we learn to model treatment response, and as we apply new technology, like cellular barcoding, single-cell transcriptomics, and mutational profiling. Together, these approaches will help to reveal how complex, multifunctional tumor suppressors, like CUX1, safeguard the blood system. Cytotoxic chemotherapy remains a mainstay of cancer therapy, indicating that we will be dealing with this problem for some time to come. By modeling treatment response, I am hopeful that we can learn to rekindle the hematopoietic system safely and avoid starting a raging inferno.

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Wang et al, page 806

Live or die: PD-L1 delays neutrophil apoptosis

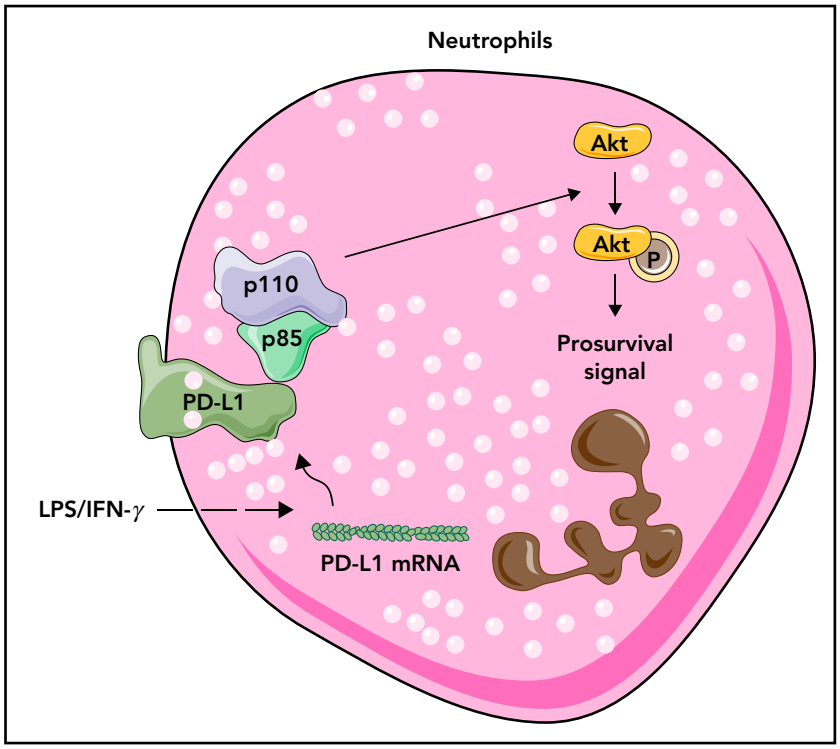
Jamel El-Benna and Pham My-Chan Dang | INSERM-U1149

In this issue of *Blood*, Wang et al uncover a key role that programmed death ligand 1 (PD-L1) plays in delaying neutrophil apoptosis at the site of inflammation by activation of the phosphatidylinositol 3-kinase (PI3K)-AKT survival pathway.[1]

Polymorphonuclear neutrophils (PMNs, or neutrophils) are the most abundant circulating leukocytes, constituting 60% to 70% of circulating white blood cells. PMNs are terminally differentiated and have a short lifespan, but are essential for innate immunity and host defense against microbes.[2] They are the first cells to be massively recruited at the site of infection where they recognize microbes via different receptors, inducing engulfment of the microbe into a phagosome.[3,4] Killing of microbes by PMNs occurs through the release, into the phagosome, of toxic agents such as reactive oxygen species and the content of granules (myeloperoxidase, glucosidases, proteases, and antibacterial peptides, etc). Microbes can also be trapped and killed by neutrophil extracellular traps (NETs).[5] Many processes such as apoptosis, NETosis, pyroptosis, and necroptosis can induce the death of neutrophils,[5] upon which they are phagocytized and eliminated by macrophages through a process called efferocytosis, resulting in the cleaning of the infection site. Thus, PMNs are critical anti-inflammatory components of the innate immune system as their physiological role is to resolve both infection and inflammation. Nevertheless, excessively activated or delayed apoptosis results in PMNs becoming harmful to surrounding tissues due to cell injury and continued inflammatory reaction, the driving factors for inflammatory disorders such as sepsis.[4]

Tissue neutrophils are believed to have longer lifespans than circulating PMNs, due to the presence of survival factors at the inflammatory site. Extended neutrophil lifespan through apoptosis suppression has been reported in patients with several inflammatory diseases and is associated with increased disease severity.[6] Neutrophils isolated from blood die through constitutive apoptosis, which can be either accelerated or inhibited by several agents. Inhibition of neutrophil apoptosis can contribute to inflammation; however, the factors leading to dysregulation of neutrophil apoptosis in inflammation are still not completely identified. In this issue, Wang et al demonstrate that PD-L1 plays a key role by delaying neutrophil apoptosis at the site of inflammation through the PI3K-AKT pathway (see figure).

Programmed cell death 1 (PD-1) protein, expressed on immune cells, is an immune checkpoint inhibitory receptor that triggers immunosuppressive signaling pathways.[7] PD-1 binds to PD-L1 or PD-L2 and blocks activating signals from T-cell receptors. PD-1/PD-L1 function as brakes to limit the adaptive immune response and the beneficial T-cell functions in cancer. PD-L1 is expressed on the plasma membrane of T and B cells and antigen-presenting cells.[7] Cancer cells also express PD-L1, which binds to the T-cell surface via PD-1 allowing them to escape host immune response. Thus, anti-PD-1/anti-PD-L1 antibodies have been used to treat various types of cancer. Previous studies have shown that PD-L1 expression on neutrophils increases in various inflammatory conditions.[8,9] In this new study, Wang et al show that PD-L1 overexpression in neutrophils from septic patients correlates with neutrophil survival. Silencing PD-L1 expression using small interfering RNA (siRNA) accelerated apoptosis



LPS plus IFN-γ and a septic environment induce PD-L1 expression in human neutrophils. PD-L1 binds to p85, the PI3K regulatory subunit, and PI3K activates AKT kinase to induce prosurvival signals in neutrophils making them live longer. mRNA, messenger RNA; p (p110 and p85), protein; P (Akt), phosphorylation.

of septic neutrophils. Interestingly, neutrophils challenged with interferon γ (IFN-γ) plus lipopolysaccharide (LPS) and neutrophils from septic patients exhibited increased AKT phosphorylation, which was reversed by PD-L1 siRNA. The authors found that PD-L1 coimmunoprecipitates with the p85 subunit of PI3K, the upstream regulator of AKT. In vivo, neutrophil PD-L1 deletion reduced neutrophil lung infiltration in a cecal ligation and puncture murine model and attenuated lung injury. Thus, increased PD-L1 expression on human neutrophils during inflammation delays cellular apoptosis via the PI3K-AKT pathway, driving lung injury and mortality.

The study by Wang et al brings significant new information to the neutrophil field. The first important message is that PD-L1 is not only a key player in cancer immunomodulation, but also in inflammation, raising the possibility that it could be a novel pharmacological target. The second message is that PD-L1 upregulation in neutrophils during sepsis promotes their survival. Finally, this study highlights the novel and important role of PD-L1 as an inducer of the PI3K-AKT signaling pathway, which regulates many neutrophil inflammatory functions. Thus, this study adds another critical step to our understanding of neutrophil biology.

As is always the case with novel mechanisms, several questions have been raised. In cancer, PD-L1 is expressed on the plasma membrane, but its localization in neutrophils is not addressed by the authors. Knowing whether PD-L1 is expressed on the plasma membrane or on the membrane of neutrophil granules would be interesting. Expression of PD-L1 on the plasma membrane would make it an easy target for immunotherapies with blocking antibodies. Indeed, while this study was under revision, Thanabalasuriar et al[10] reported the role of PD-L1$^+$ neutrophils in airway inflammation in mice and showed that an anti-PD-L1 antibody protected mice from inflammation. Patera et al[9] have also shown that inflammatory neutrophils express PD-1; the intercellular PD-1/PD-L1 engagement on PMNs could induce PI3K-AKT activation in neutrophils. This possibility could be checked using blocking antibodies. Wang et al showed that IFN-γ plus LPS induced PD-L1 expression in neutrophils; however, it would be of interest to know whether other inflammatory stimuli also induce PD-L1 expression in PMNs and by which mechanisms.

Although this study suggests a fundamental role of the PI3K-AKT pathway in mediating PD-L1–induced neutrophil survival, the mechanism by which PD-L1 activates PI3K was not investigated as the authors only show its ability to bind p85 when expressed in HEK293 cells. This pathway should be checked in inflammatory neutrophils. In addition, the PI3K-AKT pathway could modulate several other important neutrophil functions including superoxide production, degranulation, and chemotaxis, raising the question of whether these functions are also impacted in PD-L1$^+$ neutrophils. Furthermore, evaluating the role of PD-L1 in other inflammatory diseases such as rheumatoid arthritis and inflammatory bowel diseases would be of interest.

In summary, the study by Wang et al represents a major advance in our understanding of neutrophil biology. Notably, their work uncovers the important function of the PD-L1–PI3K–AKT axis in delaying neutrophil apoptosis in inflammatory situations, opening new avenues for novel therapeutic approaches in several immunological and inflammatory diseases and raising intriguing questions about PD-L1 to be explored in the future.

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CLINICAL TRIALS AND OBSERVATIONS

Comment on Ghione et al, page 811

The next wave: immunizing the immunosuppressed

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In this issue of *Blood*, Ghione et al report important early results on the differential development of antibodies to SARS-CoV-2 after vaccination in patients with lymphoma who were receiving B-cell–directed therapies.[1] These data add to information recently reported by Terpos et al[2] on antibody response to vaccination in older patients with multiple myeloma (MM).

Unfortunately, the results confirm what we feared—that many of our patients will not achieve immunoglobulin G (IgG) antibody responses from the coronavirus vaccination.[3] The letters both emphasize that we still have much to learn about the complex interactions between preventative inoculation strategies in patients with disease or treatment-related immunosuppression.

Clinical researchers have been highly motivated to quickly determine the efficacy of current vaccination efforts in patients with diseases such as MM and in patients who are immunocompromised. The clinical question posed by Terpos et al[2] was: how much response one can expect from a single dose of the BNT162b2 messenger RNA (mRNA) vaccine? With the supply of vaccines in question and international pressure to defer second doses for people who were not in priority groups until members of priority groups had received at least one dose,[4,5] researchers wondered whether just 1 dose would generate an adequate response in patients with MM. By using the 50% neutralizing antibody titer as a threshold for clinically relevant viral inhibition, these investigators demonstrated that only about 10% of patients with MM reach an adequate level of protection after the first vaccination. Their data suggest that immunoparesis of at least 1 uninvolved immunoglobulin may be the reason for failure to respond to the initial vaccination. Indeed, as the authors pointed out, hypogammaglobulinemia has been associated with inferior antibody response to coronavirus among patients with chronic lymphocytic lymphoma (CLL). Notably, the older individuals who served as controls in their study were also poorly protected after a single vaccination; only 20.2% achieved clinically relevant viral inhibition before they received the second dose. A

The article by Ghione et al focuses on patients with lymphomas and assessed antibody levels after full vaccination. The patients were divided into 4 cohorts based on time since treatment with B-cell–directed therapy, with health care workers and nursing home residents serving as controls. The researchers found that IgG responses were significantly different, depending on the length of time since treatment. Of the 52 patients with B-cell lymphoma who were vaccinated within 9 months of B-cell–directed treatment, only 6 (11%) developed a humoral response, whereas 22 of 25 patients who had a treatment-free interval of 9 months or more before they were vaccinated were able to develop IgG antibodies. The takeaway here is that there may well be a minimum interval for immune reconstitution after B-cell–directed therapy, an interval that could be used in an effective revaccination protocol.

Immunosuppressed individuals have faced special peril with this pandemic all along. The severe infection rates and morbidity for patients with hematologic malignancies are higher than those with other forms of malignancy.[6] Whether this vulnerability is a result of higher rates of infectivity, disproportionately poor response to therapy, comorbidities, or provider nihilism remains an open question. With the excellent efficacy rates of most of the approved vaccines, no one is advocating against vaccination, even in those who may not adequately respond.[7] Rather, these data emphasize the importance of maintaining infection control practices even after our patients have been vaccinated.

For years, there have been reports of inadequate immune response to vaccination in patients with CLL, MM, and other conditions associated with immune deficiency.[8] After autologous allogeneic transplantation, patients have severely reduced antibody titers, and they subsequently undergo broad spectrum vaccinations after transplantation. Consensus guidelines have regularly been published to help manage this population, but even those guidelines point out the significant holes in the data.[9]

What we don't know is evident in the letter from Terpos et al[2] and the article by Ghione et al. What are the best predictors of response in patients? How much antibody is enough to prevent severe infection? In the absence of humoral response, can cellular response provide protection? In regions where herd immunity has not yet been achieved, which treatments should be deferred? Should titers be measured in everyone? Will revaccination or booster shot strategies work?

Large-scale studies designed to provide answers to some of these questions are underway, although it is anticipated that the lessons from Ghione et al and Terpos et al[2] will prove true even in much broader populations. Meanwhile,