ABSTRACT—Since the definition of systemic inflammatory response syndrome/sepsis was originally proposed, a large amount of new information has been generated showing a much more complex scenario of inflammatory and counterinflammatory responses during sepsis. Moreover, some fundamental mechanisms of sensing and destroying invading microorganisms have been uncovered, which include the discovery of TLR4 as the lipopolysaccharide (LPS) gene, implications of innate immune cells as drivers of the adaptive response to infection, and the modulation of multiple accessory molecules that stimulate or inhibit monocyte/macrophage and lymphocyte interactions. The complexity of the infection/injury-induced immune response could be better appreciated with the application of genomics and proteomics studies, and LPS was a useful tool in many of these studies. In this review, we discuss aspects of bacterial recognition and induced cellular activation during sepsis. Because of the relevance of endotoxin (LPS) research in the field, we focus on LPS and host interactions as a clue to understand microorganisms sensing and cell signaling, then we discuss how this response is modulated in septic patients.

KEYWORDS—LPS, tolerance, Toll-like receptor, monocytes, alternatively activated macrophages, Th1, Treg, Th17

INTRODUCTION

Severe sepsis and septic shock occur with a high incidence and prevalence in emergency rooms and intensive care units. An elegant study in the United States, which evaluated hospital admissions between 1979 and 2000 using a representative sample of American hospitals, depicted an increase in sepsis incidence from 82.7 episodes per 100,000 inhabitants in 1979 to 240.4 episodes per 100,000 inhabitants in 2000 (1). Another study projected the occurrence of 750,000 cases of severe sepsis in 2001, with approximately 210,000 deaths and an annual increment of 1.5% (2). Despite improvements in therapy, sepsis remains, with high mortality rates, ranging from 22% to 56% (3).

Several decades ago, it was suggested that sepsis is markedly driven by the host response; this idea was further supported in the late 1980s as knowledge concerning the role of inflammatory mediators, such as tumor necrosis factor α (TNF-α), in the pathogenesis of sepsis emerged. This knowledge led to the concept of sepsis as a systemic inflammatory response syndrome (SIRS) triggered by infection (4) and prompted a search for diagnostic and prognostic biomarkers, as well as therapeutic interventions, to inhibit the presumed inflammatory response.

Sepsis may be caused by bacteria, fungi, parasites, or virus (4). The etiology is related to the place of acquisition (community- or hospital-acquired infection) and the primary source of infection. Interestingly, an increase of bloodstream infections was observed during the last century, with dynamic changes in their etiology. Bloodstream infections were predominantly caused by gram-positive bacteria in the beginning of the century, followed by increased proportion of gram-negative bacteria and finally by the emergence of fungi as relevant pathogens (5). Nowadays, gram-positive and gram-negative bacteria with increased antimicrobial resistance are common source of nosocomial infections in developed and developing countries (6, 7). Recent worldwide survey of prevalence and outcomes of infections in intensive care units showed that among the patients with positive cultures 62% presented gram-negative organisms; 47%, gram-positive; and 19%, fungi (3).

In the last 20 years since the definition of SIRS/sepsis was proposed, a huge amount of new information has been generated, showing a much more complex scenario of inflammatory and counterinflammatory responses during sepsis. Moreover, fundamental mechanisms of sensing and destroying invading microorganisms have been uncovered.

In this review, we discuss aspects of bacterial recognition and induced cellular activation during sepsis. Because of the relevance of endotoxin (lipopolysaccharide [LPS]) research in the field, we will focus on LPS and host interactions as a
model to understand microorganism sensing and cell signaling. Finally, we discuss how this response is modulated in septic patients.

LPS sensing and signaling

Endotoxins are present in the outer membrane of gram-negative bacteria. Chemically, endotoxins are LPSs, which comprise three structural and functional subregions: lipid A (the innermost region), the core, and the O polysaccharide. Lipid A is composed of a β-d-glucosaminyl-(1-6)-α-d-glucosamine disaccharide, which possesses two phosphoryl groups (one at the 4’ position and one at position 1) and is acylated by four R-3-hydroxy fatty acid residues. Lipid A is highly conserved among different pathogenic gram-negative bacteria and represents the toxic moiety of LPS. The core oligosaccharide consists of a hetero-oligosaccharide, with limited variability within different bacterial species. The O-specific chain is composed of repeating oligosaccharide units that determine the serological specificity of the LPS and thereby the bacteria cell wall (8). Rough (R) mutants of gram-negative bacteria synthesize LPS lacking O-polysaccharides and are designated Ra-Re in the order of the decreasing complexity of the core oligosaccharide (8). Most wild-type bacteria synthesize smooth (S)- and R-form LPS, and thus, LPS preparations represent mixtures of S- and R-form LPS, despite their designation as the S-form (9).

It has long been recognized that the biological activities of the LPS were mediated by the host response. In an elegant text on endotoxin research published almost 30 years ago, Westphal (10) mentioned the mediator theory for inflammation of Valy Menkin and the concept stressed by L. Thomas, another pioneer in endotoxin research, that it is not endotoxin that is so highly active or toxic per se, it is the higher animals with its affine cells and their receptors and the following sequences of endogenous reactions via mediators that make a species more or less sensitive to endotoxin. Then, he pointed out that “one of the most important fields of investigations is the search for mediators elicited by endotoxin signals and for the type of cells producing such highly active secondary products, with the hope to finally purify and identify and even synthesize these biologically most interesting agents” (10).

Histocompatible mice strains that are sensitive and resistant to LPS were used early in LPS research to clearly illustrate this idea: the sensitive mice (C3H/HeN and C57BL/10ScSn) presented a wide range of biological responses, such as cell proliferation and activation, which were absent in the resistant mice (C3H/HeJ and C57BL/10ScCr) [for a review, see Galanos et al. (8) and Morrison (11)]. A putative LPS gene, located on chromosome 4 in mice, was inferred to be responsible for the differences in cellular responses and LPS susceptibility between congenic mouse strains. Michalek et al. (12) performing cell transfer experiments that demonstrated the cells from the reticulum-endothelial system were involved in LPS bioactivity. Freudenberg et al. (13) transferred macrophages from the sensitive mouse strain (C3H/HeN) to the LPS-resistant strain (C3H/HeJ) and rendered the latter sensitive to the toxic effects of LPS, suggesting that macrophages were the primary targets of LPS.

Knowledge of the LPS mechanism of activation emerged, demonstrating a role for soluble LPS-binding protein (LBP) and CD14 in LPS sensing, but it was not until 1998 that the Toll-like receptor 4 (TLR-4) was identified as the receptor that senses LPS. Poltorak and coworkers (14) demonstrated that the TLR4 gene has a point mutation and a deletion in C3H/HeJ- and C57BL/10ScCr-resistant mice, respectively, results also found by other investigators (15). Toll was first described in *Drosophila melanogaster* as a gene that is involved in dorsoventral embryo development (16), and its role in innate immunity was characterized in the J. Hoffmann laboratory, demonstrating that *Drosophila* lacking the Toll receptor were highly sensitive to fungi (17). The first TLR in mammals was identified in C. Janeway’s laboratory in 1997 (18) while working on the concept that pathogen sensing is mediated by a set of germline-encoded pattern recognition receptors (PRRs) that detect the conserved products of microbial biosynthesis pathways, called pathogen-associated molecular patterns (PAMPs) (19, reviewed in 20). Notably, Nüsslein-Volhard was honored with the Nobel Prize in 1995 and B. Beutler and J. Hoffmann in 2011 for their work with Toll and TLR.

The LPS cell interaction is complex and involves LBP as an opsonin and CD14 as the acceptor of LPS-LBP on the cell membrane (mCD14), which subsequently complexes with TLR-4 (21). Cells lacking mCD14 can be activated with LPS with the aid of the secretory form of CD14 (sCD14) present in plasma. It has recently been shown that R-form LPS can induce cell activation through TLR-4 in a CD14-independent manner (22, 23). Myeloid differentiation factor 2 (MD-2) is an 18- to 25-kd soluble protein that occurs in two forms: either bound to TLR-4 in the Golgi and transported in this complex form to the cell surface or secreted as a soluble molecule. The TLR-4–MD-2 complex is required for LPS signaling (24). Crystal structure studies demonstrated that LPS interacts with a large hydrophobic pocket in MD-2 and induces the formation of an m-shaped receptor multimer composed of two symmetrically arranged copies of the TLR-4–MD-2–LPS complex (25). Once LPS binds to TLR-4–MD-2, the signaling process involves the recruitment of Toll–interleukin 1 resistance (TIR) domain–containing adaptor molecules to the cytoplasmic face of the TLR-4 cluster via homophilic interactions with the TIR domain of TLR-4. Two pathways are activated, depending on the adaptors involved: (a) an early myeloid differentiation factor 88 (MyD88)–dependent response, involving the adaptors MyD88 and MyD88 adaptor-like (Mal), which is also called TIR domain–containing adaptor protein (TIRAP), and (b) a delayed MyD88–independent response, involving TIR domain–containing adaptor, which induces interferon β (TRIF) and the TRIF-related adaptor molecule. The MyD88-dependent pathway induces the early activation of nuclear factor κB (NF-κB), which leads to the production of inflammatory cytokines, and the MyD88-independent pathway induces the activation of interferon (IFN)–regulatory factor 3 (IRF3) and the late activation of NF-κB, which leads to the production of IFN-β and IFN-inducible genes (reviewed in Akira and Takeda (26)). It has been shown that TLR-4 sequentially activates these pathways. Beginning at the plasma membrane, TLR-4 activates...
In the context of sepsis, it is important to consider that there are many other PAMPs, TLRs, and PRRs involved. Currently, 13 TLRs have been identified in mammals: TLR-1, TLR-2, TLR-4, TLR-5, and TLR-6 are expressed on the cell surface, where they specialize in the recognition of bacterial products, including bacterial lipoproteins and lipoteichoic acids (TLR-2 as a heterodimer with TLR-1 or TLR-6), LPS (TLR-4), or flagellin (TLR-5). TLR-3, TLR-7, TLR-8, and TLR-9 are localized in intracellular compartments and specialize in viral detection and the recognition of nucleic acids, including double-stranded RNA (TLR-3), single-stranded viral RNA (TLR-7), or the unmethylated CpG DNA of bacteria and viruses (TLR-9) [reviewed in Akira and Takeda (26)]. There are other receptors that recognize specific microbial components, which include nucleotide binding and oligomerization domain–like receptors, retinoic acid–inducible gene I–like receptors, and C-type lectin receptors. The activation of different receptors occurs during infection and might be important for the recognition of a diverse range of microorganisms. This activation might also result in complementary, synergistic, or antagonistic effects, thus modulating innate and adaptive immunity [see Ishii et al. (28) for review].

**Modulation of LPS activity—Induction of hypersensitivity and tolerance**

The biological activity of LPS may be modulated in vivo and in vitro, and hypersensitivity and hyposensitivity might be induced under experimental conditions. It is known that susceptibility to LPS is also controlled by genetic and environmental factors and is thereby subject to variation. Whereas the hyporesponsive and tolerance to LPS have achieved a great deal of interest, hypersensitivity has been overlooked and may be equally important.

**Hypersensitivity to LPS**—Hypersensitivity to LPS has been demonstrated in vivo by the administration of hepatotoxic agent (N-galactosamine, N-GalN), in the presence of growing tumors and infection [reviewed in Galanos et al. (29)], N-GalN, administered prior to or concomitant with LPS, increases the sensitivity to LPS by a factor of up to 100,000-fold (30). In addition, the presence of Lewis growing tumors rendered animals hypersensitive to LPS. Previous exposure to gram-positive and gram-negative bacteria, such as Propionibacterium acnes and Salmonella typhimurium, respectively, also sensitizes mice to the lethal effects of LPS. In these cases, the underlying condition led to increased LPS-induced TNF-α production and an increased sensitivity to the toxic effects of this mediator (29). Accordingly, the pretreatment of mice with live or heat-killed *P. acnes* increases LPS-induced inflammatory cytokine production by a factor of 1,000-fold and reduces the LPS LD50 production by a similar factor in LPS-responder C3H/HeN and C57BL/10ScSn mice (29). Interestingly, pretreatment with *P. acnes* rendered the LPS-nonresponder C3H/HeJ susceptible to LPS, an effect that was not observed in C57BL/ScCr mice (29). The analysis of this finding led to the discovery of IFN-γ as a key mediator of LPS hypersensitivity [reviewed in Freudenberg et al. (31)], suggesting that the sensitized host is both hypersensitive to LPS and to all agents that activate TLRs in general [reviewed in Freudenberg et al. (9)].

The development of LPS hypersensitivity is mediated by IFN-γ or IFN-α/β, depending on the inducing microorganism. Interferon γ–mediated LPS hypersensitivity is induced by gram-positive and gram-negative bacteria and is mainly dependent on IL-12 but also occurs in an IL-12–independent pathway based on the activation of the signal transducer and activator of transcription (STAT) 4 by IFN-α/β and IL-18 signaling. Interferon α/β–mediated hypersensitivity to LPS occurred in mice during the early stages of viral infection, including lymphocytic choriomeningitis virus [LCMV; reviewed in Freudenberg et al. (9)] or adenovirus infection (32).

Hypersensitivity to LPS could be beneficial or deleterious in the course of an infection (33). Thus, in an otherwise lethal model of infection with *S. typhimurium*, *P. acnes*–mediated LPS hyporesponsivity might increase the resistance of mice to low bacterial challenge (10⁵ colony-forming units [CFUs]) or accelerate lethality to high bacterial challenge (10⁶ and 10⁷ CFUs). The protective effect of priming is explained by the enhanced LPS sensitivity of the host, which enables innate defense mechanisms to sense minute amounts of LPS contained in the invading bacteria and to mobilize an efficient antibacterial defense. The damaging effect of hypersensitivity was caused by the enhanced toxicity of the LPS already present in the relatively high numbers (10⁶ and 10⁷ CFUs) of infecting bacteria (33). Accordingly, this damaging effect was absent after superinfection with the gram-positive, LPS-lacking *Listeria monocytogenes*.

This situation seems to be more complicated in the case of sensitizing viral infections. Infection with LCMV resulted in susceptibility or resistance to secondary bacterial infections depending on the time course of the LCMV infection. During the early course of LCMV infection (3 days), when IFN-α/β is the key mediator of sensitization, accelerated lethality due to secondary infections with *S. typhimurium* or *L. monocytogenes* was observed. Although IFN-α/β is a well-known antiviral protein that protects against secondary viral infections, the IFN-α/β–improved sensing of bacterial TLR ligands did not make the host more resistant, but rather more susceptible to bacterial superinfections. At present, the reason for this phenomenon is still unclear. However, later in the course of LCMV infection (after 12 days), when IFN-γ drives the LCMV-induced sensitization, protection from both murine typhoid fever and *L. monocytogenes* infection was observed (34).

**LPS tolerance**—The hyporesponsive to LPS, also known as tolerance, has long been recognized but has received renewed interest because it resembles the modulation of cellular functions observed in septic patients (35). The induction of tolerance might be achieved in vitro and in vivo by previous exposure to small amounts of LPS, which renders animals resistant to an otherwise lethal LPS-challenge or hyporesponsive to LPS. There are basically two mechanisms of LPS tolerance in vivo: an early phase tolerance, which is lipid A–dependent and lasts for a few days, and a late phase, which is mediated by specific O-antibodies and is transferable with serum from LPS-tolerant animals [reviewed in Johnston and Greisman (36)].
Using the d-GalN model (30), we have shown that LPS-responsive macrophages are required for the induction of early tolerance to LPS (37).

Protection against LPS lethality and the inhibition of inflammatory cytokines (primarily TNF-α) are considered as the paradigms of LPS-induced tolerance. Different strategies have been used to study tolerance, and the results might depend on LPS conditioning, the cell types, or the in vivo models used (29). It is important to emphasize that the modulation of LPS biological activities could be achieved through pretreatment with low concentrations of LPS, indicating that tolerance does not occur because of cell exhaustion but rather by modulating the cellular response.

Pioneering studies have shown that rabbits become refractory to LPS-induced fever when injected daily with the typhoid vaccine (38). Some decades thereafter, it was shown that the priming of rabbits with LPS resulted in an inability to release TNF-α in response to a secondary LPS injection (39).

The effect of repeated LPS administration on TNF-α, IL-6, IL-8, and granulocyte colony-stimulating factor production was studied in cancer patients. Patients re-exposed to LPS at 2 weeks after the first intravenous LPS injection displayed significantly lower serum levels for all cytokines measured. Subsequently, a second LPS challenge performed 2 weeks later resulted in a partial restoration of the TNF-α response, whereas the level of IL-6, IL-8, and G-CSF responses remained low. A further LPS injection, which followed 2 weeks after the second challenge, produced nearly the same level of cytokines as that obtained after the first exposure to LPS, indicating that the effect of LPS tolerance had partially subsided. Not only did pretreatment of the patients with IFN-γ before a 12-h challenge prevent the induction of tolerance, but also the levels of TNF-α, IL-6, and G-CSF were increased above the levels observed after the first exposure to LPS. In contrast, the administration of IFN-γ could not prevent or revert the downregulation of IL-8 (40). Cytokine production and cellular changes were also investigated in cancer patients in response to the repeated daily intravenous administration of highly purified LPS. A single injection of LPS induced high levels of circulating TNF-α, IL-6, IL-8, G-CSF, and macrophage colony-stimulating factor. Repeated injections at daily intervals resulted in the downregulation of TNF-α, IL-8, G-CSF, and macrophage colony-stimulating factor responses to baseline level. In contrast, increased levels of IL-6 were observed up to day 5 of repeated LPS injections. Lipopolysaccharide caused a reduction in white blood cells during the 5 days of repeated injections, whereas the granulocyte overshoot recovered gradually diminished. Interestingly, the peripheral blood mononuclear cells (PBMCs) of patients treated with LPS for 5 consecutive days displayed no tolerance after ex vivo restimulation with LPS. In contrast, their capacity to produce TNF-α, IL-1β, and IL-8 was strongly enhanced (41). The downregulation of the proinflammatory cytokine response (TNF-α, IL-1β, IL-8, IL-6, and G-CSF) to endotoxin was also observed at 3 days after pretreatment with a nontoxic lipid A analog in cancer patients (42).

In addition to LPS and TLR-4, other TLR ligand family members could trigger the induction of tolerance to endotoxins, even though controversial results have been reported. The ability of a stimulus, other than LPS, to initiate tolerance is called cross-tolerance or heterotolerance. Cross-tolerance to LPS has been shown with the TLR-2 agonists MALP-2 (43), lipoteichoic acid (44), and endogenous mediators, such as IL-1β (45) and TNF-α (29). In contrast, Dobrovolskaia and collaborators (46) demonstrated that conditioning Escherichia coli LPS was not able to reduce the production of TNF-α upon challenge with LPS from Porphyromonas gingivalis, a TLR-2 agonist, in the peritoneal macrophages of mice. Conversely, reduced TNF-α production was observed when the cells were conditioned with LPS from P. gingivalis and challenged with LPS from E. coli. They did not observe cross-tolerance between LPS and the TLR-2/1 agonist Pam3Cys. We also did not observe tolerance to LPS in bone marrow–derived mouse macrophages pretreated with MALP-2, a TLR-2/6 agonist, or lipoteichoic acid (unpublished). However, the addition of LPS to human whole blood rendered monocytes tolerant to LPS and MALP-2 but not to flagellin, a TLR-5 agonist (47). The controversial results obtained by different researchers in different experimental models are at least in part due to insufficient purities of the microbial TLR ligands used. In biological tests, LPS contaminants present in agents used for testing or bacterial non-LPS contaminants in LPS preparations are responsible for misleading results.

Multiple mechanisms might be involved in tolerance to LPS: the presence of circulating mediators, such as IL-10 and transforming growth factor β (TGF-β); the excessive upregulation of LBPs and/or soluble CD14; the downregulation of LPS receptors on the cell surface of tolerant cells; and the downregulation of TLR signaling pathways. The latter is accompanied by an increase in the expression of the inhibitors IRAK-M, SOCS1, MyD88s, and SIGIRR and the inactive p50p50 homodimer of NF-kB, as well as a decrease in the expression of the p50p65 active heterodimer [for a review, see Cavaillon and Adib-Conquy (35) and Dobrovolskaia and Vogel (48)].

One important aspect of tolerance to LPS considers that it is not a true downregulation of the cellular response to LPS. Thus, despite the usual terms used to define this phenomenon (e.g., tolerance, desensitization, hyporesponse, and deactivation), Cavaillon and Adib-Conquy (35) proposed the use of the term “cellular reprogramming,” previously suggested by Zhang and Morrison (49), to better define LPS-induced functional modulation and its relevance in experimental and clinical sepsis. Zhang and Morrison (49) evaluated the effect of LPS pretreatment on the LPS-induced production of TNF-α and nitric oxide (NO) in mouse peritoneal macrophages. They observed both hyporesponse and hyperresponse to LPS challenge depending on the amount of LPS used for pretreatment and the type of response (TNF-α or NO production) investigated. These authors concluded that the LPS-induced NO and TNF-α responses are differentially regulated. Furthermore, the LPS-pretreated macrophages were not truly refractory but modulated in their capacity to respond to subsequent LPS activation (49).

Functional changes in human monocytes induced by LPS pretreatment in vitro further support the idea of cell
reprogramming rather than tolerance induction. We observed that although human monocytes pretreated with LPS exhibited reduced IL-6 production, they retained their ability to phagocytose bacteria (47). Moreover, consistent with del Fresno and coworkers (50), we observed the enhanced production of reactive oxygen species (ROS) upon bacterial uptake (47). Furthermore, the increased expression of cell surface CD64 and the decreased gene expression of HLA-DR and its regulator CIITA were observed in LPS-tolerant monocytes. Accordingly, such cells exhibited an increased ability to phagocytose pathogens, with impaired antigen presentation (50, 51).

The pretreatment of animals with LPS has been related to protective effects to injuries, such as thermal injury, ischemia/reperfusion, and hemorrhagic shock [reviewed in Cavaillon and Adib-Conquy (35)]. It has been long recognized that LPS can either raise or lower resistance to infection. More recently, Lehner and coworkers (52) found increased resistance to Salmonella enterica serovar typhimurium in endotoxin-tolerant mice, despite an attenuated cytokine response, and Rayhane and coworkers (53) showed that LPS-induced tolerance protects mice from fungal infections. Nevertheless, increased susceptibility to infections has also been described. The injection of LPS into the portal vein of rats resulted in increased circulating TNF-α levels and a concomitant impaired clearance of aerosol-inoculated Pseudomonas aeruginosa. Interestingly, this negative effect on the pulmonary host defense against P. aeruginosa could be mimicked through the prior intravenous administration of recombinant TNF-α (54).

**Inflammatory response during clinical sepsis**

As shown in the previous section, in addition to the impact of genetic background, LPS susceptibility is greatly influenced by experimental conditions. It is known that human susceptibility to LPS is also controlled by genetic and environmental factors and is thereby subject to variation. Therefore, concerning this interesting aspect of LPS biology, we should consider that sepsis does not usually occur in a healthy patient, and his/her underlying disease and previous exposure to infection might influence his/her inflammatory response to the ongoing insult. As discussed above, changes of LPS susceptibility in either direction might be both advantageous and disadvantageous for the infected host (55).

Sepsis has been defined as a proven or suspected infection with a concomitant systemic inflammatory response that is characterized by hypothermia or hyperthermia, tachycardia, tachypnea, leukocytosis, or leukopenia (4). Accordingly, early studies have shown that increased levels of inflammatory mediators were mostly related to poor outcomes in septic patients (56, 57), but protective association has also been observed (58, 59). The lack of success using anti-inflammatory agents in the therapy of septic patients in clinical trials and the emerging knowledge concerning the regulatory mechanisms of the host response during sepsis led Bone (60) (a renowned leader in sepsis research and member of the consensus conference that defined sepsis and SIRS) to review a “fundamental flaw” in the concept: it was one-sided. Thus, he noted “that in response to the original inciting event (the inflammatory response), the body mounts a ‘compensatory anti-inflammatory response syndrome.’” Although the balance of these opposite forces would restore homeostasis, the predominance of one of them would be related with sepsis sequela (60).

Several studies have been conducted to characterize the dynamics of the inflammatory response during sepsis and the mechanisms governing proinflammatory and anti-inflammatory responses. It is currently accepted that the initial inflammatory response in septic patients is followed by a state of hyporesponse or immune paralysis that is related to the susceptibility to new infections and lethality (61, 62). Hotchkiss and Opal (63) stated that “sepsis-induced immunosuppression is increasingly recognized as the overriding immune dysfunction in these vulnerable patients.” This state of hypo-responsiveness is characterized by the low production of inflammatory cytokines in peripheral blood cells from septic patients upon restimulation in vitro (64–66) and the decreased expression of membrane-bound CD14 (mCD14) and HLA-DR on the surface of blood monocytes (67–69). Furthermore, reduced numbers of circulating lymphocytes, increased lymphocyte apoptosis, and a shift from T helper 1 and 2 (T_{H1} to T_{H2}) subpopulations also occur in sepsis [reviewed in Hotchkiss and Karl (61)].

The extent of the inflammation and immunosuppression during sepsis might depend on host and pathogen variables (61). Although functional changes in the inflammatory/immune response during sepsis involve many cell types, we focus our discussion on blood monocytes and lymphocytes and their interactions. The important role of neutrophils in sepsis has been reviewed elsewhere (70, 71).

**Peripheral blood mononuclear cells**—Although the biphasic character of sepsis (i.e., initial inflammatory stage followed by immunosuppression) is generally accepted, most clinical studies with peripheral blood cells of septic patients do not show an initial increase in the production of inflammatory cytokines in vitro but rather consistently show downregulated inflammatory cytokine responses to ex vivo stimulation (64–66). It is possible that hyper-responsiveness, which appears during the onset of sepsis, had already subsided and was no longer present in the patients under investigation. This idea is supported by our observation that the PBMCs from septic patients without organ dysfunctions produced higher levels of TNF-α and IL-6 upon LPS stimulation, whereas patients with severe sepsis and septic shock produced strongly decreased levels compared with healthy human volunteers (68). Notably, we previously observed that enhanced responses to heat-killed Staphylococcus aureus were concomitantly elicited with strongly suppressed TNF-α or IFN-γ responses to LPS in the blood cells of septic patients (72).

**Monocytes**—We evaluated whether the change in LPS-induced cytokine production during sepsis was related to the amount of LPS bound to the surface of monocytes and if this amount was modulated by changes in the expression of cell surface receptors on monocytes from septic patients [reviewed in Salomao (73)]. Using flow cytometry, we demonstrated that the LPS binding to monocytes from patients with severe sepsis and septic shock was similar to that in monocytes from healthy volunteers; however, in the patient group, TNF-α production was dramatically reduced (74).
As highlighted in the previous section, LPS-induced cell activation involves a complex mechanism in which LPS, in the presence of LBP, binds to CD14 and TLR-4-MD-2 and triggers intracellular signaling. In addition, CD11b/CD18 plays an important role in the optimal LPS response (75). Given that the expression of these receptors is, in fact, modulated by exposure to LPS or during infection, one could envisage that changes in monocyte function in patients with sepsis might be caused by changes in the expression of these receptors.

The LPS-induced expression of cell surface receptors in healthy volunteers and the evaluation of their expression on the surface of the monocytes of septic patients have led to conflicting results. We found that, in the whole blood from healthy volunteers, LPS induces a rapid increase in the expression of CD11b and CD11c on the surface of monocytes, as well as an initial increase followed by a decrease in the expression of mCD14. There were no changes in the expression of TLR-4 and TLR-2; a slight increase in the expression of TLR-4 was observed at 6 h after LPS addition (76). In septic patients, we found the decreased expression of mCD14 and increased levels of soluble CD14 (sCD14), likely due to proteolytic cleavage, compared with healthy volunteers. There were no observed differences between the two groups in the expression of TLR-2 and TLR-4 (68) or CD11b (Silva et al., submitted). There are reports showing the decreased (77) and preserved expression of mCD14 (78) and CD11b (79), Silva et al., submitted, as well as the enhanced, preserved, and diminished expression of TLR-2 and TLR-4 in septic patients compared with healthy volunteers. There are reports showing the decreased (77) and preserved expression of mCD14 (78) and CD11b (79), Silva et al., submitted, as well as the enhanced, preserved, and diminished expression of TLR-2 and TLR-4 in septic patients compared with healthy volunteers.

Thus, despite data supporting an unequivocal role for these receptors in LPS sensing and signaling, it is likely that changes in their expression levels on the cell surface might not account for the changes in monocyte functions observed in septic patients. Accordingly, we observed the unaltered expression of TLR-2, TLR-4, and similar downregulated expression of mCD14 on the surface of monocytes in patients, independently of the severity of sepsis (68). Therefore, the changes in the expression of these relevant receptors could not explain the upregulation and downregulation of the LPS-induced cytokines in the early and late stages of sepsis (68). Interestingly, we found a similar pattern of IL-6 production by the PBMCs from patients with sepsis, severe sepsis, and septic shock when we used LPS, IL-1β, or TNF-α as agonists (68). This evidence suggests regulatory mechanisms below the surface receptors consistent with the postulated intracellular control of LPS-induced activities in tolerant cells [reviewed in Cavaillon and Adib-Conquy (35), Dobrovolskaia and Vogel (48), and Biswas and Lopez-Collazo (51)]. Accordingly, the LPS-induced TLR signaling pathway was modulated in tolerant human monocytes without affecting TLR-4 and CD14 expression on the surface of monocytes (83).

In addition to their ability to produce proinflammatory cytokines, other functions of monocytes, such as the production of ROS or NO, participate in antimicrobial defense. Interestingly, monocytes from septic patients who were hyporesponsive with respect to the production of inflammatory cytokines (68) displayed an enhanced production of ROS and NO in response to LPS and gram-negative or gram-positive bacteria in our recent studies (84, 85). This result indicates that the modulation of monocyte function during sepsis was differently regulated for inflammatory cytokines and reactive oxygen and nitrogen species [reviewed in Salomao (73)] (Fig. 1). A similar phenomenon, coincident with the depressed production of cytokines and an enhanced production of ROS and NO, was observed in an in vitro model of LPS tolerance (see the above section).

Two major functions of monocytes/macrophages are to present antigen and activate T cells through costimulatory molecules. Decreased HLA-DR expression was associated with infectious complications in trauma patients (86). We observed a decrease in the expression of HLA-DR on the monocytes of septic patients, both constitutively and after stimulation with LPS in vitro (74). Importantly, the persistence of low HLA-DR expression was reported as an independent mortality factor in septic patients (69). The increased expression of programmed cell death 1 (PD-1), which is a member of the extended C28/CTLA-4 family of receptor regulators, was observed on the macrophages/macrophages of mice with severe bacterial infections and on monocytes of shock patients (87). Because the close association between the upregulated expression of PD-1 on monocytes and the functional decline of these cells during sepsis has been observed, the authors proposed that PD-1 could be used as a marker of macrophage/monocyte deterioration. A recent study evaluating the expression of costimulatory molecules on antigen-presenting cells (APCs) in the spleens from patients dying of sepsis demonstrated the increased expression of the inhibitory ligand PD-L1, the decreased expression of the stimulatory ligand CD86, and the reduced expression of HLA-DR (88).

Thus, the phenotype and function of monocytes in human sepsis, e.g., the reduced expression of HLA-DR and costimulatory molecules and the increased expression of CD64, the reduced production of inflammatory cytokines, and the preserved ROS and NO generation, resemble those observed in LPS-tolerant monocytes. These changes might indicate the reprogramming of functions to control inflammatory damage...
and preserve the ability to phagocytose and kill infecting microorganisms. In septic patients, these changes might lead to immunosuppression, with impaired innate and adaptive immune responses.

The inflammatory cytokine responses of monocytes from septic patients to LPS resemble, in part, those of alternatively activated macrophages (AAMs). Alternatively activated macrophages, which differ from classically activated macrophages (89, 90), can be generated under different conditions. Alternatively activated macrophages induced by the TH2 cytokines IL-4 and IL-13 exhibit increased arginase activity (repair activity) and enhanced mannose receptor expression. Alternatively activated macrophages induced by IL-10, TGF-β, glucocorticoids, or a combination of LPS and immune complexes produce high levels of IL-10 and low levels of IL-12 compared with other macrophage types [reviewed in Gordon (89) and Mosser and Edwards (90)]. Alternatively activated monocytes/macrophages were also induced by the coculture of human monocytes/macrophages with regulatory T cells (Tregs) (91). These cells exhibited an increased expression of CD206 (mannose receptor) and CD163 (hemoglobin-haptoglobin receptor), which are typical markers of AAMs. In addition, these cells exhibited increased phagocytosing activity and decreased HLA-DR expression. Furthermore, these monocytes/macrophages produced, upon LPS stimulus, strongly reduced TNF-α, IL-6, and IL-1 responses. Macrophage heterogeneity is likely to reflect the plasticity and versatility of these cells in response to microenvironmental signals (92).

We evaluated the expression of markers of AAMs in monocytes from septic patients and found a profound increase in the percentage of monocytes expressing CD163 and the mannose receptor CD206 compared with healthy volunteers (93). Previous studies determining the levels of circulating sCD163 showed that these soluble receptors were enhanced in patients with sepsis. Here, the degree of sCD163 enhancement paralleled the degree of reduction of the proinflammatory response and the severity of sepsis (94, 95).

Human and mice monocytes have also been characterized as classic and nonclassic, or inflammatory and resident monocytes, based on CD14/CD16 (for humans) and Ly6C/CX3CR1/CCR2 (for mice) expression (96). Because of their ability to produce proinflammatory cytokines, human CD14+CD16+ cells are also referred to as proinflammatory monocytes [reviewed in Serbina et al. (96)]. Fingerle et al. (97) characterized CD14+CD16+ monocytes as “small monocytes” and found this population to be increased in septic patients.

Lymphocytes—The adaptive immune response has long been recognized as relevant for the control of bacterial infection, and decreased lymphocyte TCD4+, TCD8+, and natural killer cell counts and a decreased proliferative response of blood lymphocytes have been found in sepsis (61). Recently, a renewed interest in lymphocyte dysfunction during sepsis emerged from elegant studies by Hotchkiss and coworkers (98) demonstrating increased apoptosis in humans and experimental sepsis (99). Blocking lymphocyte apoptosis in a murine model of sepsis (cecal ligation and puncture [CLP]) resulted in a marked improvement in survival of the animals (99). Accordingly, they demonstrated a dramatic reduction in lymphocyte populations in patients dying of sepsis (61).

Adaptive immunity is driven by innate immune cells through sensing microorganisms and presenting antigens in the context of major histocompatibility complex class II (MHC class II) and costimulatory molecules. As previously discussed, antigen presentation and costimulation are impaired in monocytes/macrophages from septic patients. T cell function is further suppressed by decreased CD28 expression (whose ligand in APC is CD80/CD86) and increased PD-1 and CTLA-4 expression, which are inhibitors of CD28 activity on CD4 and/or CD8 T cells, as shown in patients dying of sepsis (88).

T cells may differentiate into various subsets, which play a major role in inflammatory and infectious diseases [for a review, see Mosmann (100)]. It has been shown that T cell differentiation into T_{H1} or T_{H2} cells is modulated through APCs and the milieu of secreted cytokines (100). It is also well known that type 1 cytokines (IFN-γ) exert a positive feedback on APCs, whereas type 2 cytokines (IL-4 and IL-10) promote downregulation of the immune response. Previous work showed a shift from T_{H1} to T_{H2} cytokine profiles following trauma, burns, and infection. Accordingly, O’Sullivan et al. (101) observed the decreased production of IFN-γ and increased production of IL-4 in patients with burns and trauma, whereas Heidecke et al. (102) observed decreased T lymphocyte production of IL-2, TNF-α, and IFN-γ and the unaffected production of IL-4 and IL-10 in patients with peritonitis.

Two other TCD4+ lymphocyte subpopulations, Treg and T_{H17} cells, have been characterized, and they exert major antagonistic effects on the inflammatory response, which may play a role in sepsis. Natural Tregs, which specifically express the forkheads/winged-helix family transcription factor Foxp3, are fundamental for maintaining self-tolerance and immune homeostasis (reviewed in 103). Regulatory T cells suppress the proliferation of naive T cells and their differentiation to effector T cells, the effector activities of differentiated TCD4+ and TCD8+ cells, and the function of natural killer cells, macrophages, and dendritic cells by mechanisms that include the secretion of immunosuppressive cytokines, cell contact–dependent suppression, and the modulation of APC function.

An increased percentage of TCD4+CD25+ cells (Tregs) among CD4+ lymphocytes was found in septic patients compared with healthy individuals because of a decreased proportion of TCD4+CD25− cells (104). The increase in circulating Treg numbers correlated with a decrease in the lymphoproliferative response (105). From these investigations, Venet et al. concluded that increased circulating Tregs contribute to lymphocyte anergy in patients with septic shock. Another study evaluating Tregs in septic patients did not demonstrate an increased proportion of Tregs at admissions. Instead, an increase was observed in follow-up samples obtained 5 days after admission (106). We evaluated the presence of Tregs in septic patients and found that the percentages of Tregs did not differ between patients and healthy volunteers (93). It is possible that the increased percentage
of Tregs observed in initial studies is not a general finding in different cohorts of septic patients. In experimental studies, the adoptive transfer of in vitro-stimulated CD4+CD25+ Tregs increased bacterial clearance and improved survival in mice with CLP-induced polymicrobial sepsis (107). Moreover, Scumpia et al. (108) found that increased natural CD4+CD25+ Tregs and their suppressor activity did not contribute to mortality in murine polymicrobial sepsis. Recent evidence supports a role of Treg in sepsis-induced immunosuppression and late susceptibility. In these experiments, mice submitted to CLP were susceptible to an otherwise sublethal challenge of Klebsiella pneumoniae, an effect that was prevented by the depletion of Tregs (109). Thus, more studies are needed to elucidate the role of these cells in the sepsis-induced inflammatory response and induced immune suppression.

T(H)17 has recently been characterized as a new distinct TCD4+ subset (110). Naive TCD4+ cells differentiate into the T(H)17 subset in the presence of IL-6 and TGF-β, and their proliferation and differentiation are supported by IL-23 and IL-1 secreted from myeloid APCs (111, 112). T(H)17 development relies on the activity of a lineage-specific transcription factor identified as the orphan nuclear receptor ROR-γt. T(H)17 cells produce IL-17A, IL-17F, IL-21, and IL-22; IL-17 plays a major role in linking adaptive and innate immunity. It induces proinflammatory cytokines and chemokines, increases the recruitment of neutrophils, and increases the production of prostaglandin E2 and NO [reviewed in Bettelli et al. (113)]. Protective and detrimental roles for IL-17 have been shown in experimental infections, but information on IL-17 in clinical sepsis is scarce.

Protective role for IL-17 has been shown in mice infected with bacteria and fungi. Interleukin 17R knockout (IL-17R−/−) mice presented impaired neutrophil recruitment into the alveolar space and reduced levels of G-CSF and MIP-2 mRNA and protein in the lung, as well as increased lethality in a model of Klebsiella pneumoniae lung infection in mice (114). Increased susceptibility was also observed in IL-17R−/− mice infected with Candida albicans, whereas the expression of IL-17A protected wild-type mice from a lethal challenge (115). In contrast, detrimental role for IL-17 was shown in a model of CLP-induced sepsis, in which the intravenous injection of anti-IL-17 improved survival in mice submitted to CLP and decreased the serum levels of IL-6 and TNF-α (116). In humans, increased IL-22 plasma levels were found in patients with abdominal sepsis (117).

We evaluated CD4+ T differentiation into T(H)1 cells (IFN-γ-producing cells), T(H)17 cells (IL-17-producing cells), and Tregs (CD4+Foxp3+CD127− and CD4+CD25+CD127−) in septic patients. As previously described (67, 98), we observed a profound lymphopenia and decreased CD4+ T cell counts in septic patients (93). This low CD4+ T cell number concerns the three subpopulations we evaluated, i.e., T(H)1, T(H)17, and Tregs. No difference in the percentage of Tregs was found between healthy volunteers and septic patients upon admission or between patients’ samples from admission and follow-up (93). Furthermore, we observed an increased proportion of CD4 T lymphocytes producing IL-17 in septic patients. These T(H)17 cells exhibited higher reactivity to PMA/Io and produced higher levels of IL-17 compared with those produced by similarly activated T(H)17 cells from healthy donors. The hyperreactive T(H)17 might be responsible for the unpaired functions of neutrophils, such as phagocytosis, and the generation of ROS in septic patients (84, 118). In addition, it represents one exception in the overall downregulation of T cell immune functions observed in septic patients.

In contrast to our study and previous studies showing different changes in T cell subsets in septic patients, the profound lymphopenia observed in septic patients was shown to equally affect the diverse T lymphocyte subpopulations in a recent study. Evaluating CD4+ T cell–specific transcription factors for T(H)1 (T-bet), T(H)2 (GATA-3), Treg (Foxp3), and T(H)17 (ROR/T) in peripheral blood cells of patients with septic shock, Venet et al. (119) found all subpopulations to be dramatically decreased compared with healthy volunteers.

One should keep in mind that the changes in circulating human blood monocytes and lymphocytes may not correspond to those ongoing in tissues. Nevertheless, similarities between blood cells and tissue cell dysfunctions were recently reported in patients dying of sepsis (88).

**Gene expression: LPS, tolerance, and sepsis**

Genomic and proteomic studies revealed a wide spectrum of biological responses to LPS and bacterial infection that encompasses multiple cell functions. Numerous challenges have been faced to integrate the new and emerging knowledge from high-throughput, multiplexed assays, improving our understanding of the disease process and addressing the gap between basic science knowledge and therapy for sepsis. Efforts to develop tools have been made to reach this goal, and terms, such as physiologic genomics or translational systems biology, have been coined to characterize this field of research [for review, see Vodovotz and An (120) and Cobb et al. (121)]. In this review, we focus on our results and on a few fundamental studies contributing to our understanding of sepsis. These results support many of the observations reported in the previous sections and open new perspectives to understand the complexity of injury-induced immune activation.

The wide range of biological activities induced by LPS suggests that a large number of genes would be activated during endotoxemia. However, the number and the tight control of LPS-induced gene expression or repression found by Calvano et al. (122) following a single LPS injection in human volunteers were impressive. Their network analysis of the inflammatory response to endotoxin comprised 1,556 genes and their interactions, 1,214 genes responsive to endotoxin, 1,201 genes responsive to endotoxin, and 342 additional highly interconnected genes. Interestingly, over half of the genes displayed reduced abundance. The authors presented a prototypical inflammatory cell illustrating the temporal response of gene expression in the innate immune system and showing the progression from an acute proinflammatory phase to counterregulation and homeostasis. Notably, they found dysregulation of functional modules in mitochondrial bioenergetics, protein synthesis, and degradation (122).

Studies evaluating gene expression during LPS-induced tolerance were insightful to understand the modulation of
inflammatory response in LPS-tolerant cells and the mechanisms regulating gene expression during inflammation. Foster and colleagues (123), evaluating in vitro experiments in LPS-induced tolerance with mice bone marrow-derived macrophages, found two groups of differentially regulated genes. The “tolerable” (T) genes were not reinduced or induced to a lesser level in macrophages upon second LPS exposure, whereas the “nontolerable” (NT) genes were reinduced more rapidly and often more efficiently. Of paramount biological relevance is that proinflammatory genes are found in the T class, and antimicrobial genes are found in the NT class. These findings support their hypothesis that TLR-induced gene expression with different biological functions would have distinct regulation, which was demonstrated to occur through chromatin modifications at the level of individual promoters (123).

In addition, del Fresno and coworkers (50) observed T and NT genes in human monocytes made tolerant to LPS. Consistent with the data of Foster and coworkers (123), they found that class T genes encompass proinflammatory cytokine genes and genes regulating antigen presentation, whereas NT genes consisted of anti-inflammatory factors and antimicrobial effectors. Of interest, an IL-12\textsuperscript{low}, IL-23\textsuperscript{low}, and IL-10\textsuperscript{high} profile was found in tolerant monocytes, which is characteristic of the M2 phenotype of macrophages. The tolerant human monocytes exhibited enhanced CD64 expression, a marker for phagocytosis, and decreased expression of MHC class II molecules. Accordingly, tolerant cells displayed elevated phagocytic activity and decreased antigen presentation (50).

We focused our studies on the TLR signaling pathway and used polymerase chain reaction (PCR) array to evaluate gene expression in a model of LPS-induced tolerance using human PBMCs (83) and sepsis in humans (124). In these experiments, we analyzed the expression profile of 84 genes related to TLR-mediated signal transduction. Of the genes evaluated in the tolerance study, approximately one third was upregulated, one third was downregulated, and one third remained unchanged after LPS challenge. Thus, the biological significance of LPS-induced tolerance was also considered in our study in genes downregulated by LPS. The persistence of this negative gene regulation was included as nontolerant genes, and the reversal of this downregulation was included as tolerant genes, where tolerance indicates upregulation of gene expression. In LPS-tolerant human monocytes, we confirmed the differential

![Toll-like receptor signaling pathway in LPS-tolerant mononuclear cells.](image-url) Tolerance was induced by pretreatment with 1 ng/mL of LPS for 48 h, and cells were challenged with 100 ng/mL LPS for 2, 6, and 24 h. Gene expression was compared with nontolerated cells. The genes shown in red were induced, and the genes shown in blue were repressed (fold change ≥2). Genes not influenced by LPS stimulation are shown in white. Tolerant genes are represented with bold boundaries, whereas nontolerant genes are represented with normal boundaries. Modified from Mendes et al. (83), with permission. Copyright (c) 2011 by Elsevier. All permission requests for this image should be made to the copyright holder.
FIG. 3. Toll-like receptor signaling pathways in PBMCs from patients with sepsis (A), severe sepsis (B), and septic shock patients (C) are compared with healthy volunteers. The genes shown in red were induced (fold change ≥2), the genes shown in blue were repressed (fold change ≤2), and the genes shown in white were not differently expressed (fold change ≤2). Based on data from Salomao et al. (124).
regulation of gene expression upon LPS challenge, the down-regulation of inflammatory cytokines and chemokines genes (e.g., TNF-α, IL-12, CCL2, and CXCL10), and the upregulation of IL-10 (83). Consistent with previous studies (50, 125), we found disrupted activation of adaptive immunity, i.e., inhibited expression of genes coding the costimulatory receptors CD80 (T) and CD86 (NT), and the inhibition of IL-12 (T). Furthermore, genes encoding NF-κB were “tolerizable” and downregulated, whereas the inhibitor NF-κBIA was not tolerated and induced (Fig. 2). Impaired NF-κB activation was also reported in earlier studies, an effect attributed to increased expression of the p50 subunit (126).

There is controversy regarding TRIF-dependent pathway regulation during LPS tolerance. Biswas and Lopez-Collazo (51) demonstrated that, in LPS-tolerant mouse embryonic fibroblasts, the induction of MyD88-dependent proinflammatory genes encoding TNF-α and CCL3 is suppressed, whereas the induction of TRIF-dependent IFN-α/β and CCL5 genes is enhanced. These authors reported the involvement of TRIF/IRF3-induced type I IFN in the elicitation of LPS tolerance in mouse embryonic fibroblasts [reviewed in Biswas and Lopez-Collazo (51)]. In contrast, Foster et al. (123) did not find a correlation between IFN-α/β receptor dependence and class T and NT gene expression in tolerant bone marrow–derived macrophages, indicating that class NT genes are not regulated through IFN-α/β feedback. In addition, Piao et al. (127) found that LPS-induced association of TLR-4 with TRIF and TRIF with TBK1, as well as activation of TBK-1 kinase and IRF3, was inhibited in LPS-tolerant human monocytes. Accordingly, although the TRIF pathway is underrepresented in the human TLR signaling pathway RT profiler PCR array used to analyze human tolerant PBMCs, we could see that TRIF and IFN-β are downregulated upon restimulation with LPS (83).

Using the above PCR array (83), we evaluated the expression of TLR pathway genes in PBMCs and neutrophils (polymorphonuclear leukocytes [PMNs]) from septic patients (124). Considering the potential dynamics of gene expression along the stages of sepsis, we divided the patients into three groups: sepsis (patients without organ dysfunction), severe sepsis (patients with sepsis and organ dysfunction), and those developing septic shock, as described in a sepsis consensus conference (4). Five genes were differently expressed (≥2-fold change and P [analysis of variance and posttest] ≤ 0.05) in PBMCs from septic shock patients compared with healthy volunteers: PRKRA, IL-1α, NFKB1, NFKB2, and REL. Using fold change as reference, other genes concerning the TLR signaling pathway are possibly upregulated and downregulated (≥2-fold change) in PBMCs in all groups of septic patients (Fig. 3). Upregulated genes mostly consisted of TLR receptors (TLR-1, TLR-2, TLR-4, TLR-6, and TLR-8) and adaptors or TLR interacting proteins (CD14, HSPA1A, and LY96). The downregulated genes largely consisted of downstream pathways and target genes, including the NF-κB pathway (CCL2, IKKKB, IL1A, IL1B, IL2, IL6, NFB1, NFB2, NFKKB, and REL), JNK/p38 pathway (MAPK8), and the effectors IRAK2, NR2C2, and PRKRA. Interestingly, in patients with more severe disease (severe sepsis and septic shock), a trend to have low numbers of upregulated genes and/or higher numbers of downregulated genes was observed in PBMCs (124) (Fig. 3). The expression pattern of downregulated genes in such patients resembled the expression pattern that we found in LPS-tolerant PBMCs (Fig. 2). In contrast, a higher number of upregulated genes was found in the PMNs of all septic patients. The upregulated genes were found in all functional groups evaluated (124).

A number of studies evaluating genome-wide expression have been performed in septic patients and were recently reviewed (128). Evaluating 12 studies with 784 individuals, Tang et al. (128) observed that sepsis induces an immediate activation of pathogen recognition receptors, accompanied by activation of signal transduction cascades. However, changes in inflammation-related genes were highly variable among the studies. They concluded that neither a distinctive proinflammatory/anti-inflammatory phase nor a clear transition from a proinflammatory to anti-inflammatory phase could be observed during sepsis (128).

Studies evaluating injured patients with or without infections have provided relevant and consistent information. A genome-wide expression analysis of circulating leukocytes in trauma patients found a wide range after injury and provided interesting insights into the interactions of monocytes with lymphocytes (129). Of interest, the monocyte- and T lymphocyte–enriched populations revealed unique gene expression patterns. Reduced expression of costimulatory genes, such as HLA-DR and CD86, on monocytes, and increased expression of genes involved in the T cell inhibitory pathway led to the proposition of an imbalanced interaction of monocytes and lymphocytes, driving T cell inhibitory pathway leading to the proposition of an imbalanced interaction of monocytes and lymphocytes, driving T cell apoptosis, reduced proliferation, and the inhibition of T cell and monocyte activation (129). Finally, an elegant prospective study was conducted evaluating the genome-wide expression patterns of blood leukocytes from 167 patients with severe blunt trauma with hypotension or acidosis and from 133 severe burn patients followed up for 28 days. They found significant changes in leukocyte mRNAs over 28 days following blunt trauma, representing greater than 80% of the human genome, with the greatest changes observed in the first 12 h after the injury. The term “genomic storm” was used to capture this magnitude of transcriptome reorganization. Genes with increased expression included those related to innate immunity, pathogen recognition, or inflammation, and genes with decreased expression included those related to antigen presentation and T cell activation. Interestingly, the direction and magnitude of peak perturbations did not differ between patients with uncomplicated clinical recovery. The difference in gene expression was not qualitative but quantitative, related to the magnitude and the time to return to normal expression. The authors concluded that the temporal nature of the current SIRS/compensatory anti-inflammatory response syndrome paradigm is not supported at the level of the leukocyte transcriptome but, instead, that alterations in the expression of classical inflammatory and anti-inflammatory and adaptive immune genes occur simultaneously, not sequentially, after severe injury. Complicated recoveries are delayed, resulting in a prolonged, dysregulated immune-inflammatory state (130).
Coupling genomic with proteomic studies will certainly improve our understanding of the inflammatory ongoing process in sepsis.

CONCLUSIONS

A large amount of information concerning the immune response to infection has emerged in the last 20 years since sepsis was first defined as an SIRS triggered by infection. This knowledge encompasses how the host senses and controls infection, including the discovery of TLR4 as the LPS gene, the implications of innate immune cells as drivers of the adaptive response to infection, the modulation of multiple accessory molecules that are stimulatory or inhibitory of monocyte/macrophage and lymphocyte interactions, and finally the complexity of infection/injury-induced immune response could be better appreciated with genomic and proteomic studies. Lipopolysaccharide was a useful tool in many of these studies. This new information allows a better understanding of sepsis pathogenesis and might be useful in designing new therapeutic strategies.

Soon after the concept of SIRS was established, the role of the counterregulatory, anti-inflammatory response emerged as pivotal for the outcome of sepsis (60). A biphasic model was proposed, with an inflammatory phase followed by an anti-inflammatory phase. This anti-inflammatory phase, also described as immunosuppression, emerged as a major dysfunction in septic patients (61, 63, 88). It is not clear whether the biphasic response indeed occurs or if both responses are present from the beginning of sepsis, one predominating over the other. Genomics studies did not support the two phases of sepsis, in that no reorganization of the genome response was found following sepsis [reviewed in Tang et al. (128)] and in trauma patients complicated or not with sepsis (130). In any

![Bacterial sensing and inflammatory response during sepsis.](image)

FIG. 4. Bacterial sensing and inflammatory response during sepsis. (A) Bacterial sensing and induced cellular activation. In initial infectious process, microorganisms and their products (PAMPs, such as LPS) are recognized by innate immune cells, such as macrophages, through the PRRs, triggering intracellular pathways of inflammatory response. (B) Amplification of inflammatory and immune response. Activated macrophages will synthesize mediators, such as TNF-α, IL-12, and chemokines, which coupled with increased expression of antigen-presenting receptors (HLA/MHC) and costimulatory molecules, such as CD80 and CD86, induce lymphocyte activation, neutrophil recruitment, and interaction with endothelial cells. Monocytes and PMNs will generate ROS and NO, among other mechanisms of microorganisms’ killing. This systemic response will induce systemic signs and symptoms. (C) Control of inflammatory response. Monocytes/macrophages reduce the production of inflammatory cytokines and the efficiency of T cell activation, by decreasing the expression of HLA/MHC and increasing the expression of coreceptors with inhibitory activity, such as PD-L1. They retain the ability of phagocytosing and microorganism’s killing through the expression of Fc receptors (CD64) and generation of ROS and NO. This phase represents the return to homeostasis in cases of spontaneous infection control, successful antimicrobial therapy, and recovery of underlying disease. On the other hand, lack of monocytes/macrophages to present a robust inflammatory response will represent a status of immunosuppression in patients worsening underlying disease, with failure of antimicrobial treatment, persistent organ dysfunction, and needs for invasive support therapy. These patients may be susceptible to new or recrudescent infection.
case, it seems clear that immunosuppression is a major problem in patients with nonresolving sepsis (63).

In this context, it is also important to consider that immune paralysis might comprise the reprogramming of cell function to control inflammation (35). In LPS tolerance models, gene expression modulation prevents tissue damage caused by excessive inflammation, whereas the mechanisms involved in antimicrobial defense are maintained (50, 83, 123). Functionally, LPS-tolerant monocytes produce low amounts of inflammatory cytokines and present preserved phagocytic activity and intracellular killing, coupled with impaired antigen presentation (47, 50). Similarly, monocytes from septic patients also produce low amounts of inflammatory cytokines in vitro, coupled with ROS and NO production upon bacterial uptake (84, 85).

A model integrating these findings would be that, early in the infection process, an initial inflammatory response is raised when innate immune cells sense bacterial products and activate the adaptive immune response. This mechanism would implicate an organized immune response with restriction of the initial infectious focus. In cases with an overwhelming inflammatory response, which may be due to host and bacterial factors, as observed in meningococemia and some patients with S. aureus bacteremia, this initial response might be deleterious. In sequence, monocytes/macrophages would repress the synthesis and release of inflammatory mediators and reduce antigen presentation and stimulatory accessory molecules, halting the amplification of the immune response, while maintaining control over the infectious. This response would be effective during the course of a self-controlled infection or in a successfully treated patient. In those patients not resolving the initial insult, both because of microorganism factors (e.g., bacterial challenge and resistance to antimicrobial agents) or host factors (e.g., immune deficiency or persistence of predisposing factors, such as patients in coma and under mechanical ventilation), the lack of inflammatory cytokines and activation of adaptive immunity will result in an ineffective response to control a persistent infection or a new event, as seen in more severe patients and those dying of sepsis (Fig. 4).

Another important issue raised in this review is that the LPS-induced host response is dramatically modulated by environmental factors, and the resulting hypersensitivity or tolerance to LPS has ambiguous consequences for resistance or susceptibility to infection. Accordingly, during normal life, humans might also have different susceptibility to infections as a result of previously experienced infections or underlying diseases. The role of underlying disease in the prognosis of septic patients was settled approximately 50 years ago by McCabe and Jackson (131) but is seldom considered in studies concerning the pathogenesis of sepsis.

**Future directions**

Experimental models have been of paramount importance to improve our understanding of the pathophysiology of sepsis. They have, however, been less suitable to predict rationale for pathogenesis-oriented target therapy. This gap should be overcome by the use of experimental models more closely related to human disease as well as by clinical studies focusing on pathogenesis. There are, among others, three challenges to be faced in human studies evaluating the pathophysiology of sepsis: the duality, e.g., protective and detrimental; the complexity; and the dynamics of inflammatory response. One may foresee that, in the future, every approach based on adjunctive immune therapy will be driven by a previous immune evaluation of the patient.

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