Commentary

A Most Important Annexation

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While extensive efforts have focused on deciphering molecular mechanisms that activate pro-inflammatory signaling pathways thought to drive sepsis, relatively little is known regarding the endogenous mechanisms that normally serve to prevent runaway inflammation. Thus, the finding by Damazo et al¹ in this issue of *The American Journal of Pathology* that annexin 1 plays a protective role in systemic inflammatory responses provides insight into one such counter-regulatory mechanism and has implications for novel treatment strategies.

Sepsis and the systemic inflammatory response syndrome (SIRS) result from generalized activation of the immune system associated with well-known clinical symptoms (fever, tachycardia, leukocytosis, etc) and an inability to regulate such responses.^{2,3} Despite more than two decades of research, sepsis and SIRS remain the leading cause of death among critically ill patients with mortality rates reported between 30 and 70%.³ Sepsis and SIRS appear to be driven in large part by host responses to pathogen-derived molecules, often referred to as pathogen-associated molecular patterns (PAMPs), such as endotoxin (lipopolysaccharide; LPS), flagellin, and peptidoglycan. Such PAMPs trigger intracellular signaling events through interactions with pattern recognition receptors such as Toll-like receptors.⁴ Expressed on a variety of cell types, these pattern recognition receptors induce changes in gene expression that result in the release of an extensive panel of soluble mediators and up-regulation of surface expression of a variety of cell adhesion molecules. Some such mediators include cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1, and IL-6, which can themselves recapitulate some of the symptoms of SIRS (eg, fever), as well as others, such as IL-8 and intercellular adhesion molecule-1, that are mechanistically known to drive some of the histopathological events associated with SIRS.

The end result of these PAMP-driven changes in host gene expression is the activation and emigration of leukocytes to inflammatory tissues.⁵ The release of granular

enzymes and production of reactive oxygen species by leukocytes play a pivotal role in combating infectious processes. However, leukocyte activity also contributes to the development of tissue damage that can lead to increased vascular permeability and organ injury, potentially progressing to multiorgan failure and death.⁶ It is not yet clear whether these immunological events represent a heroic, albeit futile, last chance effort to ward off the infecting pathogen and/or a microbial strategy to obfuscate the host immune system. Regardless, it is important to note the clinically relevant point that once SIRS has been initiated, it displays strong persistence, generally resulting in the death of the host even if antibiotic therapy has been successful at clearing the offending pathogen(s). Therefore, it remains a major challenge to develop therapies aimed at down-regulating the inflammatory response to be used in combination with antibiotics to treat sepsis.

In light of this view that the “hyperactivity” of the pro-inflammatory response is the primary mechanism driving SIRS, most therapeutic interventions have been aimed at blocking pro-inflammatory mediators or the microbial stimuli driving activation of the immune response, particularly LPS. However, clinical trials of these drugs have failed to make a significant impact on the outcome of sepsis and SIRS. For example, studies targeting mediators such as TNF- α have resulted in worse outcomes, suggesting that they also play a protective role.^{6–8} A possible mechanism underlying this phenomenon is that activation of pro-inflammatory gene expression is generally accompanied by activation of anti-inflammatory mediators that may be needed to down-regulate inflammatory responses that are appropriately activated in response to perturbing pathogens. While some of these anti-inflammatory mediators such as IL-10, IL-13, and TGF- β have been defined,^{5,9} the fact that pro- and anti-inflammatory mediators are often induced with similar kinetics and generally have a complex array of bioactiv-

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ities in different tissues can make it difficult to discern whether the induction of a particular gene is beneficial or detrimental to the septic host.

The relatively recent development of methods to genetically engineer mice to express or lack specific genes has substantially improved abilities to mechanistically dissect the role of individual genes. This notion is exemplified in the paper by Damazo et al¹ in this issue of the *Journal*, which examines the role of the endogenous anti-inflammatory protein annexin 1 in experimental endotoxemia. Annexin 1 is a 37-kD protein identified as a glucocorticoid-inducible element with inhibitory actions on phospholipase A2 function.^{10,11} Annexin 1 is expressed in a variety of cell types, and, since its discovery, it has been shown to play important roles in diverse cellular functions including membrane trafficking, cell division, and differentiation.¹¹ Annexin 1 had also been suggested to have anti-inflammatory properties that were attributed to its inhibition of phospholipase A2 activity and thus production of eicosanoids. However, annexin 1 was also found to exhibit a profound regulatory role in leukocyte adhesion and transmigration across endothelia.^{12–15} Expression of annexin 1 on the surface of leukocytes, up-regulated by glucocorticoid treatment, impairs the ability of leukocytes to adhere to endothelia and thus affects their recruitment to sites of tissue injury.¹⁶ Annexin 1 has also been shown to mediate the response of other cell types in the innate immune system, such as epithelial cells, to glucocorticoids. However, up until now, little was known about the spatial and temporal regulation of annexin 1 expression and the significance of its expression in SIRS.

Damazo et al¹ used mice in which the annexin 1 gene was replaced with a “reporter” gene, thus making it possible to analyze both the regulation of annexin 1 expression and its role in endotoxemia. They observed that after LPS challenge, annexin 1 gene expression is turned on in a variety of organs including the liver, lung, and kidney. While such expression of annexin 1 could be consistent with driving or down-regulating inflammation, wild-type mice challenged with LPS exhibited only a controlled and steady leukocyte extravasation. However, mice lacking functional annexin 1 exhibited a rapid, marked activation of leukocytes that occurred within the microvasculature compartment and resulted in organ injury, thus suggesting a counter-regulatory role for this protein. Moreover, that annexin 1 null mice exhibited a substantially prolonged and more intense elevation of cytokines and plasma markers of organ injury that was accompanied by death indicates a critical protective role for annexin 1 in endotoxemia. While most studies of sepsis have generally focused on leukocytes, it is interesting that multiple organ systems and cell types, notably epithelial cells, expressed increased levels of annexin 1 in response to LPS challenge, influencing the degree of organ injury. This suggests that annexin 1 function is of major importance in regulating innate immune responses in these somatic cells. This finding not only provides insight into counter-regulatory mechanisms in systemic inflammatory responses but also highlights the potential importance of such mechanisms in the outcome of SIRS.

While the mechanism by which annexin 1 expression results in lessening LPS-induced inflammation and ultimately protects against sepsis is not yet clear, the elevated responses of the annexin 1-deficient mice to LPS could be significantly reduced by administration of recombinant annexin 1. This suggests that the hyperinflammatory response is not due to inherent defects in annexin 1-deficient cells but rather may result from the inability of the deficient cells to activate anti-inflammatory signaling events on cells expressing receptors for annexin 1. Consistent with this concept, recent reports suggest that anti-inflammatory effects of annexin 1 may be mediated by the lipoxin A₄ receptor, ALXR,^{15,17} previously termed formyl-peptide like receptor 1. Originally defined as the high affinity receptor that mediates the anti-inflammatory effects of some members of the lipoxin family of eicosanoids, it has since been found to be a relatively “promiscuous” receptor with several reported seemingly unrelated ligands albeit at much lesser affinities than seen for lipoxin A₄.¹⁸ Thus, it is intriguing to consider the idea that anti-inflammatory effects of annexin 1 may also be mediated by this G-protein coupled receptor as it suggests some commonality in mechanism between these two endogenous anti-inflammatory pathways.

While this study by Damazo et al¹ appears to represent a major stride toward developing effective treatments for sepsis, additional basic research should help further this possibility. For example, as the annexin 1 gene expression assayed in their study was performed in mice lacking functional annexin 1, it will be important to define annexin 1 expression in mice with normal annexin 1 levels and thus not subject to dysregulated pro-inflammatory gene expression. It will also be important to determine whether annexin 1 plays an important role in host defense and thus whether annexin 1 treatment might be likely to worsen some instances of SIRS. Another important consideration will be to determine whether annexin 1 expression is limiting in inflammation in terms of whether exogenous annexin 1 can further enhance the anti-inflammatory activity of endogenous annexin 1. Lastly, it will be essential to determine whether these mechanisms are applicable to humans. Nonetheless, the description of annexin 1 expression and its critically protective role in model sepsis by Damazo et al¹ is a major advance in understanding endogenous down-regulation of inflammation and may ultimately lead to novel therapies to treat this deadly problem.

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