CLINICAL IMPLICATIONS OF BASIC RESEARCH

Sepsis and Endothelial Permeability

Warren L. Lee, M.D., Ph.D., and Arthur S. Slutsky, M.D.

Sepsis, derived from the Greek verb *sepo* (meaning "I rot"), has been recognized for millennia and refers to the disseminated inflammatory response elicited by microbial infections. Despite its ancient etymology, sepsis remains a current challenge: it is increasing in frequency, expensive to treat, and lethal, with an associated rate of death as high as 70%.[1] Despite intensive research over decades, few new therapies have been developed, and the mainstay of treatment remains nonspecific supportive care. Indeed, sepsis has been described as the "graveyard" of pharmaceutical discovery[2] because most drugs that appeared promising on the basis of in vitro and animal models have proved to be ineffective in humans. This failure to develop new therapies suggests that our understanding of sepsis in humans is inadequate.

Research into the pathogenesis of sepsis has traditionally focused on leukocytes. The current thinking is that sepsis leads to a state of immunosuppression characterized by lymphocyte apoptosis and susceptibility to nosocomial infections. However, clinicians also know that progressive subcutaneous and body-cavity edema typically develops in patients with sepsis, suggesting widespread increases in vascular permeability. Tissue edema is not benign: the accumulation of parenchymal and interstitial fluid could impair organ function by increasing the distance required for the diffusion of oxygen and by compromising microvascular perfusion because of increased interstitial pressure. It is no coincidence that a well-recognized feature of recovery from septic shock is a spontaneous diuresis with reduction in edema, consistent with restoration of vascular integrity. Given that all blood vessels are lined with endothelial cells, vascular leak and tissue edema in sepsis suggest endothelial dysfunction. Indeed, it is easy to imagine how widespread damage to the microvascular endothelium could predispose not only to vascular leak and edema, but also to shock, microvascular thrombosis, and organ failure — common events preceding death in patients with severe sepsis. Yet, it is not clear whether endothelial damage or dysfunction and the resultant edema of sepsis are simply epiphenomena or are important from a pathophysiological standpoint. Resolving this issue is crucial. If the loss of endothelial integrity contributes to the disease and death associated with sepsis, understanding the underlying mechanisms might lead to new therapeutic approaches.

A study by London and colleagues[3] has shed light on this issue. They were interested in the role of a signaling pathway involving the Slit and Robo proteins in the regulation of vascular permeability. Although the Slit protein and its receptor, Robo, were implicated in neuronal development, a couple of years ago, the same investigators found that these proteins also have a role in angiogenesis. In their recent study, London et al. showed that recombinant Slit can attenuate the endothelial permeability caused by endotoxin activity and cytokines in vitro. This protective effect required the Robo4 receptor, and the effect appeared to be mediated by enhanced localization to the cell membrane of a key adhesion molecule known as VE-cadherin.

VE-cadherin is the major component of adherens junctions, tightly regulated protein complexes that join adjacent endothelial cells and prevent leukocyte emigration and vascular leak. The displacement of VE-cadherin from the cell membrane to the interior of the cell is sufficient to induce gaps between endothelial cells, leading to increased permeability. This removal of VE-cadherin is normally prevented by another protein, p120-catenin, which binds to and stabilizes VE-cadherin at the membrane. Inflammatory mediators such as vascular endothelial growth factor are known to cause p120-catenin and VE-cadherin to dissociate, leading to internalization of VE-cadherin. London et al. found that the Slit protein prevents this dissociation (Fig. 1).

Most importantly, the ability of Slit to reduce the incidence of vascular leaks was confirmed in three different mouse models of infection, in-

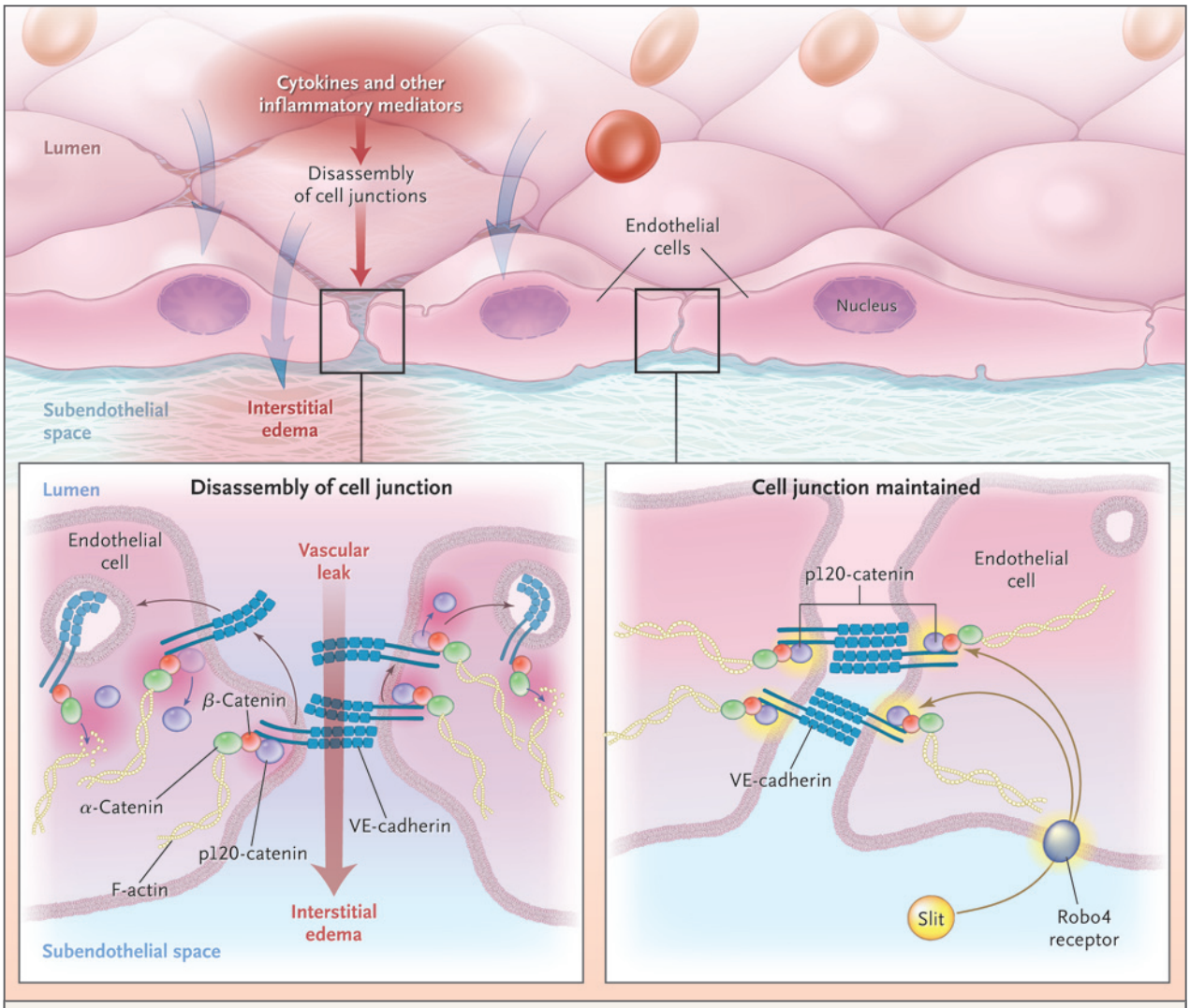

Figure 1. Elucidating Edema.

Cytokines and other inflammatory mediators induce gaps between endothelial cells by disassembly of intercellular junctions, by altering the cellular cytoskeletal structure, or by directly damaging the cell monolayer. This creation of gaps can result in microvascular leak and tissue edema, which are characteristic of sepsis. A study by London et al.[3] showed that by binding the Robo4 receptor, the Slit protein prevents the dissociation of p120-catenin from VE-cadherin in response to inflammatory mediators, with the result that VE-cadherin remains on the plasma membrane. Thus, the disassembly of intercellular junctions is prevented, and barrier integrity is maintained. A potential therapeutic approach would be to stabilize or enhance Slit-mediated signaling through the Robo4 receptor. Another approach would be to directly stabilize the association between p120-catenin and VE-cadherin.

cluding infection with intratracheal endotoxin (a model of gram-negative bacterial pneumonia), cecal ligation and perforation (a clinically relevant model of intraabdominal sepsis), and infection with the highly virulent avian influenza (H5N1) virus. Crucially, intravenous injection of Slit before the induction of disease dramatically reduced the mortality among infected animals. Remarkably, treatment with Slit did not lower the elevated levels of lung and serum cytokines among infected animals, indicating that its enhancement of vascular integrity and reduction in mortality were accomplished even in animals with continued widespread inflammation. Furthermore, its beneficial effects in multiple animal models suggest that preventing or ameliorating vascular leak may be a widely applicable therapeutic strategy for sepsis.

The broader implication of this study, the results of which require confirmation in humans,

is that the endothelium is critical to the vascular leak and subsequent shock that contribute to mortality among patients with sepsis. The study by London et al. is only one of several that have begun to investigate the pivotal role of the microvascular barrier as a therapeutic target in sepsis.[4,5] An understanding of the function of the endothelial barrier, endothelial repair, and endothelial regeneration may one day lead to new therapies. For frustrated pharmaceutical companies, for clinicians, and most importantly for patients with sepsis, this day cannot come a moment too soon.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

From the Keenan Research Center of the Li Ka Shing Knowledge Institute, St. Michael's Hospital; and the Divisions of Respirology and Critical Care Medicine, University of Toronto — both in Toronto.

1. Russell JA. Management of sepsis. N Engl J Med 2006;355:1699-713. [Erratum, N Engl J Med 2006;355:2267.]
2. Riedemann NC, Guo RF, Ward PA. The enigma of sepsis. J Clin Invest 2003;112:460-7.
3. London NR, Zhu W, Bozza FA, et al. Targeting Robo4-dependent slit signaling to survive the cytokine storm in sepsis and influenza. Sci Transl Med 2010;2:23ra19.
4. Ye X, Ding J, Zhou X, Chen G, Liu SF. Divergent roles of endothelial NF-kappaB in multiple organ injury and bacterial clearance in mouse models of sepsis. J Exp Med 2008;205:1303-15. [Erratum, J Exp Med 2008;205:1509.]
5. Gröger M, Pasteiner W, Ignatyev G, et al. Peptide Bbeta(15-42) preserves endothelial barrier function in shock. PLoS One 2009;4(4):e5391.