

JOURNAL OF INTERFERON & CYTOKINE RESEARCH  
Volume 00, Number 0, 2011  
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DOI: 10.1089/jir.2011.0062

## Interleukin-6 in Sepsis and Capillary Leakage Syndrome

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Bacterial sepsis is one of the most frequent and dreaded causes of death in intensive care units. According to the current understanding of sepsis, bacterial components activate innate immune responses via pattern-recognition receptors that stimulate signaling pathways, thereby leading to activation of NF- $\kappa$ B and the release of cytokines, alarming the organism and coordinating appropriate defense mechanisms. The resulting “cytokine storm” not only restricts bacterial invasion; it also harms the host by triggering a hemodynamic collapse with a drop in blood pressure, which could lead to death. One of the cytokines released during sepsis is interleukin-6 (IL-6). Originally described as a B-cell-stimulating factor, this cytokine has since been shown to have multiple additional functions. Interestingly, there is emerging evidence of IL-6 trans-signaling in the pathogenesis of sepsis. We review recent findings and discuss whether therapeutic interference with IL-6 trans-signaling may be beneficial in this important clinical scenario.

**AU1** ▶ **AU2** ▶ **S**EPSIS (ETYMOLOGICALLY DERIVED from the Greek word “σψις” meaning “decomposition”) is a leading cause of death in intensive care units. Sepsis is responsible for 9% of deaths per year in the United States and Germany, despite multiple novel therapeutic approaches tested over the past several decades (Stearns-Kurosawa and others, 2011). Because bacteria predate humans, sepsis probably predates modern humans as well (Baron and others, 2006).

Operational terms used by clinicians to describe different stages of sepsis are systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock (Stearns-Kurosawa and others 2011). Systemic inflammatory response syndrome is caused by increased levels of pro-inflammatory cytokines in the blood and is formally defined by altered body temperature ( $>38^{\circ}\text{C}$  or  $<36^{\circ}\text{C}$ ), increased heart rate ( $>90$  beats per minute), altered respiratory rate ( $>20$  breaths per minute), and a white blood count  $>12,000$  cells/ $\text{mm}^3$  or  $<4,000$  cells/ $\text{mm}^3$ . Interestingly, SIRS may also be caused by noninfectious diseases, such as trauma, pulmonary emboli, and myocardial infarction. In contrast, sepsis is defined as SIRS plus infection, whereas severe sepsis involves sepsis with dysfunction of at least one organ. Finally, septic shock is severe sepsis with hypotension (systolic blood pressure  $<90$  mm Hg or mean arterial pressure  $<65$  mm Hg after an adequate fluid infusion). Frequent features in septic shock are a high cardiac output and a low systemic vascular resistance state associated with diminished myocardial function. Blood volume is continually lost into the interstitial space (due to capillary leakage; see below) and intracellular locations, and blood vessels are clogged by perturbed coag-

ulation cascades (disseminated intravascular coagulation). Eventually, systolic hypotension and diffuse vasoconstriction lead to a fatal therapy-refractory ischemia of multiple organs and to organ necrosis.

Usually, sepsis originates from serious infections, such as pneumonia, or by indwelling medical devices, such as intravenous lines, that undergo colonization by microorganisms (e.g., bacteria), enabling these invasive pathogens to access the blood stream. The spectrum of bacteria isolated from blood cultures of patients with sepsis has changed profoundly over the past decades. Whereas gram-negative bacteria (such as *Pseudomonas aeruginosa*) were the most frequently isolated culprits in the 1970s, gram-positive bacteria (such as *Staphylococcus aureus*) and fungi (such as *Candida albicans*) are becoming increasingly relevant. The rapid transmission of plasmid-based antibiotic-resistance genes among bacteria (Krüttgen and others 2011), the existence of virulence genes, and the propensity of certain bacteria to adhere on indwelling catheter surfaces, forming biofilms, are contributing to the increases in hospital-acquired infections; such infections are of serious concern for clinicians (Seifert 2009).

One component of the gram-negative membrane is of paramount significance for the pathogenesis of sepsis: the bacterial endotoxin lipopolysaccharide (LPS). Release of LPS into the circulation triggers a strong systemic pro-inflammatory response reminiscent of septic shock. Most interestingly, it is actually the host response to LPS (not the intrinsic properties of endotoxin) that induces the potentially lethal consequences. This overwhelming response—to which the human species

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might be especially prone—is associated with dramatically increased serum concentrations of pro-inflammatory cytokines, commonly known as “cytokine storm.” Although LPSs are intrinsic components of the outer membrane of gram-negative bacteria, lipoteichoic acids and peptidoglycans serve a similar role in gram-positive sepsis (Schmidt and others 2011).

Both LPS and lipoteichoic acids bind to Toll-like receptors (TLRs), an important family of pathogen-associated molecular-pattern-recognition receptors alerting the innate immune response system to the presence of dangerous microorganisms. Whereas TLR4 is the primary LPS receptor, TLR2 is regarded as the receptor for lipoteichoic acids (Beutler and Rietschel 2003). Other TLRs recognize an extensive array of other microbial components from fungi, viruses, and parasites. If pathogens may escape the extracellular detection by TLRs and manage to invade into the cytoplasm of their human host cells, their presence may still be detected by nucleotide binding oligomerization domain-like receptors and intracellular TLRs (Stearns-Kurosawa and others 2011).

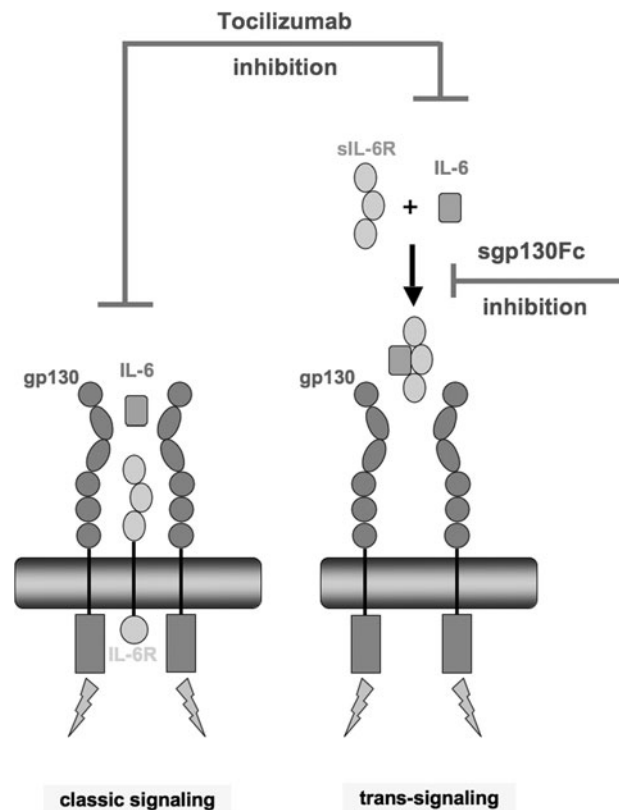
TLRs trigger intracellular pathways involving the signaling molecules MyD88 or TRIF and leading to the activation of the transcription factors NF- $\kappa$ B and c-Jun N-terminal kinase, thereby initiating the transcription of pro-inflammatory cytokine genes (Stearns-Kurosawa and others 2011). One of the major NF- $\kappa$ B target genes is the cytokine interleukin-6 (IL-6). Intriguingly, the plasma concentration of this key cytokine may rapidly increase dramatically. Plasma levels of IL-6 normally range between 1 and 5 pg/mL and have been reported to reach levels  $>1 \mu\text{g/mL}$  during sepsis (Waage and others 1989). Therefore, IL-6 is frequently used as a biomarker for sepsis in intensive care units, especially in the case of diseased children.

### IL-6 and Its Soluble Receptors

Interleukin-6 is a member of the 4-helical cytokine family, which signals via an 80-kDa cytokine receptor (IL-6R, glycoprotein [gp] 80). Once IL-6 binds to IL-6R, the complex of IL-6 and IL-6R associates with the signaling receptor subunit gp130, which thereupon dimerizes and induces intracellular signaling via the Jak/STAT pathway as well as via the Ras/Map-kinase pathway (Grotzinger and others 1999). Importantly, only few cells in the body—hepatocytes and some leukocytes—express IL-6R, whereas gp130 is expressed ubiquitously (Rose-John and others 2006). Because IL-6 has no measurable affinity to gp130, it follows that cells, which do express gp130 but not IL-6R, are not responsive to IL-6 (Scheller and others 2011). However, IL-6 signals by 2 mechanisms: (1) via the ubiquitous transmembrane gp130: “classic” signaling using membrane-bound IL-6R (gp80) and (2) via trans-signaling using soluble IL-6R (sIL-6R) (Fig. 1). Therefore, cells expressing only gp130 and not IL-6R are responsive to IL-6 in complex with the sIL-6R (Chalaris and others 2011).

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Even more interestingly, trans-signaling is selectively inhibited by soluble gp130. Guided by seminal initial structure-function analysis of IL-6 (Brakenhoff and others 1989; Krüttgen and others 1990), designer cytokines such as the agonist Hyper-IL6 (consisting of IL-6 fused by a linker to sIL-6R) and the inhibitor sgp130Fc have been genetically engineered, adding to our repertoire of tools to elucidate or manipulate IL-6 signaling *in vitro* and *in vivo* (Grotzinger and



**FIG. 1.** Interleukin-6 (IL-6)/soluble IL-6R (sIL-6R) trans-signaling and its inhibition by sgp130Fc. Blood-borne IL-6 and sIL-6R form a complex that binds to and activates glycoprotein (gp) 130 at the plasma membrane of target cells (trans-signaling), whereas classic signaling describes binding of IL-6 to cells co-expressing gp80 and gp130. Recombinant soluble sgp130—consisting of the extracellular domain of gp130 fused with the Fc part of an antibody—acts as a potent scavenger by competing with surface membrane gp130 for the binding of the naturally occurring IL-6/sIL-6R complex. Whereas the monoclonal antibody tocilizumab inhibits both classic signaling and trans-signaling, sgp130Fc selectively inhibits trans-signaling.

others 1997). The sgp130Fc protein has been used as a molecular tool to define which gp130-driven murine disease models are driven by classic and trans-signaling (Rose-John and others 2007). It turned out that the pro-inflammatory activities of IL-6 are mainly driven by IL-6 trans-signaling via the sIL-6R, whereas anti-inflammatory or regenerative functions rely on classic IL-6 signaling via the membrane-bound receptor (Scheller and others 2011). Of note, it has been shown that dying neutrophils, which are the first line of defense of the body during infectious diseases, release the sIL-6R and thereby lead to an attraction of mononuclear cells, which are needed for the resolution of the inflammatory process (Chalaris and others 2007).

### Sepsis and IL-6

Pro-inflammatory cytokines play a pivotal role in the pathogenesis of sepsis. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-1 $\beta$  are probably the best-examined pro-inflammatory cytokines. In animal studies, the sole injection of high concentrations of TNF- $\alpha$  or IL-1 $\beta$  has lethal consequences (Stearns-

Kurosawa and others 2011). Unexpectedly, the blood levels of these key cytokines turned out to be of limited use as clinical markers for at-risk patients. Even more disappointingly, numerous clinical studies failed to demonstrate robust clinical benefits from pharmacologic inhibition of TNF- $\alpha$  and IL-1 $\beta$  (Stearns-Kurosawa and others 2011). As discussed below, this might have been caused by the complexity of sepsis with—perhaps simultaneously—hyper-inflammatory and anti-inflammatory processes, seriously complicating the clear-cut stratification of patients and thereby hampering the decision about which patients profit at which time point from which therapeutic regimen.

Besides IL-1 $\beta$  and TNF- $\alpha$ , a few other interleukins are also of paramount importance. Among these, IL-6 is firmly established as a clinically suitable biomarker for sepsis. In seminal studies, Waage and coworkers (1989) observed high levels of IL-6 and its association with fatal sepsis in patients with meningococcal infection. Since then, many other studies confirmed and extended these results. For instance, evaluation of postoperative patients with severe sepsis showed that in survivors, IL-6 significantly decreased during the first 2 weeks; in nonsurvivors, IL-6 mostly increased within the second week (Frink and others 2009; Tschaikowsky and others 2011). Although IL-6 is an established prognostic marker for mortality in sepsis because of its higher diagnostic discriminative ability, procalcitonin has overtaken IL-6 in terms of clinical significance (Stearns-Kurosawa and others 2011).

The increased levels of the key cytokine IL-6 correlating with mortality raised the intriguing hypothesis that IL-6 signaling plays a mechanistic role in human sepsis. Indeed, many studies in mice and cell cultures have corroborated this idea. Remick and coworkers established that measuring IL-6 concentrations 6 hours after injury infliction is an accurate predictor of mortality from experimental sepsis in a mouse model (Remick and others 2002). However, initial work with IL-6 knock-out mice was rather disappointing because it turned out that IL-6 *per se* might not be of lethal importance: These mice had no altered mortality in sepsis (Remick and others 2005). Later on, however, IL-6 signaling via the gp130 receptor was found to be of paramount importance because mice lacking this receptor in the liver were protected against an infection with the common sepsis pathogen *Streptococcus pyogenes* (Klein and others 2007). In line with these results, gp130 (F/F) knock-in mice expressing a gp130 receptor variant with an overactive STAT3 response were hypersensitive to LPS-induced sepsis (Greenhill and others 2011). Moreover, it was shown that trans-signaling in vascular endothelial cells seems to play a major role in sepsis because IL-6 trans-signaling in these cells modulated TLR4-dependent inflammatory responses (Greenhill and others 2011).

During shock, the refractory drop in blood pressure is a dire clinical problem. Interestingly, a link between IL-6 signaling and blood pressure was established; peripheral vasodilatation in human patients is most strongly associated with increased IL-6 levels in blood (Hartemink and Groeneveld 2011). In addition, another interesting study found that serum IL-6 correlated with endothelial dysfunction (Esteve and others 2007). Therefore, an emerging question was the mechanism by which IL-6 signaling decreased blood pressure and endothelial function, and whether there was a relation to the development of septic shock.

### Sepsis, Endothelial Leakage, and IL-6

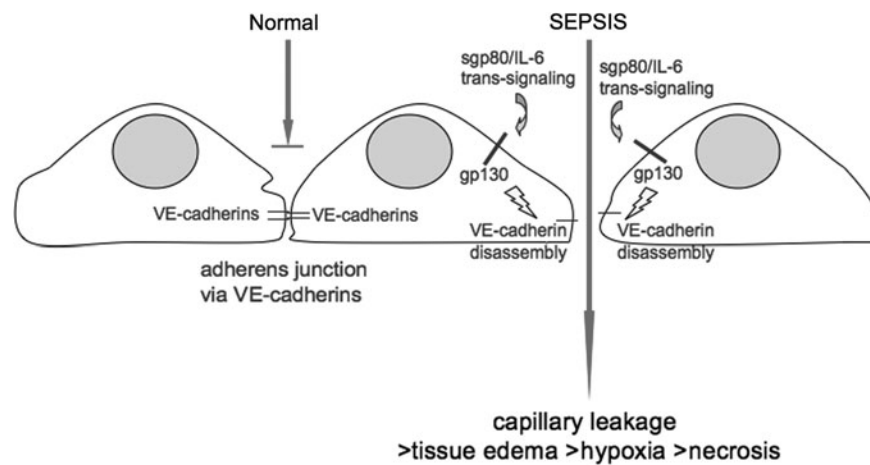
Blood vessels are lined with endothelial cells that are involved in the regulation of blood pressure. Cell adhesion molecules, such as vascular-endothelial (VE) cadherin, form cell–cell adherens junctions by connecting neighboring endothelial cell membranes. Clinically, sepsis is associated with tissue edema caused by vascular leakage through endothelial cells (endothelial leakage) that impairs oxygenation of tissues (Lee and Slutsky 2010). Signal transduction pathways triggered by inflammatory mediators lead to phosphorylation and endocytosis of VE-cadherin in endothelial cells, giving rise to gaps in the endothelial barrier and resulting in serious loss in barrier function. An important recent paper demonstrated the decisiveness of capillary leakage for mortality in murine sepsis: London and Li (2011) showed that sealing vascular leaks by infusion of recombinant slit (a soluble protein best known for its effects in axon guidance of nerve cells) completely protected mice from death induced by multiple infectious agents. In line with this, the late Judah Folkman demonstrated that therapeutic application of tetracycline might fulfill a similar role (Fainaru and others 2008).

Is there a link between IL-6 and vascular leakage during sepsis? In a visionary paper published in 1997, Ciliberto and coworkers showed that vascular endothelial cells express gp130 but not IL-6R and respond to trans-signaling by IL-6/sIL-6R (Romano and others 1997). In sepsis, considerable amounts of both IL-6 and sIL-6R are present in the blood (Frieling and others 1995), which is separated from organ tissues by gp130-expressing vascular endothelial cells. IL-6 is produced in high amounts upon bacterial infection (Waage and others 1989), whereas sIL-6R protein is generated by neutrophils, which are likely to be the first cells to encounter invading bacteria (Chalaris and others 2007). Therefore, blood-borne IL-6/sIL-6R complexes are bound to interact with gp130-expressing endothelial cells lining blood vessels. What are the likely signaling consequences?

A recent paper from the field of cancer biology provided us with a possible answer. Lo and others (2011) showed that IL-6/sIL-6R signaling on human umbilical vascular endothelial cells triggered phosphorylation and redistribution of VE-cadherin, leading to vascular leakage. Because human umbilical vascular endothelial cells are a standard model for the study of endothelial cells in sepsis, one effect of IL-6 trans-signaling in sepsis might be a capillary leakage syndrome (Fig. 2). This term describes the breakdown of the vascular-endothelial barrier function that normally regulates the delicate distribution of nutritional compounds, fluids, and leukocytes between blood and tissues. Increased endothelial permeability by disruption of VE-cadherins function results in trans-endothelial flow of fluid and interstitial edema. This dramatically impairs tissue oxygenation due to increased viscosity of the blood and increased tissue pressure, contributing to therapy-refractory shock (Stearns-Kurosawa and others 2011).

Besides the unexpected role of IL-6 in triggering vascular leakage in vascular endothelial cell, another recent study provided an equally important rationale for interfering with IL-6 trans-signaling in sepsis to increase survival outcome (Barkhausen and others 2011). Intestinal epithelial cells provide a primary physical barrier against bacteria, and the integrity of this barrier is damaged when pathologic events

**FIG. 2.** Presumed function of interleukin-6 (IL-6)/soluble IL-6R (sIL-6R) trans-signaling on vascular endothelial (VE) cells during sepsis. Normally, adherens junctions between neighboring endothelial cells do not permit uncontrolled influx of fluid from the blood into tissues. During sepsis, IL-6/sIL-6R trans-signaling triggers the disassembly of these adherens junctions by inducing the phosphorylation of VE-cadherin. Influx of blood into tissues leads to edema, thereby contributing to therapy-refractory necrosis.



lead to a drop in blood pressure with ensuing necrosis. This is a very serious consequence of shock-induced ischemia: The resulting necrosis of the gut-blood barrier leads to an irresistible invasion of the countless gut-resident commensal bacteria, to which resistance is futile. Interestingly, Barkhausen and others (2011) recently showed in a sepsis model that blocking IL-6 trans-signaling using sgp130Fc blocked intestinal epithelial cell apoptosis in the gut and decreased mortality. Global blockade of IL-6 by a monoclonal antibody had no beneficial effect. Thus, by keeping enteric epithelial cells alive, blocking IL-6 trans-signaling protects patients with sepsis by keeping the important barrier to enteric bacteria sealed, protecting from a “second front” against those opportunistic pathogens that threaten to invade the hosts’ blood from within the gut.

### Human Sepsis and IL-6 Antagonists?

Studies guided by a detailed understanding of the structure-function relationships of IL-6 with its receptors led to the development of potent IL-6 inhibitors, such as monoclonal neutralizing antibodies against IL-6 and its gp80 receptor, as well as a soluble gp130 Fc fusion protein that inhibits IL-6/sIL-6R trans-signaling (Brakenhoff and others 1990; Brakenhoff and others 1994; Grotzinger and others 1997). Previous studies that applied IL-6 inhibitors in murine disease models of arthritis and colitis were encouraging and paved the way to clinical studies (Rose-John and others 2007). As a result of such persistent efforts, tocilizumab, a humanized monoclonal antibody that acts as an IL-6 receptor antagonist, has been approved for the treatment of rheumatoid arthritis (Mima and Nishimoto 2009).

Although this seems to be good news for clinicians hoping that IL-6 antagonists will also be approved for the treatment of sepsis, caution is warranted. So far, the history of the search for drugs to treat sepsis is so discouraging that this field has even been called the “pharmaceutical graveyard” (Riedemann and others 2003). Many of these failed endeavors were aimed at interfering with cytokines known to exert detrimental effects in sepsis. Why did so many previous expeditions—based on good basic science—eventually fail? In addition, regarding IL-6, what could still hold up the view that this time (i.e., with research on modulation of IL-6 trans-signaling) might be different and that these studies could lead to a panacea for this lethal disease?

Numerous explanations have been brought forward for the disappointing clinical studies.

First, interfering with a complex system is complex (Stearns-Kurosawa and others 2011).

Second, lack of appropriate stratification of patients might have played a role in disappointing statistical effects; sepsis encompasses many different entities that have different prognoses and different therapeutic needs at different time points (Bone 1995). Support for “personalized sepsis medicine” is based on the finding that blunting inflammation improves survival only of animals at a high risk of dying, whereas low-dose glucocorticoids are effective in patients with adrenal malfunction (Remick 2007). The need for a tailored therapy in sepsis is already indicated by the observation that IL-6 levels are significantly higher in gram-negative bacteremia than in gram-positive bacteremia (Alexandraki and Palacio 2010). Thus, if IL-6 indeed turns out to be a valid therapeutic target in bacterial sepsis, the microbiological origin of sepsis surely needs to be taken into account, most likely resulting in differential dosing of IL-6 antagonists according to the Gram state of the invading bacteria.

Third, too late is too late: As a result of therapeutic interference with first-line cytokines during the initial phase of pathogenesis, therapy may have no benefit when initiated too late (e.g., during the subsequent late phases of the clinical course when patients enter the hospital). Indeed, prospective studies on patients presenting with sepsis showed that in most patients, peak levels of key cytokines were present on the day of admission and decreased on the following days (Damas and others 1997). Thus, the best time point for interfering with first-line cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ , might have been missed. Encouragingly, IL-6 levels remain high and stable during the course of sepsis (Damas and others 1997). The study by Barkhausen and others (2011) demonstrated that a therapeutic application of the sgp130Fc protein 24 hours after the onset of the sepsis model still yielded a therapeutic benefit.

Fourth, there is a lack of appropriate animal models; mice are mice, and humans are humans. As a species, humans are known to be particularly susceptible to lethal effects LPS. Thus, identification of mechanisms in animals and pharmaceutical blockade of these pathways might not translate into clinical efficacy in septic humans (Rittirsch and others 2007). Most dramatically, the shortcoming of animal models for

human sepsis became evident in human volunteers in a phase I study of an experimental anti-CD28 antibody (Suntaralingam and others 2006); although this antibody was well tolerated in mice and nonhuman primates, all human volunteers given this antibody developed a cytokine storm and shock within a few hours, eventually leading to multi-organ failure.

### Conclusions

Bacterial sepsis is highly fatal, and its pathophysiology is complex. There is a great need for novel treatments. Successful treatment probably requires biomarker-based stratification of patients with sepsis into subgroups, resulting in some degree of personalized sepsis medicine. One of the most relevant biomarkers of sepsis is the multifunctional cytokine IL-6. Many studies suggest that IL-6 is not only a suitable marker but also a key actor in the molecular choreography of sepsis pathogenesis. Most recently, IL-6 trans-signaling was found to increase endothelial permeability by phosphorylation of VE-cadherin. We consider this finding, emerging from the field of cancer biology, as very important for the sepsis field: Because of spatial considerations, vascular endothelial cells are bound to be the most likely physiologic target of blood-borne IL-6/sIL-6R complexes. Thus, vascular leakage caused by IL-6 trans-signaling (resulting in tissue edema and hypoxia) represents a valuable novel target for pharmaceutical intervention and basic science. Despite the hope that this novel opportunity will be fruitful, disappointing experiences with different cytokines lower our expectations and prompt caution regarding the value of animal study-derived concepts for human immunology. Nevertheless, the novel IL-6 trans-signaling inhibitor is a welcomed addition in our long quest for a much-needed cure for human sepsis.

### Acknowledgments

S.R.-J. was supported by the Deutsche Forschungsgemeinschaft (SFB 877, TP A 1) and by the Cluster of Excellence "Inflammation at Interfaces." S.R.-J. is inventor on patents describing the function of sgp130Fc and is a shareholder of the CONARIS Research Institute (Kiel, Germany).

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Received 9 July 2011 / Accepted 9 September 2011

**AUTHOR QUERY FOR JIR-2011-0062-VER9-KRÜTTGEN\_1P**

AU1: Headings are usually omitted at the beginning of an article. After the introductory paragraphs about definitions of sepsis, can "Signal Transduction" be inserted as a heading?

AU2: Please use English transliteration of Greek word rather than symbols.

AU3: Please provide an issue number for each journal reference.

AU4: Is the Barkhausen reference in press? Couldn't find this one on PubMed.