Lymphocyte activation occurs early during sepsis and is a key component of the proinflammatory response to infection. This response includes a "storm" of cytokines released into the circulation and a cascade of immunologic events directed at containing and killing pathogens (1). This early reaction to infection includes the mobilization of immature granulocytes, or band forms, from the bone marrow. The presence of bands has long been identified as a sign of infection and remains an inflammatory criterion in the definition of sepsis (2).

However, identifying the exact mechanisms that lead to organ dysfunction and death in sepsis has remained elusive. In addition to a proinflammatory response, evidence suggests that simultaneous anti-inflammatory processes lead to durable immunosuppression and increased risk for secondary infections (3–5). Elevated levels of anti-inflammatory cytokines and a suppressed adaptive immune response, including T-cell dysfunction and lymphopenia, have been widely observed (6–8). Moreover, the relationship between the proinflammatory and anti-inflammatory pathways in sepsis is poorly characterized, and whether the cytokine storm is linked to the development of immunosuppression is not known.

In this issue of *Critical Care Medicine*, Guerin et al (9) use multicolor flow cytometry to identify two leukocyte subsets, consistent with immature granulocytes, that predict clinical deterioration in septic adults. Furthermore, these data present intriguing support for the hypothesis that immature granulocytes, classically linked to the inflammatory pathway in sepsis, may be directly involved in immunosuppression of the adaptive immune response as well.

The investigators evaluated 23 leukocyte subsets, including immature and mature granulocytes, dendritic cells, macrophages/monocytes, plasma cells, and T- and B-cell subsets, using 10-color flow cytometry in blood samples from 177 septic adults (2). Presurgical, noninfected patients and infected patients without evidence of systemic inflammatory response were included separately as controls. Compared with controls, diminished expression of CD16 (CD16dim) and CD10 (CD10dim) on granulocytes was diagnostic of sepsis. When limited to just the sepsis cohort, CD16dim/CD10dim emerged as the most predictive markers of clinical deterioration and 30-day mortality.

In addition to identifying a subset of leukocytes that predicted clinical deterioration and mortality, the authors used multicolor flow sorting to identify and then isolate a subpopulation of immature granulocytes that were CD14neg/CD24pos within the CD16dim compartment from seven patients with septic shock. These cells were then cocultured with activated autologous T cells in vitro and exhibited a dose-dependent cytotoxic effect on these T cells, suggesting that these cells might also lead to T-cell death in vivo. Furthermore, interrogation of the clinical dataset demonstrated that increased granulocyte CD16dim in septic patients was associated with T-cell lymphopenia. Notably, band forms were identified on manual differential among all septic CD16dim/CD10dim patients, suggesting that these markers may identify the subpopulation of immature granulocytes that are commonly referred to as "bands."

The authenticity of CD16dim/CD10dim as a novel immune marker of sepsis and disease progression is bolstered by comparisons with previously described immunophenotypes in sepsis. Neutrophil expression of CD64 is a well-characterized diagnostic marker of neonatal sepsis (10). Consistent with these findings, CD64 expression was elevated in septic patients compared with controls in the current study. CD64 positivity did not, however, predict clinical deterioration among septic patients. Decreased monocyte expression of human leukocyte antigen (HLA) complex-DR, previously associated with secondary infections, shock, and mortality in intensive care populations (11, 12), was associated with progression to septic shock and sepsis mortality in this study. Interestingly, however, neither neutrophil CD64 expression nor monocyte HLA-DR was associated with lymphopenia. The latter finding is noteworthy since HLA-DR expression, a marker of monocyte functionality, has been postulated as a potential biomarker of sepsis-related immunosuppression although the mechanism may be independent of T-cell suppression (12). Overall, these findings are consistent with prior studies and provide credence to the methodology of the current study.

Thus, this study presents two main findings: 1) flow cytometry measures of immature granulocytes may aid in the prognosis of patients presenting with sepsis and 2) these cells, known mediators of the sepsis inflammatory response...
response, may also be directly linked to sepsis-related immunosuppression.

However, several important uncertainties remain in translating these results to the management of sepsis in clinical practice. First, this is a single-center, modest-sized cohort study. External validation of these findings will be necessary. Second, it is not clear that CD16<sup>dim</sup>/CD10<sup>dim</sup> cells are equivalent to bands. Although band forms were observed in all CD16<sup>dim</sup>/CD10<sup>dim</sup> patients in this study, prior data suggest that the CD16<sup>dim</sup>/CD10<sup>dim</sup> cell surface signature may encompass a broader population of newly emerged granulocytes including segmented neutrophils (13). Reliable measures of bandemia must be correlated with the presence of granulocyte CD16<sup>dim</sup>/CD10<sup>dim</sup> and clinical outcomes in septic populations. Third, whether flow cytometry is practical for clinical use in sepsis remains to be seen. Immunophenotyping has historically suffered from lack of standardization and reproducibility (14, 15). Standardized assays are being developed and should be applied in future research. Furthermore, although flow cytometry is increasingly available in clinical laboratories, it will be difficult to use as a point-of-care test. In addition, further study will be required to determine whether flow cytometry can provide additive prognostic information over other accurate, potentially more cost-effective measures of bandemia.

Finally, despite intriguing in vitro evidence, it is not clear that CD14<sup>neg</sup>/CD24<sup>pos</sup> immature granulocytes cause T-cell death in vivo, and the association between CD16<sup>dim</sup> and T-cell lymphopenia could be due to separate distal effects inherent in a more severely ill population. A detailed mechanistic approach determining the link between immature granulocytes and immunosuppression is warranted and, if established, could provide a potential therapeutic target in the future.

We therefore return to our title: “From storm to suppression in sepsis: Are bands the link?” The answer is that we do not yet know, but the current work by Guerin et al (9) provides intriguing leads for additional study to eventually answer this important question.

REFERENCES