



Long hidden sepsis subgroup may benefit from immune therapy

Mark Tidswell

Division of Pulmonary and Critical Care Medicine, Baystate Medical Center, Springfield, MA, USA

Correspondence to: Mark Tidswell, MD. Division of Pulmonary and Critical Care Medicine, 759 Chestnut Street, Baystate Medical Center, Springfield, MA 01199, USA. Email: mark.tidswell@baystatehealth.org.

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Sepsis syndrome includes an immediate inflammatory, or innate immune, response to infection that may be followed by mild or severe organ failure, excessive or depressed inflammation, survival, or death in more than 25% of patients. Research in animal models indicates that excessive inflammation increases early mortality, and targeted anti-inflammatory therapies reliably decrease mortality (1). Unfortunately, targeting inflammatory pathways in human clinical trials has not reduced patient mortality. Possible explanations for lack of response in humans include the heterogeneity of the sepsis syndrome in terms of cause and patient responses: micro-organism, site of infection, chronic patient illness, genomic program of inflammation, or timing of therapy in relation to onset of infection (2). An important element for future clinical trials will be to better characterize patients and match with one or more therapies most likely to reduce mortality.

Research over the past 25 years has described the innate response to infection caused by pathogen-associated molecular pattern (PAMP) molecules (3,4). PAMPs bind to toll-like receptors (TLRs) and NOD-like receptors that activate inflammatory signaling pathways and release of pro- and anti-inflammatory cytokines. The innate immune response is followed by an adaptive immune response (T cell proliferation), eradication of infection and return to normality, sustained inflammation, or immune suppression (5,6). The failure of immune-modulating agents in clinical trials of sepsis suggest that the complexities of immune responses are not adequately understood, particularly for the individual patient. In clinical practice, cytokine levels are unknown and timing of onset of infection is

uncontrolled so that initial therapy is based on signs and symptoms. A clinical trial of anti-inflammatory medication in a population of patients with sepsis might benefit a subgroup, increase risk for another subgroup, or have no effect for another subgroup, so that taken as a whole the medication could appear to offer no benefit. Determining that there is heterogeneity of treatment effect in a clinical trial population could lead to more precise and personalized treatment for patients. One approach to investigating heterogeneity of effect in a trial population is to divide the population into subgroups based on characteristics, such as cellular or plasma biomarkers, thought to influence response. A recent study by Meyer and colleagues (7) re-evaluated data from a previous trial in sepsis patients (8) to identify subgroups and determine the response of these subgroups to recombinant human interleukin-1 receptor antagonist (rhIL1RA). Meyer and colleagues categorized subgroups from the 1994 trial of rhIL1RA based on the levels of the plasma biomarkers plasma interleukin-1 β (IL1 β) and interleukin-1 receptor antagonist (IL1RA). Their results suggest that treatment differentially impacted mortality related to baseline plasma IL1RA levels. Their study will be described following an overview of IL-1 biology.

The interleukin-1 (IL-1) family of receptors and cytokines plays a major role in innate immunity. The IL-1 cytokines include 11 members with pro- or anti-inflammatory functions. These include pro-inflammatory (IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β , IL-36 γ), anti-inflammatory (IL-37), or antagonists to pro-inflammatory cytokines (IL-1RA, IL-36Ra, IL-38) (9).

Two IL-1 innate and pro-inflammatory cytokines, IL-1 α and IL-1 β , bind to the IL1 receptor. Generally, IL1 α is not present in the plasma, but is found within epithelial and endothelial cells in multiple organs, to be released in response to inflammatory conditions and during cell death. IL1 β is not typically present in plasma or cells, but is synthesized by mononuclear cells in response to stimuli such as PAMPs, cytokines, and IL1 β itself can induce IL1 β synthesis. IL1 β causes fever, vasodilation and hypotension, and along with tumor necrosis factor (TNF), is a major inflammatory molecule in septic shock.

IL1 β is synthesized as an inactive pro-cytokine until cleaved by caspase-1, and caspase-1 must first be activated by an intracellular protein complex known as the inflammasome. The inflammasome is activated by PAMPs such as lipopolysaccharide (LPS) binding to the TLR 4 (10,11). The cytokine IL1 β binds to the extracellular domain of IL-1 receptor type I. The IL1 receptor contains an intracellular domain, known as Toll-IL-1 receptor (TIR) which is shared with the innate immune system TLRs. As a result of cytokine binding to IL1 receptor, intracellular signaling via the MyD88 and NF-kappaB pathway leads to transcription of pro- and anti-inflammatory gene products, in steps similar to TLR signaling. IL1 β also facilitates adaptive immunity by mechanisms such as increasing production of T-cell growth factor IL-2.

IL1 β upregulates its own production via the IL-1 receptor and has the potential to cause chronic auto-inflammatory disease. IL1 β is implicated in chronic inflammation and diseases: type 2 diabetes mellitus, rheumatoid arthritis, after tissue ischemia (stroke or myocardial infarction), macrophage activation syndrome, and colitis (10,12).

IL-1 receptor antagonist (IL1RA) is an IL-1 family cytokine that competitively inhibits IL1 β binding to the IL-1 receptor and reduces intracellular signaling. Plasma IL1RA is secreted by monocytes, macrophages and neutrophils (13). IL1RA is produced in response to cytokines (including IL1 β and IL-4), LPS, GM-CSF and other stimuli. IL1RA binds to IL-1 receptor I without inducing a conformational change in the receptor, so that no signal for gene transcription is generated. The observation that IL1RA production increases in response to IL1 β has been interpreted as a homeostatic mechanism for attenuating the inflammatory response to IL1 β [reviewed in (10)]. IL1RA must be present at 100-fold or higher concentration than IL1 β to block the IL1 receptor (14).

In animal models of acute and chronic inflammation

IL1RA effectively reduces inflammation (10). For humans, IL1RA is available as the recombinant human protein Anikinra (Kineret[®], Swedish Orphan Biovitrum) to treat conditions in which IL1 β is implicated. Anikinra has been used by over 150,000 patients. It is clinically effective and approved in the United States for rheumatoid arthritis and for cryopyrin-associated periodic syndromes (CAPS). CAPS are genetic diseases that lead to excessive IL1 β production or deficiency of IL1RA (15). While not FDA-approved for other uses, reports suggest benefit in other rheumatological conditions including adult-onset Still's disease (AOSD), gout, and Behcet's disease. IL1RA is the subject of investigation in patients with acute or chronic inflammatory diseases attributed to IL1 β (12,15).

In humans, a large phase III multicenter trial of recombinant human IL1RA (rhIL1RA) for treatment of sepsis (the source of plasma for the Meyer study) was carried out in the early 1990's (8). There was no significant difference in 28-day mortality (34% placebo versus 31% in the treatment group). Post-hoc analysis showed an increase in survival time with rhIL1RA among patients with organ dysfunction and/or predicted risk of mortality of $\geq 24\%$. A confirmatory trial of 1,300 patients (16) was then conducted but stopped early for futility (observed 33.1% mortality in IL1RA compared to 36.4% in placebo). The differences in mortality in subgroups observed in the earlier phase III trial were not replicated.

The lack of effect of a single immunotherapy in sepsis may not be surprising (2). Human mononuclear cells change expression of thousands of genes in response to infection (17). Scores such as SIRS, SOFA, qSOFA (18) assess risk of mortality but do not describe the underlying inflammatory responses. Better understanding of which pathways are activated and change over time may facilitate precisely targeted therapy or combinations of immune modulators. Studies suggest that leukopenia or lymphopenia may identify patients with an immunosuppressive phenotype (6), which might benefit from immunostimulatory therapy such as interferon (5), anti-PD-1 antibody, or IL-7 (19). Rapid assays could be used to enrich a clinical trial population with subjects more likely to respond to a test therapy. For examples: an assay of endotoxin activity to determine suitability for endotoxin removal (20); measurement of cell-free hemoglobin as an eligibility criterion for acetaminophen inhibition of lipid peroxidation (21). Additionally, understanding that many inflammatory pathways are activated in sepsis, concurrent clinical trials of multiple agents should be considered. Might

gene expression profiling lead to advances in sepsis research as it has with oncology precision medicine trials? Oncology platform trials match genetically characterized tumors to multiple treatments that target the genetic changes in tumors, with an outcome of tumor response (22). Similar trials for patients with sepsis will be challenging, as the time course of sepsis may not allow time to process tissue for genotyping, characterization of genotype or phenotype may be limited to circulating inflammatory cell and plasma markers, and more research to determine which markers are beneficial, harmful, or interact, will be necessary. A recent study provides an example of discovery of genome-wide blood gene expression profiles associated with increased mortality in patients with sepsis (23). Distinct sepsis response signatures included over 3,000 genes that were up- or down-regulated. A genotype associated with greater risk of death independent of APACHE severity score was based on 140 genes. This genotype was associated with genes related to hypoxia response, metabolism, as well as genes involved with innate and adaptive immunity, endotoxin tolerance, cytotoxicity, cell death, apoptosis, T-cell activation that produce an immunosuppressed phenotype; but not by TNF, IL6 or IL1 β cytokine genes. These results offer a new means to stratify risk of death, and perhaps new potential therapeutic targets and sepsis subgroups for targeted therapy.

Meyer and colleagues (7) analyzed baseline plasma samples from a 1994 sepsis trial (8) to identify a subgroup of patients who had an activated IL1 β axis, based on IL1 β and IL1RA levels, and who may have benefited from rhIL1RA. In the 1994 trial 893 patients with sepsis syndrome received an intravenous loading dose of rhIL1RA, 100 mg, or placebo, followed by continuous 72-hour intravenous infusion of rhIL1RA (either 1.0 or 2.0 mg/kg per hour) or placebo. Twenty-eight-day all-cause mortality was not different for rhIL1RA treatment compared to placebo among all patients (8).

Meyer *et al.*, made use of banked plasma from the earlier trial, which had been frozen at -70°C for over 20 years. Plasma IL1RA and IL1 β levels were measured using enzyme linked immunosorbent assay with laboratory personnel blinded to clinical data including treatment status and survival. The primary endpoint was to test for heterogeneity in rhIL1RA treatment effect by plasma baseline biomarker concentration. Both plasma and sufficient clinical data were available for 529 (59%) of patients in the original study. Baseline characteristics in this limited group of patients was both similar to the overall population in the study and

similar between subjects treated with rhIL1RA or placebo [Tables E1 and E2, On Line Data Supplement (7)].

The primary analysis was treatment effect of rhIL1RA based on baseline plasma biomarker concentration. They selected a plasma concentration cut point to optimize area under the mortality receiver operating curve. In order to ensure the heterogeneity observed was a function of baseline plasma IL1RA level rather than severity of illness measures such as APACHE III score, the authors tested treatment related to biomarker levels across tertiles and deciles of predicted mortality.

The baseline IL1RA plasma concentration was significantly higher in patients that died, and the distribution appeared to be skewed [Table 1 (7)]. High plasma levels of IL1RA correlated with high APACHE III predicted mortality, septic shock, acute respiratory distress syndrome (ARDS), biliary dysfunction, macrophage activation syndrome, ATN, and higher IL1 β concentration [Table E3 (7), P values not corrected for multiple testing]. A cut point to optimize the mortality ROC was determined using the Youden method, presumably one of the non-parametric approaches (24). Patients with baseline IL1RA above an optimal cut point of 2,071 pg/mL demonstrated a significantly reduced mortality ($P=0.044$) when rhIL1RA was administered. The adjusted risk difference in this subgroup was -0.12 (95% CI, -0.23 to -0.01). Statistical analysis indicated that the treatment effect was similar across the different doses of rhIL1RA administered and throughout tertiles or deciles of baseline IL1RA plasma level. In subjects with IL1RA $<2,071$ pg/mL, rhIL1RA treatment had a mild but non-significant increase in mortality. There was not a statistically significant interaction between IL1 β level and mortality [Tables 1, E6 (7)]. Note that determination of a cut point in baseline plasma IL1RA with which to predict mortality assumes plasma IL1RA is not responsive to other factors unrelated to risk of mortality such as age or site of infection. The authors tested for interactions, and demonstrated a consistent rhIL1RA treatment effect across different groups. The authors did not report the Youden index, a number between 0 and 1 analogous to area under the receiver operating curve which describes the discriminant value of the cut point.

To explain the findings of increased risk of mortality for subjects whose plasma IL1RA is elevated and a mechanism whereby further rhIL1RA reduces mortality, Meyer and colleagues point out that IL1RA is a marker for persistent inflammation. Pro-inflammatory cytokines, including IL1 β ,

increase IL1RA levels. They suggest that higher levels of IL1RA during sepsis likely represent a response to higher levels of IL1 β , and that treatment with rhIL1RA may shift the balance toward less IL1 β activity and less inflammation. These findings are consistent with observations that inhibition of IL1 β requires IL1RA at 100-fold or greater excess (13,14). Compared to patients with high IL1RA, in those patients with low levels of IL1RA (and presumably less IL1 β) further suppressing IL1 β activity was not beneficial and trended toward harm.

The authors addressed many of the issues of their post hoc study design. They did not have complete plasma and clinical data for the entire research cohort; however, they showed that the samples were representative. The patients varied in age, co-morbidities, site of infection, and severity of sepsis, but the variation appeared to be evenly distributed. Statistical analyses indicated that heterogeneity of treatment effect was related to baseline plasma IL1RA. Other issues could not be addressed by study design. Multiple comparisons were necessary and the P values were not adjusted; while appropriate for exploratory analyses the potential for a type I error is underestimated. Long-term storage of samples may lead to degradation of cytokine levels. Genetic differences may lead to differences in baseline constitutive IL1RA expression independent of response to sepsis. During the last 20 years since the phase III trial of rhIL1RA, treatment of sepsis has changed and mortality rate has declined, while the population now includes more patients with chronic co-morbidities. Additionally, while 28-day mortality remains an important clinical study endpoint, longer-term outcomes may be affected by immunosuppression (6). In the current era of sepsis management, plasma IL1RA levels may have a different predictive value.

In summary, IL1 β and IL1RA imbalance has a role in inflammation, as seen in animal models of sepsis and human diseases. The study under discussion (7) set out to identify a subgroup of patients with sepsis who might show decreased mortality in response to rhIL1RA. Using stored plasma and statistical analyses they were able to identify patients with high baseline plasma IL1RA concentration as such a subgroup. These results offer encouragement that the goal of treating the complex patterns of inflammation in sepsis with personalized therapies may be one day achievable. Individual patients identifiable by serum markers, such as IL1RA, may be responsive to specific anti-inflammatory therapies.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References

1. Fink MP. Animal models of sepsis. *Virulence* 2014;5:143-53.
2. Marshall JC. Why have clinical trials in sepsis failed? *Trends Mol Med* 2014;20:195-203.
3. Wiersinga WJ, Leopold SJ, Cranendonk DR, et al. Host innate immune responses to sepsis. *Virulence* 2014;5:36-44.
4. Angus DC, van der Poll T. Severe Sepsis and Septic Shock. *N Engl J Med* 2013;369:840-51.
5. Döcke WD, Randow F, Syrbe U, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. *Nat Med* 1997;3:678-81.
6. Drewry AM, Samra N, Skrupky LP, et al. Persistent lymphopenia after diagnosis of sepsis predicts mortality. *Shock* 2014;42:383-91.
7. Meyer NJ, Reilly JP, Anderson BJ, et al. Mortality Benefit of Recombinant Human Interleukin-1 Receptor Antagonist for Sepsis Varies by Initial Interleukin-1 Receptor Antagonist Plasma Concentration. *Crit Care Med* 2018;46:21-8.
8. Fisher CJ Jr, Slotman GJ, Opal SM, et al. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. *Crit Care Med* 1994;22:12-21.
9. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev* 2018;281:8-27.
10. Palomo J, Dietrich D, Martin P, et al. The interleukin (IL)-1 cytokine family--Balance between agonists and antagonists in inflammatory diseases. *Cytokine* 2015;76:25-37.
11. Dinarello CA. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood* 2011;117:3720-32.
12. Dinarello CA, van der Meer JW. Treating inflammation by blocking interleukin-1 in humans. *Semin Immunol* 2013;25:469-84.

13. Arend W, Malyak M, Guthridge C, et al. Interleukin-1 Receptor Antagonist: Role in Biology. *Immunol. Annual Rev* 1998;16:27-55.
14. Arend WP, Welgus HG, Thompson RC, et al. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest* 1990;85:1694-7.
15. Schett G, Dayer JM, Manger B. Interleukin-1 function and role in rheumatic disease. *Nat Rev Rheumatol* 2016;12:14-24.
16. Opal SM, Fisher CJ Jr, Dhainaut JF, et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. *Crit Care Med* 1997;25:1115-24.
17. Goh C, Knight JC. Enhanced understanding of the host-pathogen interaction in sepsis: new opportunities for omic approaches. *Lancet Respir Med* 2017;5:212-23.
18. Raith EP, Udy AA, Bailey M, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *JAMA* 2017;317:290-300.
19. Francois B, Jeannet R, Daix T, et al. Interleukin-7 restores lymphocytes in septic shock: the IRIS-7 randomized clinical trial. *JCI Insight* 2018;3.
20. Klein DJ, Foster D, Schorr CA, et al. The EUPHRATES trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock): study protocol for a randomized controlled trial. *Trials* 2014;15:218.
21. Janz DR, Bastarache JA, Rice TW, et al. Randomized, placebo-controlled trial of acetaminophen for the reduction of oxidative injury in severe sepsis: the Acetaminophen for the Reduction of Oxidative Injury in Severe Sepsis trial. *Crit Care Med* 2015;43:534-41.
22. Woodcock J, LaVange LM. Master Protocols to Study Multiple Therapies, Multiple Diseases, or Both. *N Engl J Med* 2017;377:62-70.
23. Scicluna BP, van Vught LA, Zwinderman AH, et al. Classification of patients with sepsis according to blood genomic endotype: a prospective cohort study. *Lancet Respir Med* 2017;5:816-26.
24. Fluss R, Faraggi D, Reiser B. Estimation of the Youden Index and its associated cutoff point. *Biom J* 2005;47:458-72.

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