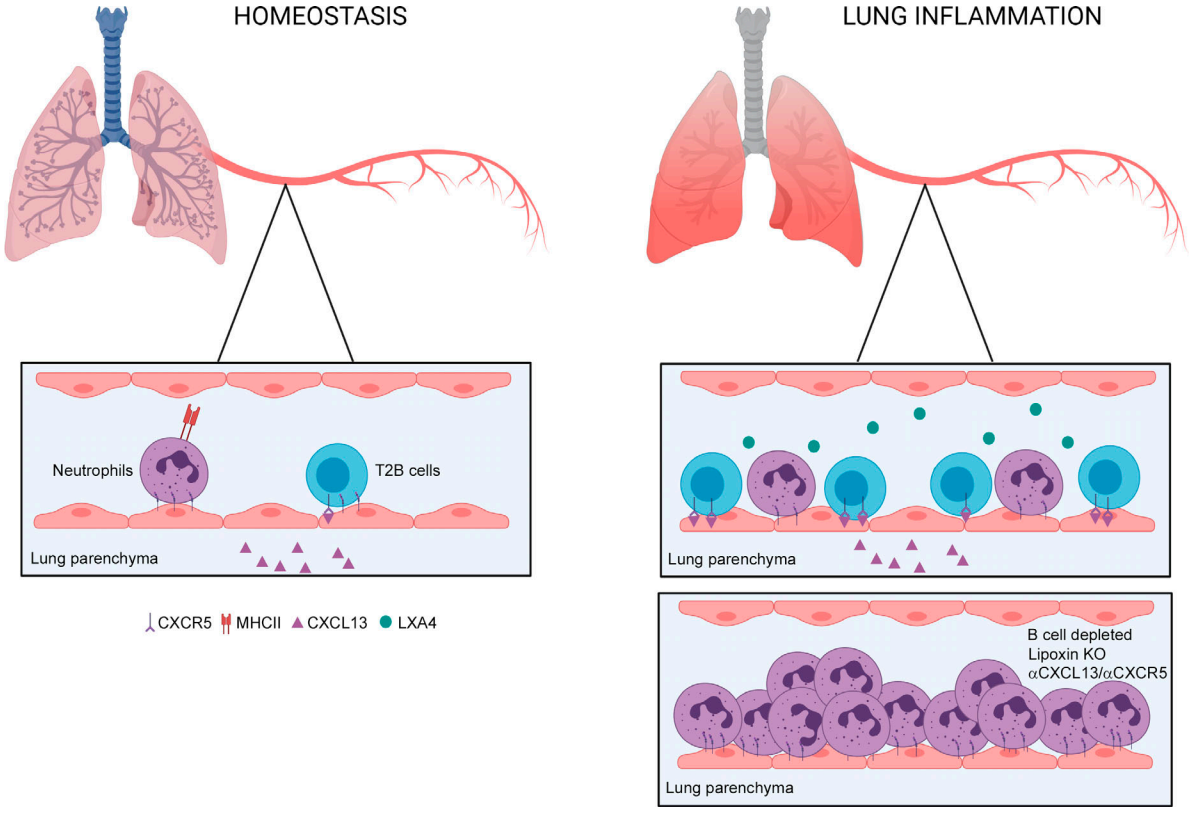

Within the lung microvasculature, aged and activated neutrophils marginate to the endothelium where they remain bound for up to 10 min. T2B cells also marginate in a similar manner in response to CXCL13. Prolonged adhesion to the endothelium is mediated by CD49e at homeostasis. These B cells can induce transfer of MHCII to neutrophils and further promote their apoptosis. In response to lung inflammation, B cell margination is enhanced, and this limits excessive neutrophil margination and activation in a lipoxin-dependent manner. Depletion of B cells, loss of lipoxin production, or inhibition of T2B cell margination (through blockade of the CXCL13–CXCR5 axis) leads to prolonged neutrophil accumulation and activity within the lung microvasculature during infection.

homeostasis. Furthermore, whether these T2B cells are able to mature while marginated and whether their differentiation is influenced by the presence of infectious agents remains to be examined. Understanding whether B cell receptor specificity plays any role in determining which T2B cells marginate in the lungs and whether this pool is distinct from the pool that migrates to and matures within the spleen also has the potential to deepen our understanding of B cell development and selection in health and disease.

Critically, this study reinforces the idea that the vascular compartment is not simply a highway, but can be a functional immune-regulatory compartment. Neutrophils have long been known to marginate and then demarginate with stimuli such as steroids, causing the neutrophilia seen with glucocorticoid administration in asthma patients, for example. Yipp and colleagues' previous work has shown that marginating neutrophils function to phagocytose microbes, and it is this unique positioning that allows them to be the primary cell type responsible for pathogen control in the lung vasculature (Granton et al., 2018). The area of margination within the blood vessels, then, serves as a functional immune compartment. This study further enhances this model by showing that B cells join neutrophils in this compartment where they serve at least in part to control neutrophil responses. While we ordinarily think of intravascular lymphocytes as being on their way to a tissue, this study makes us reconsider their function. Perhaps recirculating mature B and T cells are not only on their way to look for antigen in a secondary lymphoid organ, but could, in fact, pause and have additional functions at some intravascular niches.

The identified intravascular T2B cell–mediated neutrophil regulation could potentially exist in vascular beds of other tissues. Marginating neutrophils have also been observed in the liver (Casanova-Acebes et al., 2018) and kidney (Awad et al., 2009). Marginated pulmonary neutrophils can contribute to tissue damage in part by NETosis (Granton et al., 2018), with ensuing tissue injury and activation of other immune cells by reactive oxygen species and Toll-like receptor agonists. In systemic lupus erythematosus (SLE), the kidney is a commonly affected organ, and neutrophils are prone to NETosis (Gupta and Kaplan, 2016). There is also an increased percentage of transitional B cells in SLE patients (Dieudonné et al., 2019), so perhaps the transitional B cells actually limit the extent of kidney disease caused by NETting neutrophils. Alternatively, since transitional B cells in SLE demonstrate a high type 1 IFN signature even when compared with other circulating B cells (Dieudonné et al., 2019), perhaps these transitional B cells are dysfunctional in their abilities to limit neutrophil activity, thereby leading to or exacerbating the kidney disease. This latter scenario is interesting to consider in relation to lupus photosensitivity, a feature of lupus whereby exposure to even ambient ultraviolet radiation (UVR) triggers not only skin inflammation but also worsening of

other affected organs such as kidneys (Sim et al., 2021). Elkon and colleagues recently showed that UVR induces neutrophils activated in the skin to reverse transmigrate from the skin and enter the vasculature of the kidneys, where they promoted tissue inflammation (Skopelja-Gardner et al., 2021). UVR also induces type 1 IFN expression, and thus perhaps contributed to the neutrophil-mediated kidney inflammation in part by disrupting a protective T2B-neutrophil axis. Further hints of such an axis in kidney also comes from the correlation between lower levels of transitional B cells in the circulation and worse outcomes in renal transplantation (Svachova et al., 2016). This study opens up further questions to explore about the importance of this T2B–neutrophil axis in regulating aberrant marginated neutrophil activation in diseases that are exacerbated by NETting or aged neutrophils.

This newly identified role for T2B cells also provides a new function for CXCL13 as a mediator of margination and therefore an inhibitor of neutrophil activity. CXCL13, originally studied by Cyster and colleagues for regulating follicular positioning within secondary lymphoid organs, is also elevated in the bloodstream in inflamed mice and humans (Havenar-Daughton et al., 2016). Does this circulating CXCL13 reflect the CXL13 expressed by tissues to stimulate B cell margination at the site? Or is it expressed by circulating cells such as T follicular helper cells (Vella et al., 2019) and serves to neutralize the tissue-derived CXCL13 gradient, thus promoting neutrophil-mediated tissue injury? Serum CXCL13 has been examined as a biomarker of disease in a number of conditions. The study of Podstawka et al. (2021) has the potential to lead to functional understanding of circulating CXCL13 and ensuing therapeutic targeting.

In addition to the T2B cells, the authors show that there is an enrichment of T1B and B1 cells in the pulmonary vasculature, although T2B cells were identified as marginating. This enrichment is reminiscent of the B cell composition of the spleen. Another parallel between the spleen and pulmonary microvasculature is the interaction between B cells and neutrophils. Within the spleen, these two cell types interact in the marginal zone sinus where the blood empties from the terminal arterioles, a site that has, similar to the areas of margination within the pulmonary microvasculature, low velocity blood flow. In this context, the neutrophils regulate the proliferation and maturation of marginal zone B cells (Puga et al., 2011). There are additional B cells in the single-cell RNA sequencing from this study that await further characterization (Podstawka et al., 2021), and parallels with the spleen suggest that studies of the pulmonary microvascular niche may also teach us about the spleen and other secondary lymphoid organs. These new data also suggest that the vasculature may be an additional level of regulation for circulating immune cells outside of conventional lymphoid tissues.

This study reminds us that there remains much to be learned from studying the bloodstream. An easily accessible compartment, the blood is most often examined for biomarkers of disease—chemokines, as in the case of CXCL13, but also white blood count, the gene expression patterns of circulating cell populations, and serum enzyme or cytokine levels. However, there is a wealth of additional information contained within the blood, namely, the functional interactions between immune cells that may be critical indicators of disease progression. Importantly, differences in the extent of these interactions in different vascular beds may be indicative of organs more or less affected by inflammatory insult and, as such, suggests that we should pay better attention to the site from which blood is drawn. Sometimes, battles are waged within the waters and not on land.

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