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Increases in Soluble Protein Oligomers Trigger the Innate Immune System Promoting Inflammation and Vascular Dysfunction in the Pathogenesis of Sepsis

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Abstract

Sepsis is a profoundly morbid and life-threatening condition, and an increasingly alarming burden on modern healthcare economies. Patients with septic shock exhibit persistent hypotension despite adequate volume resuscitation requiring pharmacological vasoconstrictors, but the molecular mechanisms of this phenomenon remain unclear. The accumulation of misfolded proteins is linked to numerous diseases, and it has been observed that soluble oligomeric protein intermediates are the primary cytotoxic species in these conditions. Oligomeric protein assemblies have been shown to bind and activate a variety of pattern recognition receptors (PRRs) including formyl peptide receptor (FPR). While inhibition of ER stress and stabilization of protein homeostasis have been promising lines of inquiry regarding sepsis therapy, little attention has been given to the potential effects that the accumulation of misfolded proteins may have in driving sepsis pathogenesis. Here we propose that in sepsis there is an accumulation of toxic misfolded proteins in the form of soluble protein oligomers (SPOs) that contribute to the inflammation and vascular dysfunction observed in sepsis via the activation of one or more PRRs including FPR. Our laboratory has shown increased levels of SPOs in the heart and intrarenal arteries of septic mice. We have also observed that exposure of resistance arteries and vascular smooth muscle cells to SPOs is associated with increased MAPK signaling including p-ERK and p-P38 pathways, and that this response is abolished with the knockout of FPR. This hypothesis has promising clinical implications as it proposes a novel mechanism that can be exploited as a therapeutic target in sepsis.

Introduction

Sepsis remains a profoundly morbid and life-threatening condition despite intense research interest, evolution of our understanding of its pathophysiology, and progress in diagnosis

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and goal directed therapy (1). A 2016 Society of Critical Care Medicine and European Society of Intensive Care Medicine task force defined sepsis as “life threatening organ dysfunction caused by dysregulated host response to infection.”(8) The definitions of sepsis have evolved since the early 1990’s, and it is important to note that these are no longer diagnostic nor unanimously accepted. Perhaps in part due to the heterogeneity of the basic mechanisms underlying the septic response, there remains no current therapeutic strategy targeting a specific mechanism that has come into clinical practice despite multiple clinical trials targeting the inflammatory cascade (5, 10). The mainstay of therapy remains early, goal-directed supportive care.

The understanding of the pathophysiology of sepsis remains incomplete to date. The loss of vascular tone due to overzealous vasodilation is a hallmark of sepsis, but the final molecular mediators of this phenomenon remain unclear (13). This occurs at least in part due to endothelial cell damage (12). Another consequence of impaired endothelial function is extravascular fluid leakage leading to severe edema in various organs (12). Vascular dysfunction has been associated with hypoxia, oxidative stress, augmented TNF- α activity, increased nitric oxide production, and increases in prostaglandin and thromboxane A₂ release (14); however, the failure of pharmacologic interventions targeting these pathways suggests that other mechanisms may be responsible for the observed phenomena.

Misfolded Proteins: Lessons from neurodegenerative disease

A wide range of highly stable misfolded protein aggregates are present in a number of age-related degenerative diseases including Alzheimer’s disease (AD) and other neurodegenerative proteinopathies. The accumulation of misfolded proteins is also linked to other disease types such as hypertension and type 2 diabetes (15). A common characteristic of such aggregates is a generic architecture that predisposes these proteins to accumulate into β -pleated sheet conformation (16). The observation that there is little correlation between the quantity of fibrillar, insoluble protein deposits and disease phenotype in organ-specific amyloidoses such as AD has led to the emerging view that it is in fact the smaller, prefibrillar, and soluble oligomer intermediates that are primarily responsible for the observed toxicity (17). The cytotoxicity of soluble oligomers has been observed in a variety of cell types including cardiac myocytes (18, 19). Interestingly, oligomers derived from different proteins share a common structural epitope regardless of amino acid sequence suggesting the possibility of a common pathogenic mechanism and therapeutic target (18, 20).

Putting it together: innate immunity, sepsis, and protein homeostasis

The notion that a misfolded protein no longer resembles a self-protein and is thus subject to recognition by the immune system fits neatly into the self-non-self theory or, as an endogenous molecule, the “danger theory” of immunology. The “danger theory” suggests that immune responses may be triggered by the release of molecules within the body’s own cells acting as “danger signals” rather than merely the presence of “non-self” entities (21–23). Multiple groups have postulated that extracellular and intracellular protein aggregates represent damage-associated molecular patterns (DAMPs) and result in chronic activation of

the innate immune system through pattern recognition receptors (PRRs) (24–27). Amyloid and oligomeric assemblies of amyloidogenic proteins have been shown to bind and activate a variety of PRRs including Toll-like receptors, formyl peptide receptors, receptor for advanced glycation end products, scavenger receptors, complement and pentraxins (25, 26). Chronic activation of innate immune system signaling pathways and sustained exposure of neurons to pro-inflammatory mediators has been shown to cause cellular dysfunction and contribute to cell death in neurodegenerative disorders (28). The observation of chronic inflammation in relatively early stages of disease makes targeting the mechanisms that drive this inflammation potentially promising therapeutic and diagnostic strategies.

An immense number of proteins are required for cellular diversity and survival, and the endoplasmic reticulum (ER) is responsible for the synthesis, maturation, and folding of these proteins (29). Cellular stressors such as hypoxia, infection, inflammation, and aging can lead to the accumulation of misfolded or aggregated proteins in the ER which activate the unfolded protein response (UPR). The UPR is highly conserved and responsible for protein homeostasis. This is accomplished via three primary sensor proteins including protein kinase RNA (PKR)-like ER kinase (PERK), activating transcription factor-6 (ATF-6), and inositol-requiring protein-1 (IRE1) which act to clear out misfolded proteins through ubiquitin-proteasome degradation or targeting the cell for apoptosis (30). These mechanisms are reviewed extensively elsewhere (31, 32), and are mentioned here to provide supporting context to the present hypothesis. In summary, the UPR initiates a series of phosphorylation and translocation events aimed at clearing out the dysfunctional protein or directing the cell to undergo apoptosis if unable to restore protein homeostasis. In the setting of sufficiently severe inflammation and cellular insult, proteostasis systems may be overwhelmed leading to the accumulation of misfolded protein and loss of cellular and tissue integrity.

Specifically, UPR activation and abnormal lymphocyte apoptosis has been demonstrated in a cecal ligation and puncture (CLP) murine model of sepsis, and this contribution of ER stress to abnormal apoptosis has highlighted ER stress as a potential therapeutic target in sepsis-induced cellular injury (33–35). Tissue damage and cell death in sepsis is driven at least in part by uncontrolled inflammation and activation of the innate immune system (33). The inhibition of ER stress by 4-phenylbutyrate (PBA), an ER stress inhibitor, was shown to ameliorate lipopolysaccharide-induced lung inflammation (36). The inhibition of transcription factor CHOP, an inflammatory mediator and major inducer of apoptosis in ER stress, has been associated with improved survival and reduced lung injury in septic mice (37, 38). Activated protein C has been shown to modulate inflammation and apoptosis in monocytes through inhibition of ER stress at least partly explaining its protective effects in sepsis (39). While inhibition of ER stress and stabilization of protein homeostasis continue to be promising lines of inquiry regarding sepsis therapy, little attention has been given to the potential tissue specific and systemic effects that accumulated misfolded proteins may have in driving sepsis pathogenesis.

With this knowledge, we propose that in sepsis there is an accumulation of toxic misfolded proteins in the form of soluble protein oligomers (SPOs) that contribute to the inflammation and vascular dysfunction observed in sepsis via the activation of one or more PRRs. Supporting this notion, several studies have shown that amyloid oligomers can activate a

PRR called formyl peptide receptor (FPR). This receptor belongs to a family of G protein-coupled receptors which are activated by chemotactic peptides and expressed at high levels in neutrophils and monocytes (40–42). Previous work from our laboratory has shown FPR expression in the heart and mesenteric resistance arteries, and that FPR activation is associated with vascular leakage and hyporesponsiveness to adrenergic receptor agonists in resistance arteries which may contribute to the loss of vascular tone observed in the development of sepsis (43, 44). Therefore, we hypothesize that soluble protein oligomers accumulate in sepsis and contribute to inflammation and vascular dysfunction via FPR activation. (Figure 1)

Preliminary data from our laboratory, using a cecal ligation and puncture (CLP) murine model of sepsis, has shown increased levels of SPO in the heart and intrarenal arteries of septic mice (45). This was accomplished using a commercially available polyclonal antibody (Oligomer A11 Polyclonal Antibody, Thermofisher AHB0052) that recognizes the structural epitope common to all SPOs regardless of their amino acid sequence (15). To the best of our knowledge, we are the first to show the presence of or increase in SPOs in the context of sepsis. We have also observed increased expression of the pro-inflammatory mitogen-activated protein kinase (MAPK) signaling pathways p-ERK in healthy tissue and p-P38 in vascular smooth muscle cells exposed to SPO (unpublished data). This response was abolished in FPR knockout vascular smooth muscle cells (unpublished data).

Conclusion

Based on our findings and lessons from neurodegenerative disease suggesting a common structure and mechanisms of toxicity of soluble protein oligomers regardless of their native protein, it is reasonable to speculate that SPOs contribute to sepsis pathogenesis by promoting inflammation through FPR signaling and activation of the innate immune system as has been shown in Alzheimer's disease (25). This hypothesis has promising clinical implications in that it proposes a novel mechanism in the pathophysiology of sepsis, which can be further evaluated as a specific therapeutic target. The loss of control of vascular tone is a major pathophysiological characteristic of sepsis and improved understanding of the mechanisms underlying this dysfunction provides a foundation for the development of targeted therapies. Many questions arise for future direction. Do SPOs affect blood pressure regulation in sepsis? Do SPOs modulate the actions of other vasoactive molecules released during the septic response or contribute to vasopressor resistance? Can SPOs be used as a marker for sepsis development and/or resolution? Further investigations to test and elaborate on our hypothesis will generate a new understanding about the role of soluble protein oligomers in the pathogenesis of sepsis and may provide a link between sepsis and its devastating consequences that can be targeted in the development of novel therapeutic strategies.

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Abbreviations:

PRR	pattern recognition receptor
FPR	formyl peptide receptor
MAPK	mitogen-activated protein kinase
p-ERK	phosphorylated extracellular signal-regulated kinase
p-P38	phosphorylated P38 mitogen-activated protein kinase
CARS	compensatory anti-inflammatory response syndrome
MARS	highly mixed anti-inflammatory response syndrome
AD	Alzheimer's disease
DAMP	damage-associated molecular pattern
ER	endoplasmic reticulum
UPR	unfolded protein response
PERK	protein kinase RNA (PKR)-like ER kinase
ATF-6	activating transcription factor-6
IRE-1	inositol-requiring protein-1
CLP	cecal ligation and puncture
CHOP	C/EBP homologous protein
SPO	soluble protein oligomer

References

1. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *The New England journal of medicine*. 2001;345(19):1368–77. [PubMed: 11794169]
2. Pfuntnr A, Wier LM, Steiner C. Costs for Hospital Stays in the United States, 2011: Statistical Brief #168 Healthcare Cost and Utilization Project (HCUP) Statistical Briefs. Rockville (MD): Agency for Healthcare Research and Quality (US); 2006.
3. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, Kurosawa S, Stepien D, Valentine C, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiological reviews*. 2013;93(3):1247–88. [PubMed: 23899564]
4. Kumar G, Kumar N, Taneja A, Kaleekal T, Tarima S, McGinley E, et al. Nationwide trends of severe sepsis in the 21st century (2000–2007). *Chest*. 2011;140(5):1223–31. [PubMed: 21852297]
5. Prescott HC, Costa DK. Improving Long-Term Outcomes After Sepsis. *Critical care clinics*. 2018;34(1):175–88. [PubMed: 29149939]
6. Longo CJ, Heyland DK, Fisher HN, Fowler RA, Martin CM, Day AG. A long-term follow-up study investigating health-related quality of life and resource use in survivors of severe sepsis: comparison of recombinant human activated protein C with standard care. *Critical care (London, England)*. 2007;11(6):R128.

7. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *The New England journal of medicine*. 2003;348(16):1546–54. [PubMed: 12700374]
8. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):762–74. [PubMed: 26903335]
9. Osuchowski MF, Craciun F, Weixelbaumer KM, Duffy ER, Remick DG. Sepsis chronically in MARS: systemic cytokine responses are always mixed regardless of the outcome, magnitude, or phase of sepsis. *Journal of immunology (Baltimore, Md : 1950)*. 2012;189(9):4648–56.
10. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *The New England journal of medicine*. 2003;348(2):138–50. [PubMed: 12519925]
11. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):801–10. [PubMed: 26903338]
12. Kotsovolis G, Kallaras K. The role of endothelium and endogenous vasoactive substances in sepsis. *Hippokratia*. 2010;14(2):88–93. [PubMed: 20596262]
13. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nature medicine*. 2003;9(5):517–24.
14. Peters K, Unger RE, Brunner J, Kirkpatrick CJ. Molecular basis of endothelial dysfunction in sepsis. *Cardiovascular research*. 2003;60(1):49–57. [PubMed: 14522406]
15. Sidorova TN, Mace LC, Wells KS, Yermalitskaya LV, Su PF, Shyr Y, et al. Hypertension is associated with preamyloid oligomers in human atrium: a missing link in atrial pathophysiology? *Journal of the American Heart Association*. 2014;3(6):e001384. [PubMed: 25468655]
16. Knowles TP, Vendruscolo M, Dobson CM. The amyloid state and its association with protein misfolding diseases. *Nature reviews Molecular cell biology*. 2014;15(6):384–96. [PubMed: 24854788]
17. Klein WL, Krafft GA, Finch CE. Targeting small Abeta oligomers: the solution to an Alzheimer's disease conundrum? *Trends in neurosciences*. 2001;24(4):219–24. [PubMed: 11250006]
18. Guerrero-Munoz MJ, Castillo-Carranza DL, Kaye R. Therapeutic approaches against common structural features of toxic oligomers shared by multiple amyloidogenic proteins. *Biochemical pharmacology*. 2014;88(4):468–78. [PubMed: 24406245]
19. McLendon PM, Robbins J. Desmin-related cardiomyopathy: an unfolding story. *American journal of physiology Heart and circulatory physiology*. 2011;301(4):H1220–8. [PubMed: 21784990]
20. Glabe CG, Kaye R. Common structure and toxic function of amyloid oligomers implies a common mechanism of pathogenesis. *Neurology*. 2006;66(2 Suppl 1):S74–8. [PubMed: 16432151]
21. Tolerance Matzinger P., danger, and the extended family. *Annual review of immunology*. 1994;12:991–1045.
22. Essay Matzinger P. 1: the Danger model in its historical context. *Scandinavian journal of immunology*. 2001;54(1–2):4–9. [PubMed: 11439142]
23. Matzinger P The danger model: a renewed sense of self. *Science (New York, NY)*. 2002;296(5566):301–5.
24. Golde TE, Miller VM. Proteinopathy-induced neuronal senescence: a hypothesis for brain failure in Alzheimer's and other neurodegenerative diseases. *Alzheimer's research & therapy*. 2009;1(2):5.
25. Salminen A, Ojala J, Kauppinen A, Kaarniranta K, Suuronen T. Inflammation in Alzheimer's disease: amyloid-beta oligomers trigger innate immunity defence via pattern recognition receptors. *Progress in neurobiology*. 2009;87(3):181–94. [PubMed: 19388207]
26. McGeer PL, McGeer EG. Innate immunity, local inflammation, and degenerative disease. *Science of aging knowledge environment : SAGE KE*. 2002;2002(29):re3. [PubMed: 14602998]
27. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. *Neurobiology of aging*. 2000;21(3):383–421. [PubMed: 10858586]
28. Heneka MT, Kummer MP, Latz E. Innate immune activation in neurodegenerative disease. *Nature reviews Immunology*. 2014;14(7):463–77.

29. Caetano-Anolles G, Wang M, Caetano-Anolles D, Mittenthal JE. The origin, evolution and structure of the protein world. *The Biochemical journal*. 2009;417(3):621–37. [PubMed: 19133840]
30. Tabas I, Ron D. Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. *Nature cell biology*. 2011;13(3):184–90. [PubMed: 21364565]
31. Walter P, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science (New York, NY)*. 2011;334(6059):1081–6.
32. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. *Nature*. 2008;454(7203):455–62. [PubMed: 18650916]
33. Khan MM, Yang WL, Wang P. ENDOPLASMIC RETICULUM STRESS IN SEPSIS. *Shock (Augusta, Ga)*. 2015;44(4):294–304.
34. Ma T, Han L, Gao Y, Li L, Shang X, Hu W, et al. The endoplasmic reticulum stress-mediated apoptosis signal pathway is involved in sepsis-induced abnormal lymphocyte apoptosis. *European surgical research Europäische chirurgische Forschung Recherches chirurgicales europeennes*. 2008;41(2):219–25. [PubMed: 18509246]
35. Ma T, Han L, Hu WQ. [Study of the role of endoplasmic reticulum stress mediated apoptosis signal pathway in sepsis-induced splenic lymphocyte apoptosis]. *Zhongguo wei zhong bing ji jiu yi xue = Chinese critical care medicine = Zhongguo weizhongbing jijiuyixue*. 2009;21(1):48–50. [PubMed: 19141193]
36. Kim HJ, Jeong JS, Kim SR, Park SY, Chae HJ, Lee YC. Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-kappaB/HIF-1alpha signaling pathway. *Scientific reports*. 2013;3:1142. [PubMed: 23359618]
37. Ferlito M, Wang Q, Fulton WB, Colombani PM, Marchionni L, Fox-Talbot K, et al. Hydrogen sulfide [corrected] increases survival during sepsis: protective effect of CHOP inhibition. *Journal of immunology (Baltimore, Md : 1950)*. 2014;192(4):1806–14.
38. Endo M, Oyadomari S, Suga M, Mori M, Gotoh T. The ER stress pathway involving CHOP is activated in the lungs of LPS-treated mice. *Journal of biochemistry*. 2005;138(4):501–7. [PubMed: 16272146]
39. Toltl LJ, Austin RC, Liaw PC. Activated protein C modulates inflammation, apoptosis and tissue factor procoagulant activity by regulating endoplasmic reticulum calcium depletion in blood monocytes. *Journal of thrombosis and haemostasis : JTH*. 2011;9(3):582–92. [PubMed: 21166994]
40. Cui YH, Le Y, Zhang X, Gong W, Abe K, Sun R, et al. Up-regulation of FPR2, a chemotactic receptor for amyloid beta 1–42 (A beta 42), in murine microglial cells by TNF alpha. *Neurobiology of disease*. 2002;10(3):366–77. [PubMed: 12270697]
41. Le Y, Gong W, Tiffany HL, Tumanov A, Nedospasov S, Shen W, et al. Amyloid (beta)42 activates a G-protein-coupled chemoattractant receptor, FPR-like-1. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2001;21(2):Rc123. [PubMed: 11160457]
42. Tiffany HL, Lavigne MC, Cui YH, Wang JM, Leto TL, Gao JL, et al. Amyloid-beta induces chemotaxis and oxidant stress by acting at formylpeptide receptor 2, a G protein-coupled receptor expressed in phagocytes and brain. *The Journal of biological chemistry*. 2001;276(26):23645–52. [PubMed: 11316806]
43. Wenceslau CF, McCarthy CG, Gouloupoulou S, Szasz T, NeSmith EG, Webb RC. Mitochondrial-derived N-formyl peptides: novel links between trauma, vascular collapse and sepsis. *Medical hypotheses*. 2013;81(4):532–5. [PubMed: 23890799]
44. Wenceslau CF, McCarthy CG, Szasz T, Gouloupoulou S, Webb RC. Mitochondrial N-formyl peptides induce cardiovascular collapse and sepsis-like syndrome. *American journal of physiology Heart and circulatory physiology*. 2015;308(7):H768–77. [PubMed: 25637548]
45. Komic A, Wenceslau CF, Martinez-Quinones P, McCarthy CG, Ogbi S, Webb RC. Increased Soluble Protein Oligomer in Sepsis is Associated with the Induction of Pro-inflammatory Signal Transduction in Intra-renal Arteries. *The FASEB Journal*. 2018;32(1_supplement):843.30–30.

Figure 1.

Accumulation of soluble protein oligomers from impaired protein homeostasis in sepsis contributes to dysregulation of host immune response, vascular collapse, and organ dysfunction in sepsis via activation of formyl peptide receptor (FPR).