Commentary

Neuropeptide Modulators of High Mobility Group Box 1 Secretion as Potential Therapeutic Agents for Severe Sepsis

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Sepsis is a potentially life-threatening condition caused by infection and characterized by a poorly controlled systemic inflammatory response.¹ Severe sepsis is sepsis with the evidence of acute organ system dysfunction, and septic shock is sepsis associated with systemic arterial hypotension.¹ Severe sepsis remains a huge public health problem. In the United States alone, epidemiological data suggest that there are more than 600,000 cases annually^{2,3} and more than 200,000 people die every year from severe sepsis and its complications.³ In this issue of *The American Journal of Pathology*, Chorny and Delgado⁴ report on the results of a series of studies that suggest that treatment with endogenous neuropeptides, either vasoactive intestinal peptide or urocortin, can improve survival in a clinically relevant animal model of lethal sepsis. Treatment of septic mice with vasoactive intestinal peptide or urocortin decreased circulating levels of high mobility group box 1, a small protein that has been implicated as being an important mediator of sepsis.

Originally identified in the early 1960s,⁵ high mobility group (HMG) proteins have been isolated and characterized from a wide variety of eukaryotic species, ranging from yeast to humans.⁶ HMG proteins all have an unusual amino acid composition characterized by a high content of charged amino acids and a high content of proline.⁷ One member of this family of proteins, high mobility group box 1 (HMGB1), has a molecular mass of ~28 kDa^{7,8} and is capable of bending DNA by virtue of a conserved DNA binding domain, the HMG1 box.⁹ HMGB1 also facilitates the binding of several regulatory protein complexes to DNA, particularly members of the nuclear hormone-receptor family,^{10,11} V(D)J recombinases,¹² and the tumor suppressor proteins p53 and p73.¹³

In 1999, Wang and colleagues¹⁴ identified HMGB1 as a cytokine-like mediator of lipopolysaccharide (LPS)-in-

duced mortality in mice. Subsequently, these findings were extended by Yang and colleagues,¹⁵ who showed that HMGB1 is also a mediator of lethality in mice rendered septic by the induction of polymicrobial bacterial peritonitis. Additional studies documented that extracellular HMGB1 can promote tumor necrosis factor release from mononuclear cells¹⁶ and increase the permeability of Caco-2 cell monolayers.¹⁷

One of the most interesting features of HMGB1 as a cytokine-like mediator of inflammation is that this protein is released much later in the inflammatory process than are the classical alarm-phase cytokines such as tumor necrosis factor and interleukin-1 β . In mice, for example, injection of a bolus dose of LPS elicits a monophasic spike in circulating tumor necrosis factor that peaks in ~90 minutes of the proinflammatory challenge and is over by 4 hours.¹⁸ The peak in interleukin-1 β concentration occurs somewhat later; ie, 4 to 6 hours after the injection of LPS.¹⁹ In contrast, after injecting mice with LPS, circulating levels of HMGB1 are not elevated until 16 hours after the proinflammatory stimulus but remain elevated for more than 30 hours.¹⁴ Furthermore, treatment with neutralizing anti-HMGB1 antibodies^{14,15} or various pharmacological agents that block HMGB1 secretion, such as nicotine²⁰ or ethyl pyruvate,²¹ is effective in preventing LPS- or sepsis-induced lethality, even when therapy is started 4 to 24 hours after the initiation of the disease process. Because of the delayed release kinetics, HMGB1 is a very attractive drug target for the development of new therapeutic agents for the management of severe sepsis. The treatment window for an anti-HMGB1 therapy should be longer than is the case for therapeutic agents directed at more proximal me-

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diators of the inflammatory cascade (eg, tumor necrosis factor or interleukin-1 β).

HMGB1 is actively secreted by immunostimulated macrophages,^{14,22–24} natural killer cells,²⁵ plasmacytoid dendritic cells,²⁶ pituicytes,²⁷ and enterocytes.²⁸ Like members of the interleukin-1 family of cytokines, the primary amino acid sequence of HMGB1 lacks a signal peptide. Accordingly, secretion of HMGB1 by macrophages or monocytes presumably occurs via a nonclassical secretory pathway. Indeed, when monocytes are activated by exposure to LPS, HMGB1 relocates from the nucleus into cytoplasmic organelles that belong to the endolysosomal compartment.²³

In the past few years, numerous agents have been shown to be capable of blocking HMGB1 secretion by immunostimulated cells, including various nicotinic cholinergic agonists^{20,29}; stearyl lysophosphatidylcholine³⁰; ethyl pyruvate²¹; the serine protease inhibitor nafamostat mesilate³¹; several steroid-like pigments (tanshinone I, tanshinone IIA, and cryptotanshinone), derived from a Chinese medicinal herb, Danshen (*Salvia miltiorrhiza*)³²; and the diuretic ethacrynic acid, as well as other drugs, which are known to be phase 2 enzyme inducers.³³ Now, two endogenous neuropeptides with known anti-inflammatory activities, vasoactive intestinal peptide (VIP) and urocortin, have been shown to inhibit active secretion of HMGB1 by LPS-stimulated murine macrophages.⁴ As is the case with almost all previously identified inhibitors of HMGB1 release from immunostimulated macrophages, both VIP and urocortin interfere in some way with a key step in the secretion process, namely nuclear-to-cytoplasmic translocation of the protein. And, importantly, like several other known inhibitors of HMGB1 secretion,^{20,21,29,30,32} treatment with VIP or urocortin improves survival in mice with lethal sepsis, even when treatment is delayed for many hours after the onset of infection. However, unlike other pharmacological inhibitors of HMGB1 secretion, which have been shown to improve survival in septic mice, VIP and urocortin are endogenous substances. Thus, as pointed out by Chorny and Delgado,⁴ endogenous release of VIP and/or urocortin might serve a counterregulatory role *in vivo* to modulate the proinflammatory effects of HMGB1 secretion. Activation of the cholinergic anti-inflammatory pathway, mediated via increased efferent traffic through the vagus nerve, might serve a similar counterregulatory role.^{34,35}

Treating septic mice with either VIP or urocortin decreases circulating HMGB1 concentration and improves survival. Although it is certainly plausible that these two effects of treatment with VIP or urocortin are mechanistically related, data from the present set of experiments are insufficient to establish this causal linkage. It is conceivable, for example, that administration of the peptides triggers another process, activation of the vagal cholinergic anti-inflammatory pathway comes to mind,^{34,35} and this other process is the proximate cause of the salutary effects observed in septic mice after the administration of VIP or urocortin.

Infusion of an anti-coagulant protein, recombinant human activated protein C, has been shown to improve survival in patients with severe sepsis,³⁶ and this agent

has been approved by regulatory agencies in the United States and elsewhere for the treatment of selected cases of severe sepsis. Nevertheless, the clinical adoption of recombinant human activated protein C has been relatively slow, possibly because of concerns about its cost,³⁷ safety,^{38,39} and/or efficacy.⁴⁰ Accordingly, there remains a need for new and better therapeutic approaches for the management of severe sepsis. One approach worth considering in this regard is the therapeutic administration of the endogenous peptides, VIP or urocortin. VIP infused into normal human volunteers has been shown to cause a range of adverse effects, including diarrhea and tachycardia.⁴¹ Whether these side effects would occur in septic patients, or be tolerable in the context of treating a life-threatening illness, remains to be determined. Infusion of urocortin into normal human volunteers seems to be better tolerated,⁴² and, therefore, this neuropeptide might be a more promising candidate for further development as an adjuvant therapeutic for severe sepsis.

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