Editorial **Open Access**

Innate Immunity and Sepsis: MyD88 as a Target for Therapeutics

Kamal U. Saikh

Department of Immunology, U.S Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Frederick, MD 21702

Sepsis is one of the most challenging health problems worldwide. In the USA alone, around three-quarters of a million cases occur every year, and the fatality rate remains between 30% and 50% [1]. A recent report indicates that between 1999 and 2005, sepsis contributed to 6% of all deaths in the United States [2]. Treating sepsis remains problematic, as no effective anti-sepsis drug is currently available.

Sepsis is associated with the activation of multiple inflammatory pathways within the host, including the cytokine network and coagulation system. It is a life-threatening disorder resulting from overwhelming systemic inflammatory responses commonly triggered by bacterial infections. Generally, host responses to bacterial infections are mediated by the innate immune system, which recognizes pathogens or pathogen-associated molecular patterns (PAMPs). This leads to secretion of pro- inflammatory cytokines and other mediators that promote the elimination of infectious agents and the initiation of tissue repair [3]. However, excessive production of inflammatory mediators or other pathogen-derived factors cause dys-regulation of the innate immune responses. This aberration of host-directed pro-inflammatory response is triggered after exposure to gram-positive or gram-negative bacterial infections. This deviant hyper-production of pro-inflammatory cytokines and other factors can be due to excessive signaling over an extended period of time. The resulting systemic inflammation triggers the pathological outcomes of sepsis, such as septic shock, toxic shock syndrome (which often leads to systemic vascular leakage), tissue injury, multi-organ failure and death [4,5].

Both pro- and anti-inflammatory events may occur during sepsis [6]. However, sepsis is most commonly associated with an early phase of excessive systemic inflammation mediated by various pro-inflammatory mediators of the innate immune system (e.g., pro-inflammatory cytokines). Shortly after this initial phase, if there is survival beyond the initial phase, counter regulatory (compensatory anti-inflammatory response) pathways become activated and include anti-inflammatory cytokine release and a refractory state in which immune cells have a decreased capacity to produce pro-inflammatory cytokines. This later phase is accompanied by immunodepression or "immunoparalysis" that could leave patients more susceptible to secondary nosocomial infections and late mortality. Despite the fact that both pro- and anti-inflammatory events occur during sepsis, research on sepsis has been dominated by the assumption that this syndrome results from an early-phase excessive inflammation. This is generated by massive activation of an inflammatory cascade (excessive systemic inflammation) mediated by various pro-inflammatory mediators of the innate immune system that contribute to fulminant sepsis and death. In line with this hypothesis, it's feasible to assume that a host intracellular component predominantly regulates the early pro-inflammatory response and that this intracellular component would be a potential target for therapeutic intervention. Thus, a strategy in which the molecular component that regulates the inflammatory signaling is targeted as a means to block pro-inflammatory signaling would consequently be expected to prevent hyper-inflammation and sepsis.

Myeloid Differentiation primary response protein 88 (MyD88) is a common adaptor protein that functions to recruit signaling proteins to the several Toll-like receptors and interleukin-1 receptors (TLR/IL-1R) [7,8], as well as interferon (IFN) -γ receptor [9]. Recent results from our laboratory demonstrate that MyD88-mediated pro-inflammatory signaling is activated after staphylococcal enterotoxin (SEB) binding to MHC class II [10] and that MyD88$^{-/-}$ mice are resistant to SEB intoxication [11,12]. In addition, MyD88 gene silencing in primary human cells via siRNA prevents SEB or SEB plus lipopolysaccharide (LPS) induction of IL-1β transcriptional activation suggesting that MyD88-mediated signaling is an essential component of SEB toxicity [13], as an excessive production of IL-1β drives systemic inflammation and lies at the center of the inflammatory response [14]. Consistent with our results, a recent report also indicated that deficiency in MHC class II impairs TLR-triggered production of pro-inflammatory cytokines and protects mice from lethal challenge with TLR ligands and gram-negative bacteria [15], whereas expression of MHC class II molecules contributes to LPS responsiveness and enhances Toll-like receptor mediated innate immune responses [16,17]. These results suggest that TLR-and MHC –mediated responses both engage MyD88 [18]. Thus, in the context of attenuating the inflammatory response against gram-positive and gram-negative infections, a wider role for MyD88 is beginning to emerge that arguably defines an important opportunity for MyD88-targeted therapeutic intervention of sepsis. In fact, recent results from our laboratory demonstrate that a synthetic small molecule mimetic of the conserved BB-loop [(F/Y)-(V/L/I)-(P/G)] in the Toll/IL-1 receptor (TIR) domain of MyD88 attenuates SEB-induced pro-inflammatory cytokine production in human primary cells. Administration of a BB-loop mimetic to mice increases survivability by reducing cytokine responses following a lethal SEB challenge [13].

The most potent microbial products implicated in septic shock pathogenesis are gram-positive-derived superantigenic exotoxins and gram-negative endotoxin. The interaction of endotoxin with superantigens is highly significant in several animal models. For example, it has been reported that SEB potentiates LPS-induced hepatic dysfunction and cytokine responses in chronically catheterized rats [19]. Results from our laboratory and others firmly established that LPS and SEB have a dose-dependent synergistic effect on toxicity in mice versus SEB or LPS alone [20-22]. Both types of toxin induce comparable pro-inflammatory cytokines from human mononuclear cells *in vitro*, cause lethal shock *in vivo*, and have been identified in the bloodstream of critically ill patients [23,24]. Additionally, these

Corresponding author: Kamal U. Saikh, Department of Immunology, U.S Army Medical Research Institute of Infectious Diseases, USA; Tel: (301) 619-4807; Fax: (301)619-6724; E-mail: kamal.saikh@amedd.army.mil

results also raise the possibility that recognition of SEB by MHC class II receptors may exacerbate the pro-inflammatory response of monocytes to gram-negative infection or endotoxin through activation of a common MyD88-mediated signaling. In clinical scenarios, gram-negative/gram-positive co-infections likely lead to worse outcomes than sepsis generated by gram-positive or gram-negative alone. In both cases, from a therapeutic perspective of sepsis, targeting MyD88 –mediated signaling would be particularly pertinent. Thus, a MyD88-targeted therapy that utilizes small-molecule inhibitors to modify early pro-inflammatory cytokine signaling would be a potential approach in limiting hyper-inflammation and treating sepsis.

Sepsis may not be attributed solely to an uncontrolled inflammatory response but may indicate an immune system that is severely compromised and unable to eradicate pathogens. Clearly, this is a dangerous scenario for the host. Therefore, measurement of circulating concentrations of inflammatory mediators may prove useful in evaluating the sepsis stage and tailoring anti-inflammatory agents useful in MyD88 targeted therapy. Alternatively, use of anti-inflammatory therapeutics that target MyD88 may not be beneficial during the hypo-immune phase, but instead worsen the outcome.

References

1. Martin GS, Mannino DM, Eaton S, Moss M (2003) The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 348: 1546-1554.

2. Melamed A, Sorvillo FJ (2009) The burden of sepsis-associated mortality in the United States from 1999 to 2005: an analysis of multiple-cause-of-death data. Crit Care 13: 28.

3. Puneet P, Yap CT, Wong L, YulinL, Rhoon Koh D, et al. (2010) SphK1 regulates pro-inflammatory responses associated with endotoxin and polymicrobial sepsis. Science 328: 1290-1294.

4. Beal AL, Cerra FB (1994) Multiple organ failure syndrome in 1990s. Systemic inflammatory response and organ dysfunction. JAMA 271: 226-233.

5. Marik PE, Varon J (2001) Sepsis: state of the art. Dis Mon 47: 465-532.

6. Van der Poll T (2001) Immunotherapy of sepsis. Lancet Infect Dis 1:165-174.

7. Akira S, Takeda K (2004) Toll-like receptor signaling. Nat Rev Immunol 4: 499-511.

8. O'Neill LAJ (2003) The role of MyD88-like adapters in Toll-like receptor signal transduction. Biochem Soc Trans 31: 643-647.

9. Sun D, Ding A (2006) MyD88-mediated stabilization of interferon-gamma-induced cytokine and chemokine mRNA. Nat Immunol 7: 375-38.

10. Kissner T L, Ruthel G, Alam S, Ulrich RG, Fernandez S, et al. (2011) Activation of MyD88 signaling upon Staphylococcal enterotoxin binding to MHC class II molecules. PLoS ONE 6: e15985.

11. Kissner TL, Cisney ED, Ulrich RG, Fernandez S, Saikh KU (2010) Staphylococcal enterotoxin A induction of pro-inflammatory cytokines and lethality in mice is primarily dependent on MyD88. Immunology 130: 516-526.

12. Kissner TL, Ruthel G, Cisney ED, Ulrich RG, Fernandez S, et al. (2010) MyD88-Dependent pro-inflammatory cytokine response contributes to lethal toxicity of staphylococcal enterotoxin B in mice. Innate Immun 17: 451-462.

13. Kissner TL, Moisan L, Mann E, Alam S, Ruthel G, et al. (2011) A Small Molecule that Mimics the BB-loop in the Toll/IL-1 Receptor Domain of MyD88 Attenuates Staphylococcal Enterotoxin B Induced Pro-inflammatory Cytokine Production and Toxicity in Mice. J Biol Chem 286: 31385-3139.

14. Mills KHG, Dunne A (2009) IL-1, master mediator or initiator of inflammation. Nature Medicine 15: 1363-1365.

15. Liu X, Zhan Z, Li D, Xu L, Ma F, et al. (2011) Intracellular MHC class II molecules promote TLR-triggered innate immune responses by maintaining activation of the kinase Btk. Na Immunol 12: 416-424.

16. Piani A, Hossle JP, Birchler T, Siegrist CA, Heumann D, et al. (2000) Expression of MHC class II molecules contributes to lipopolysaccharide responsiveness. Eur J Immunol 30: 3140-3146.

17. Frei R, Steinle J, Birchler T, Loeliger S, Roduit C, et al. (2010) MHC class II molecules enhance Toll-like receptor mediated innate immune responses. PLoS ONE 5: e8808

18. Hassan GS, Mourad W (2011) An unexpected roll for MHC class II. Nat Immunol 12: 375-376.

19. Beno DW, Uhing M R, Goto M, Chen Y, Jiyamapa-Serna V A, Kimura R E (2001) Staphylococcal enterotoxin B potentiates LPS-induced hepatic dysfunction in chronically catherized rats. Am J Physiol Gastrointest Liver Physiol 280: G866-G872

20. Stiles BG, Bavari S, Krakauer T, Ulrich R G (1993) Toxicity of staphylococcal enterotoxins potentiated by lipopolysaccharide: major histocompatibility complex class II molecule dependency and cytokine release. Infect Immun 61: 5333-5338.

21. Blank C, Luz A, Bendigs S, Erdmann A, Wagner H, Heeg K (1997) Superantigen and endotoxin synergize in the induction of lethal shock. Eur J Immunol 27: 825-833.

22. Schlievert P (1982) Enhancement of host susceptibility to lethal endotoxin shock by staphylococcal pyrogenic exotoxin type C. Infect Immun 36: 123-128.

23. Danner RL, Elin RJ, Hosseini JM, Wesley RA, Reilly JM, et al. (1991) Endotoxemia in human septic shock. Chest 99: 169-175.

24. Azuma K, Koike K, Kobayashi T, Mochizuki T, Mashiko K, Yamamoto Y (2004) Detection of circulating superantigens in an intensive care unit population. Intl J Infect Dis 8: 292-298.