

# Lipopolysaccharide: An Endotoxin or an Exogenous Hormone?

**John C. Marshall**

Department of Surgery and the Interdepartmental Division of Critical Care Medicine, University of Toronto, Toronto, Ontario, Canada

Conventional models of the pathogenesis of sepsis assume that microorganisms or their products are necessarily injurious to the host. In contrast, an evolutionary perspective suggests that host-microbial interactions are a symbiotic model and that disease results from the disruption of a mutually beneficial homeostatic state. Lipopolysaccharide (LPS) from gram-negative bacteria is a prototypical trigger of sepsis and a target for the development of novel therapeutics. The biological mechanisms underlying the recognition of, and response to, LPS are more characteristic of a hormone than of a toxin. All mammals carry endogenous stores of LPS and express dedicated carrier proteins, a cellular receptor complex, and mechanisms that specifically antagonize the response to LPS. Disruption of any component of this complex recognition system jeopardizes host defenses against infection with exogenous microorganisms. Thus, LPS is not less an endotoxin than an exohormone, and its neutralization may potentially result in either benefit or harm.

*...one of the most important changes we can make is to supersede the 20th-century metaphor of war for describing the relationship between people and infectious agents. A more ecologically informed metaphor, which includes the germs'-eye view of infection, might be more fruitful...they are equally part of the superorganism genome with which we engage the rest of the biosphere.*

—Joshua Lederberg [1]

Interactions between the animal and microbial worlds are intimate, complex, and vital to the good health of both. More than this, they have been of fundamental evolutionary importance. Over the course of close to 1 billion years, the mutual accommodation of the microorganism and the multicellular host has enabled each to survive and adapt to a changing environment. Indeed, the basic processes of cellular respiration in eukaryotes, and of photosynthesis in plants, are possible only because of the presence in the cell of a microbial

parasite—the mitochondrion in the former [2, 3] and the chloroplast in the latter [4]. The evolution of multicellular organisms was possible only because of microbial intracellular parasites.

Yet, this intricate symbiotic relationship has had a darker side. Microorganisms are the trigger of a broad array of human diseases, from parasitic infections and malaria to acute infections in the traumatized host to more chronic illnesses, such as peptic ulcer disease [5], cancer, and coronary artery disease [6]. The intensity of this struggle over evolutionary time is imprinted in the human genome in genetic mutations, such as those responsible for hemoglobin S or thalassemia, which have permitted local human populations to survive in areas where malaria is endemic [7]. It is also seen in the development of complex systems of innate immunity that enable the host to respond to an acute

Reprints or correspondence: Dr. John C. Marshall, Rm. 4-007, Bond Wing, St. Michael's Hospital, 30 Bond St., Toronto, ON, Canada M5B 1W8 (marshallj@smh.toronto.on.ca).

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threat, often by exploiting unique features of that threat to achieve a survival advantage, just as microorganisms have used the same defensive, subversive strategy.

This infinitely fascinating tension between 2 superkingdoms of the living world—driven for each by the need to survive and the need to respond to external threats in order to do so—defines the biome of the planet [8]. For the physician, whose loyalties lie with the immediate interests of the species *Homo sapiens*, it is all too easy to lose sight of our evolutionary heritage and to seek victory, rather than accommodation, in approaching the ravages of human disease. Yet inherent in the concept of sepsis—defined as the host response to infection—is the nucleus of a realization that host-microbial interactions are complex and not necessarily adversarial and that, although infection poses a threat, the host may be at even greater risk as a consequence of responding to that threat. If the metaphor of a battle is appropriate to describe the interactions between humans and the microbial world, then the fight is less analogous to a battle between nations or ideologies than to the more fanciful battle of the sexes—for, despite tensions, each is dependent on the other for its ultimate survival.

Given this state of interdependence, it follows that the biological basis of this interaction bears more of the features of an endocrine interaction than of a toxic one. Thus, the cardinal endogenous microbial product that mediates host-microbial interaction—lipopolysaccharide (LPS) from the cell wall of gram-negative bacteria—might better be considered an exohormone than its more common designation as endotoxin. The distinction is not simply semantics or sophistry, but one of immediate relevance to attempts to modulate these interactions to improve human health.

## HOST-MICROBIAL INTERACTIONS IN HEALTH

**Commensal flora of the multicellular organism.** Virtually all multicellular organisms—from plants to invertebrate marine species to mammals—harbor a stable microbial flora in intimate proximity to cells of the host. This flora varies from one species to the next but, in the absence of disease, is remarkably stable over time.

The normal, healthy human being is a complex aggregation of mammalian and microbial cells. Although the interior of the human host is sterile, the epithelial surfaces are normally colonized by a complex microbial flora, established shortly after birth and persisting largely unchanged over the life span of the individual [9]. So extensive is this flora that microbial cells outnumber human cells by a factor of 10 to 1 in the healthy human [10] and represent somewhere between 500 and 1000 distinct species [11]. Because of the large number of gram-negative organisms that are included among these, the total mass of LPS, ~10% of the weight of the organism, is measured in grams. The fact that human volunteers become symptomatic

when they are administered 2–4 ng/kg LPS illustrates that the gut is a remarkable repository of microbial triggers of sepsis.

The normal state of interactions between humans and the indigenous flora is one of accommodation rather than disease. Even more than benign indifference, the concept inherent in the adjective “commensal,” the interactions between the gut mucosa and its associated immune tissues and the flora of the adjacent intestinal lumen are symbiotic in nature, each shaping the form and function of the other in fundamental ways.

Studies of gnotobiotic animals (animals that are either germ-free or colonized with a single defined microbial species) have revealed that the intestinal flora plays a critical role in normal immunologic, metabolic, and even anatomic development. Rawls et al. [12] used DNA microarrays to evaluate the impact of the intestinal flora on gut development in the zebrafish. They found that the presence of an intestinal flora alters the expression of 212 genes regulating a wide variety of processes in the gastrointestinal tract, including epithelial proliferation, nutrient metabolism, and innate immunity. Similar studies among germ-free mice confirm the impact of the gut flora on gene expression and show this influence to be greatest in the jejunum, where 267 genes are differentially regulated [13].

The indigenous flora exerts a variety of influences on intestinal structure and function. The indigenous intestinal microflora influences the rate of proliferation of the intestinal epithelium [12, 14], and the germ-free animal has increased numbers of cells secreting gastrin, motilin, and glucagon [15]. Development of the capillary network of the intestinal villi is impaired in the germ-free animal but can be restored by recolonization with *Bacteroides thetaiotaomicron* [16]. The normal flora also regulates patterns of glycosylation of gut epithelial cells [17] and influences myoelectric activity of the small bowel [18]. Intestinal healing is increased in the presence of an intact normal flora [19].

The intestinal flora exerts a potent influence on systemic immunologic homeostasis. Mice raised under germ-free conditions are highly susceptible to lethal infection with *Staphylococcus aureus* or *Klebsiella pneumoniae*; intriguingly, however, they are resistant to systemic endotoxin challenge [20, 21]. They are similarly vulnerable to infection with *Salmonella typhimurium*, and recolonization of the gut with *Escherichia coli* confers protection [22]. Lymphoid populations of the intestinal mucosa and Peyer patches are altered in germ-free animals [23, 24], and germ-free animals are unable to induce oral tolerance to enterally delivered antigens [25]. Stem cell mobilization in response to granulocyte colony-stimulating factor is impaired [26], and cytokine release by splenic or bone marrow-derived macrophages is altered [27].

The endogenous microbial flora can modify host systemic homeostasis, but the interaction is truly symbiotic. Microbial structure and function is also altered through its evolutionary

persistence within the gastrointestinal lumen. Consider the case of the intestinal obligate anaerobe *B. thetaiotaomicron*. *Bacteroides* species make up about one-quarter of the bacterial population of the human gut [28]. The genome for *B. thetaiotaomicron* includes multiple genes that have evolved to hydrolyze dietary polysaccharides [29] and to sense cues from the local intestinal environment [28], reflecting its adaptation to the local environment of the colon. At the same time, colonization of the gut of germ-free mice with *B. thetaiotaomicron* results in changes in mucosal gene expression. Hooper et al. [30] used DNA microarrays to evaluate the effects of colonization of germ-free mice with *B. thetaiotaomicron* on gene expression in the ileal mucosa. They demonstrated that ileal colonization was associated with inhibition of the expression of 23 probe sets and up-regulation of the expression of a further 95 probe sets, the latter including genes involved in nutrient absorption and metabolism (particularly lipids and glucose), mucus production, epithelial barrier integrity, toxin metabolism, neurotransmission and muscle development, and intestinal angiogenesis. Moreover, *Bacteroides* species can interact directly modify the function of host cells in the microenvironment of the gut epithelium. *B. thetaiotaomicron* elaborates a soluble factor that modifies glycosylation of intestinal epithelial cells [31] and can inhibit cellular inflammatory responses through a mechanism that involves enhancing the nuclear export of the nuclear factor (NF)- $\kappa$ B subunit, RelA [32]. Others have shown that nonpathogenic strains of *Salmonella* species can block the degradation of NF- $\kappa$ B inhibitor I $\kappa$ B and so inhibit nuclear translocation of NF- $\kappa$ B, thereby blocking the transcription of NF- $\kappa$ B-dependent proinflammatory genes [33].

**Endosymbionts.** Microbial species are found not only in stable extracellular association with host cells but also as intrinsic components of the cellular structure of eukaryotic organisms. Known as endosymbionts, they differ from intracellular pathogens because their presence is beneficial to the host cell. For example, cells of the tsetse fly harbor an endosymbiont, *Wigglesworthia glossinidia*, that is transmitted through the maternal genome and that generates vitamin B for the fly. Similarly, aphid cells are parasitized by *Buchnera aphidicola*, which plays a role in amino acid biosynthesis [34]. The coevolution of an endosymbiont and its cellular host is associated with the loss of redundant genes and exchange of DNA between the endosymbiont and the host nucleus, with the result that endosymbiotic DNA is incorporated into the host genome and the endosymbiont genome is much smaller than the smallest genome needed to sustain independent life [8, 35]. Transfer of microbial DNA to the eukaryotic nucleus is the likely explanation for the observation that the human genome contains >200 genes with high degrees of homology to bacterial proteins [36].

Endosymbiosis underlies the life and death of eukaryotic cells

and was an essential ancient development in the evolution of multicellular organisms. Mitochondria, the cellular organelles that subserve both energy production and the initiation of one pathway of programmed cell death in the eukaryote, are believed to have arisen from an ancient intracellular bacterial parasite of the alpha-Protobacterial family [3, 37].

The emergence of the eukaryotic cell was possible only because of ancient symbiotic relationships with the preexisting microbial world, and the evolution of multicellular organisms has been irrevocably shaped through intimate interactions with bacteria that have come to be an inextricable component of the larger organism. Microorganisms have been a major cause of human disease, although the most virulent tend to be those whose presence in the human environment has been relatively recent: Studies of the emergence of polymorphisms that confer protection against malaria, for example, show that these have arisen in only the past 10,000 years, concomitant with the emergence of agricultural societies [38]. The relationship between the human and microbial worlds has been predominantly one of mutual benefit, rather than enmity, however, and is sufficiently interwoven such that mammals have exploited these interactions to serve as components of the innate host defense against potential pathogens. From the perspective of the host, constituents of the endogenous flora subserve a role that is less analogous to that of a toxin than to that of a hormone.

## **TOXINS, HORMONES, AND THE CELLULAR RESPONSE TO HOMEOSTATIC CHANGES**

The ability of a fixed cell population to respond to a stimulus that may have originated at a remote anatomic site is a key requisite for the maintenance of homeostasis in multicellular organisms. This need is accomplished by 1 of 2 mechanisms: transmission of electrical impulses via the nervous system or dissemination of molecular mediators in the circulation. The terminology used to describe the latter reflects the consequences of the process for the host more than the distinct biological mechanism involved. A toxin (from the Greek word for poison) is defined as “a noxious or poisonous substance that is formed or elaborated either as an integral part of the cell or tissue, as an extracellular product (exotoxin), or as a combination of the two, during the metabolism and growth of certain microorganisms and some higher plant and animal species” [39].

A hormone (from the Greek verb meaning to arouse or set in motion) is defined as “a chemical substance, formed in one organ or part of the body and carried in the blood to another organ or part; hormones can alter the functional activity, and sometimes the structure, of just one organ or tissue or various numbers of them” [39].

The primary differences between hormones and toxins are that hormones are a product of the host, whereas a toxin arises exogenously, and that the activity of a hormone sustains ho-

meostasis, whereas that of a toxin is harmful. However, these distinctions are far from absolute. As we have seen earlier, the endogenous flora is an intimate part of the healthy host and plays a cardinal role in normal metabolic and immunologic homeostasis. At the same time, excessive levels of hormones can be harmful, as has been evident in high-profile cases of murder committed after the administration of exogenous insulin. Table 1 compares toxins and hormones.

Hormones are produced as the secretions of specialized cell populations that constitute endocrine organs and often, but not necessarily, are released into ductal systems. Their origins are endogenous, and the machinery required for their synthesis, release, and biological activity is encoded in the human genome. A classic toxin, on the other hand, is typically synthesized outside the host, although most biological molecules become toxic if their concentrations significantly exceed the range of normal values.

Hormone release occurs in a regulated manner in response to an acute change in internal homeostasis, either directly or through the intermediate action of a releasing hormone. The secretion of insulin by pancreatic  $\beta$  cells, for example, occurs through a multistep process. Increased cellular metabolism of glucose in response to increased extracellular glucose levels triggers closure of membrane ATP-sensitive potassium channels, which, in turn, activates  $Ca^{++}$  channels, resulting in an intracellular influx of calcium; this increased cytosolic calcium triggers exocytic release of insulin [40]. Cortisol secretion by the adrenal cortex occurs in response to the release of adrenocorticotrophic hormone in the hypothalamus; adrenocorticotrophic hormone release, in turn, is triggered by corticotropin-releasing hormone, whose transcription and release is regulated by neural inputs and inflammatory mediators such as IL-1 [41]. In contrast, exposure to toxins is unregulated and generally unpredictable.

Although some hormones such as insulin circulate unbound to plasma carrier proteins, others are highly bound to specific carrier proteins or albumin, and protein binding regulates their tissue availability in vivo. More than 99% of circulating thyroxine is bound to thyroxin-binding globulin, transthyretin, or albumin, and protein binding serves to direct hormone to spe-

cific tissue beds and to limit access to cellular receptors [42]. Similarly, >90% of circulating cortisol is bound to corticosteroid-binding globulin or albumin [43]; acute reductions in the levels of these proteins in trauma or critical illness confounds accurate measurement of cortisol levels and may contribute to altered end-organ responses [44, 45]. Toxins, on the other hand, lack specific, evolutionarily conserved carrier mechanisms.

The interaction of a hormone with its cellular target occurs through the engagement of a specific cellular receptor, whose engagement, in turn, generates intracellular signals that regulate gene transcription. The insulin receptor, for example, is a tetrameric, transmembrane tyrosine kinase composed of 2  $\alpha$  and 2  $\beta$  subunits. In the quiescent state, the  $\alpha$  subunit tonically represses the tyrosine kinase activity of the  $\beta$  subunit; engagement by insulin results in release of this inhibition, transphosphorylation of the  $\beta$  subunits, and downstream signaling through interactions with one or more of at least 9 distinct receptor substrates [46]. Cortisol exerts its activities through binding to an intracellular receptor, the glucocorticoid receptor, that serves as a nuclear transcription factor and modulates the activity of other DNA transcription factors [47]. The glucocorticoid receptor resides in the cytoplasm, bound to heat shock proteins. Cortisol binding results in liberation of the receptor from its molecular chaperones and its translocation into the nucleus, where it can modulate gene expression. Toxins do not share a single mode of action but generally exert their effects by usurping otherwise beneficial cellular processes. Cholera toxin, for example, binds to GM1 gangliosides in membrane lipid rafts to be taken up by the cell [48] and then activates adenylate cyclase within the intestinal epithelial cell, causing massive fluid secretion and the clinical manifestation of diarrhea [49]. Toxin A of *Clostridium difficile* inactivates Rho by monoglucosylation and so disrupts the epithelial cell cytoskeleton [50], whereas streptococcal pyrogenic exotoxins are superantigens that bind to the T cell receptor and evoke massive clonal activation and cytokine release [51].

Hormone activity is highly regulated. Cortisol, for example, can act through multiple negative feedback loops to regulate the mechanisms of the hypothalamic-pituitary-adrenal axis that lead to cortisol release [52]. In addition, hormonal activity can

**Table 1. Characteristics of hormones and toxins.**

Characteristic	Hormone	Toxin
Origin	Endogenous	Exogenous
Trigger	Releasing hormone	None
Transport	Carrier protein	None
Cellular recognition	Dedicated receptor	None
Cellular interaction	Regulates DNA transcription	Interferes with biological process
Neutralization	Counterhormone	None
Toxicity	Excess or deficit harmful	Dose-dependent toxicity

be antagonized by the release of counterhormones, for example, glucagon to counter the effects of insulin or calcitonin to counter those of parathyroid hormone. No such inhibitory influences exist for toxins, although, by evoking an immune response, they may be antagonized by specific antibodies.

Hormones mediate homeostasis, and disease results when hormone activity falls outside of an optimal range and is either insufficient or excessive. A toxin, on the other hand, has no optimal level of activity and instead demonstrates dose-dependent injury to the host.

The cardinal distinction between a hormone and a toxin derives not from differences in biological mechanism of action but from the consequences of such action for the host. In excess, a hormone can become a toxin.

### LPS: AN EXOGENOUS HORMONE

If the relationship between the healthy host and the indigenous flora can be considered a symbiotic one, then the relationship between microbial products and the host may resemble that of a hormone more than that of a toxin. The biology of the host response to LPS exemplifies this concept.

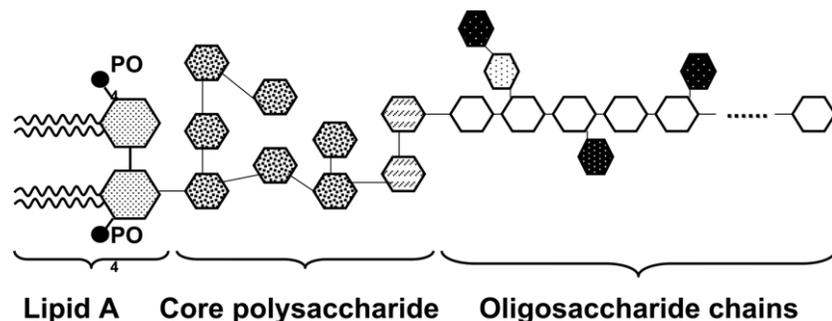
LPS is an intrinsic component of the cell wall of gram-negative bacteria, which, in turn, are ubiquitous symbionts in the gastrointestinal tracts of most animals. Originally identified by the German microbiologist Richard Pfeiffer in the late 19th century, LPS is the prototypical and best-studied trigger of the host inflammatory response that underlies the pathogenesis of sepsis [53].

The structure of the LPS molecule varies from one strain of bacteria to the next, but a constant feature of all species is the presence of a toxic lipid A moiety, covalently joined to a polysaccharide core, with or without variable oligosaccharide side chains (figure 1). Gram-negative infections result in systemic endotoxemia. However because of the presence of large amounts of LPS in the gut lumen and the environment, endotoxemia also occurs in a variety of noninfectious circumstances. Endotoxin can be detected in the blood of the majority

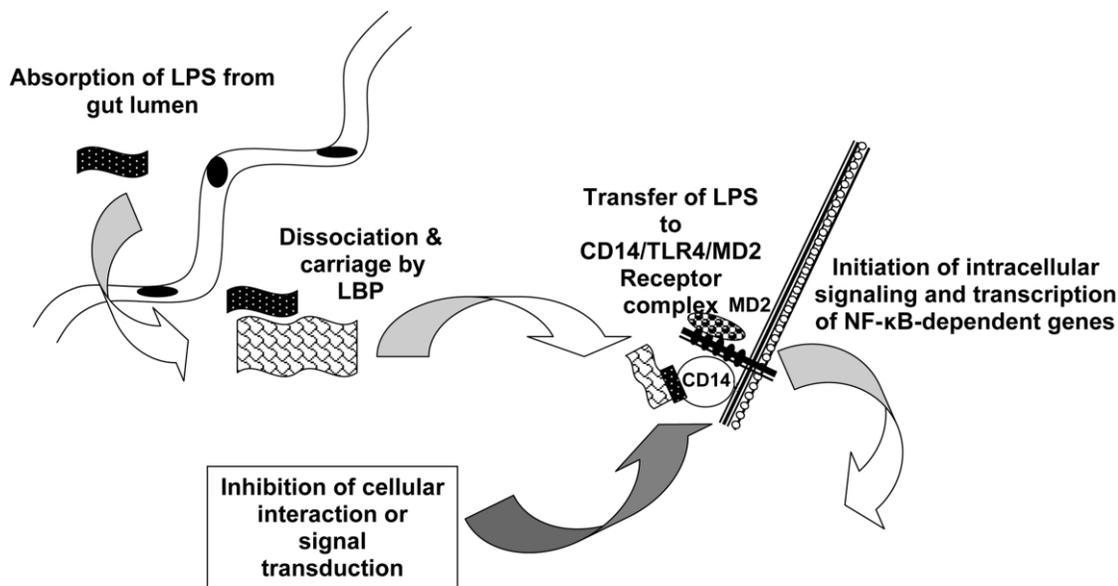
of patients with sepsis, independent of the nature of the infecting organism [54], and in most patients undergoing aortic surgery [55]. In fact, elevated levels of endotoxin are present in the majority of patients on the day of their admission to an intensive care unit, and endotoxemia is associated with non-specific illness severity, as measured by the APACHE score, as well as with the presence of infection (both gram-negative and gram-positive) and clinical manifestations of sepsis [56]. Endotoxin is ubiquitous in the external environment as well, and exposure can occur in cigarette smoke [57] or during mechanical ventilation [58]. Its ubiquity during times of acute physiological stress is reminiscent of the patterns of release of such classic stress hormones as cortisol or epinephrine.

The interaction of LPS with cells of the host innate immune system is both intricate and specific (figure 2). Aggregates of LPS bind to plasma proteins (e.g., albumin, LPS-binding protein [LBP], and soluble CD14) that serve to convert higher-molecular-weight endotoxin complexes to monomeric particles that can interact with host cells [59]. Bound complexes of LPS and LBP or soluble CD14 can interact with a specific cell surface receptor, Toll-like receptor 4 (TLR4). The complex of protein-bound LPS, TLR4, and an accessory protein, myeloid differentiation protein 2 (MD2), activates an intracellular signaling pathway utilizing kinase cascades and the transcription factor NF- $\kappa$ B to initiate the transcription of several hundred genes that contribute to the phenotype of the resulting systemic inflammatory response [53, 60, 61]. The details of this interaction are similar in many respects to that of an endogenous hormone whose evolutionarily conserved role is the support of innate immunity. In particular, disruption of the systems that facilitate recognition of LPS by the host can result in impairment of innate immune mechanisms, to the detriment of the host.

**LPS exposure arises from endogenous stores.** LPS is a microbial product; however, it is found in all mammalian hosts because of the presence of the indigenous gram-negative flora of the gastrointestinal tract. Absorption of gut endotoxin can cause endotoxemia during acute physiological stresses, such as



**Figure 1.** Schematic representation of the lipopolysaccharide molecule, comprising the phospholipid, lipid A, which anchors the molecule to the bacterial cell wall, covalently linked to a polysaccharide core and the variable oligosaccharide side chain, composed of multiple oligosaccharide repeats.



**Figure 2.** Endotoxin from gram-negative bacteria in the gut lumen enters the body either as a component of the whole organism or as polymers of free lipopolysaccharide (LPS). These polymers are dissociated by LPS-binding protein (LBP), which carries free LPS, and transfers it to the LPS receptor complex on cells of the innate immune system. CD14 is involved in recognition of LPS, whereas the Toll-like receptor 4 (TLR4)/myeloid differentiation protein-2 (MD2) complex binds CD14/LPS and results in the recruitment to TLR4 of a family of adapter proteins that initiate the process of intracellular signaling for gene transcription. NF- $\kappa$ B, nuclear factor- $\kappa$ B.

vigorous exercise [62, 63] or the resumption of feeding following periods of deprivation [64]. Systemic endotoxemia has been documented after multiple trauma [65] but also after ankle fracture [66] or colonoscopy [67], suggesting that LPS is readily absorbed from the gut. Thus, although endotoxin is not produced by human cells, it is released from stores that are present in all humans in response to a variety of acute stresses.

In contrast to classic hormones, such as cortisol, there is no known endotoxin-releasing factor that is capable of regulating endotoxin absorption. On the other hand, LPS itself might be considered a releasing hormone—absorbed from the gut in response to physiological alterations in splanchnic flow and capable of triggering the release of host-based cytokines from cells of the innate immune system.

**LPS has a dedicated carrier protein.** LBP is a 58-kDa glycoprotein synthesized as an acute-phase reactant both in the liver and by intestinal epithelial cells [68]. LBP is capable of dissociating complexes of LPS to yield LPS monomers that can interact with soluble or membrane-bound CD14, an interaction that is facilitated by albumin [69]. LPS binding to LBP occurs through the N-terminal portion of the LBP molecule (residues 91–100), whereas CD14 binding occurs through the C-terminal portion [70]. LBP increases the efficiency of LPS transfer to CD14 by 3 orders of magnitude, enabling monocytes to respond to concentrations of LPS as low as 10 pg/mL [71]. Higher concentrations of LBP blunt cellular activation in response to endotoxin by facilitating transfer of LPS from membrane-bound CD14 back to LBP [72].

**LPS interacts with a specific cellular receptor.** Cellular activation by LPS requires the coordinated engagement of a trimolecular receptor complex consisting of membrane-bound CD14, TLR4, and the adapter protein MD2 [73]. CD14, a 53-kDa glycoprotein present in both soluble and membrane-bound forms, lacks an intracellular tail and serves primarily to transfer LPS from the LPS/LBP complex to the signaling component of the receptor complex, comprising MD2 and TLR4. LPS bound to MD2 can activate TLR4 in the absence of CD14 [74] and permits cellular responses to picomolar concentrations of LPS [75]; interactions between LPS and MD2 are facilitated by clusters of basic amino acids, predominantly lysine, on the surface of the MD2 molecule [76]. TLR4 serves as the signaling component of the LPS recognition complex [77], recruiting adapter proteins through the intracellular Toll/IL-1 receptor domain that activate intracellular pathways, leading to inflammatory gene transcription [78].

**LPS signaling is specifically modulated by endogenous mechanisms.** The complex processes supporting the uptake of, and response to, LPS can be modulated at multiple levels (table 2). Bacterial LPSs differ in their ability to activate TLR4 signaling; thus, certain strains of LPS may serve as competitive antagonists of endotoxin activity [53]. The interaction of circulating LPS with its cellular receptor complex can also be inhibited by interactions with plasma proteins. For example, bactericidal/permeability-increasing protein is a neutrophil product homologous in its C-terminus with LBP; bactericidal/permeability-increasing protein can competitively bind and

**Table 2. Endogenous mechanisms inhibiting lipopolysaccharide (LPS) signaling.**

Mechanism	Example(s)
Circulating inhibitors	Bactericidal permeability-increasing protein
	LPS-binding protein; soluble CD14
	High-density lipoprotein
	Very-low-density lipoprotein
	Anti-LPS antibodies
Accelerated LPS degradation	Alkaline phosphatase
Inhibition of TLR4 signaling	TLR1
	Tollip
	ST2
	SIGIRR
Enhanced TLR4 degradation	Triad3A

**NOTE.** SIGIRR, single immunoglobulin IL-1 receptor-related protein; TLR, toll-like receptor.

neutralize LPS, blocking cellular activation and facilitating LPS clearance [79, 80]. Other plasma proteins, notably high-density lipoprotein [81] and very-low-density lipoprotein [82], can also bind and inactivate circulating endotoxin. Cell-bound LPS can be removed by LPS carrier proteins such as soluble CD14 and LBP. Finally, circulating LPS evokes a neutralizing antibody response [83].

TLR4-mediated signaling is inhibited through a variety of mechanisms. The extracellular domain of TLR1 has been shown to inhibit TLR4 signaling in endothelial cells [84], and 2 recently described members of the TLR family, known as ST2 [85] and single immunoglobulin IL-1 receptor-related protein, function in a similar role. Adapter proteins associated with the cytoplasmic regions of the TLR4 signaling complex, including Tollip [86], also play an inhibitory role in TLR4 signaling, whereas TLR4 is degraded by Triad3A [87].

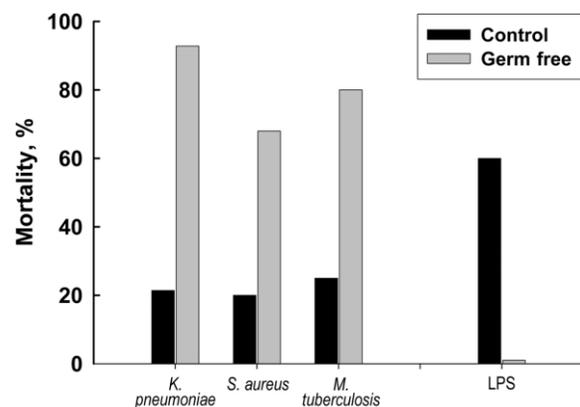
In summary, interactions between LPS and cells of the host innate immune system are regulated at multiple levels, with the result that cell signaling in response to LPS stimulation is tightly controlled. Although this complex interplay between an agonist and endogenous regulation and control systems bears the hallmarks of an endocrine interaction, the sine qua non of a hormonal effect is that the consequences of this process provide benefit to the host.

**Recognition of, and response to, LPS enhances antimicrobial immunity.** As described above, mice raised under germ-free conditions and lacking an indigenous gram-negative flora exhibit a number of immunologic derangements, including enhanced lethality following infection with *S. aureus*, *K. pneumoniae*, or *Mycobacterium tuberculosis* [20] (figure 3). Disruption of the mechanisms involved in the recognition of and response to LPS has similar consequences. In particular, although mice with targeted deletion of components of the LPS recognition pathways

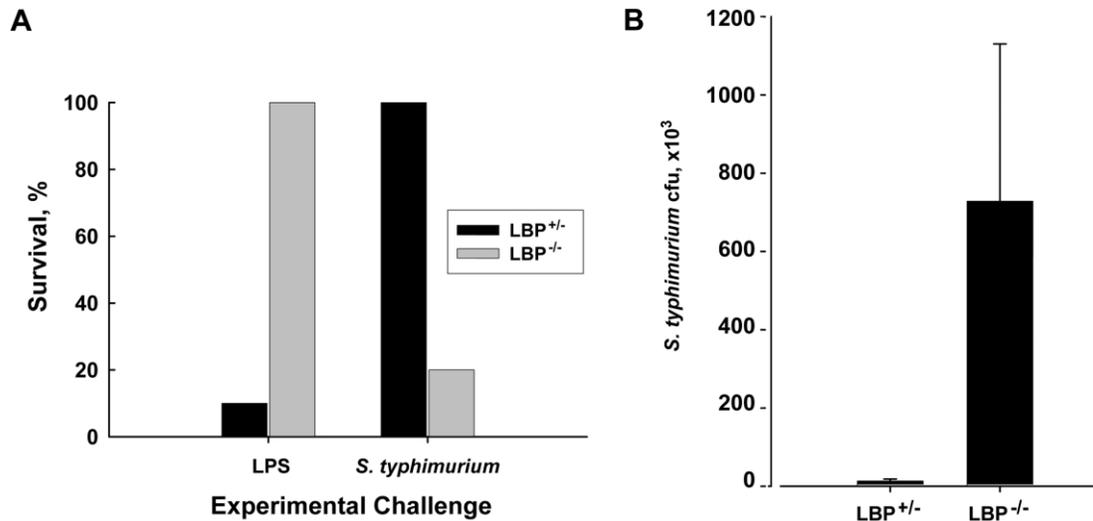
are resistant to endotoxin challenge, they exhibit increased lethality following challenge with live organisms.

LBP-knockout mice, for example, show delayed neutrophil migration [88] and dramatically increased mortality [89] in response to intraperitoneal challenge with *S. typhimurium*; however, they are resistant to intraperitoneal challenge with endotoxin (figure 4). Knockout mice show a reduced inflammatory mediator response and enhanced lethality following intraperitoneal challenge with *E. coli* [90]. Neutralization of LBP with an antibody results in reduced TNF release and increased lethality following challenge with *K. pneumoniae* [91]. CD14-deficient mice demonstrate similar abnormalities, with increased survival during endotoxemia [92] but increased lethality following infection with *K. pneumoniae* or *S. typhimurium* [93]. Yet this differential responsiveness does not appear to reflect simply the difference between challenge with bacterial products and live organisms, because CD14-knockout mice show increased survival when the challenge organism is *E. coli* O:111 [92], and the same organism has no adverse effects in LBP-knockout mice [91], whereas lethality is increased when CD14- or LBP-knockout mice are challenged with bacterial lipoprotein [93].

Finally, the TLR4 mutant C3H HeJ strain of mice is endotoxin-resistant; however, mice die after challenge with a dose of *Salmonella enterica* that is readily tolerated by their C3H HeN counterparts [94] or after intrapulmonary challenge with *Streptococcus pneumoniae* or *K. pneumoniae* [95], and clearance of *Candida* species is significantly impaired [96] (figure 5). Although the capacity to respond to LPS results in activation of a potentially lethal inflammatory mediator response, its evo-



**Figure 3.** Mortality among Swiss Webster mice raised under either conventional or germ-free conditions and challenged with live *Klebsiella pneumoniae*, *Staphylococcus aureus*, or *Mycobacterium tuberculosis* or purified endotoxin. Mortality for germ-free mice challenged with live organisms was significantly increased, although they were resistant to a dose of lipopolysaccharide (LPS) that killed 60% of conventional mice. Data are from Dubos et al. [20] and Schaedler et al. [21].



**Figure 4.** A, Survival of lipopolysaccharide (LPS)–binding protein (LBP)–deficient mice, either homozygous (LBP<sup>-/-</sup>) or heterozygous (LBP<sup>+/-</sup>), after challenge with LPS (20 h after challenge) or live *Salmonella typhimurium* (7 days after challenge) ( $P < .05$ ). B, Bacterial colony-forming units (cfu) 3 days after infectious challenge; bacteria counts were significantly higher in mice with homozygous deletion of LBP. Data are from Jack et al. [89].

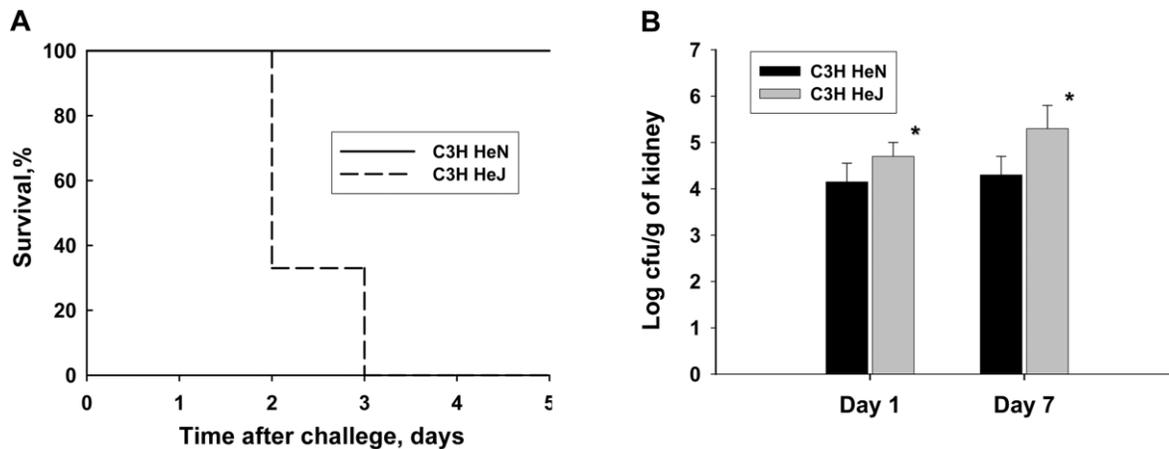
lutionarily conserved role is to support the ability to withstand an acute infectious challenge.

### MAINTAINING HOMEOSTASIS DURING HOST-MICROBIAL INTERACTIONS: AN ENDOCRINE MODEL

Multicellular organisms harbor a diverse but stable and predictable indigenous microbial flora that exerts multiple influences on immune homeostasis. Beyond this, they have evolved complex and efficient mechanisms that use microbial signals to optimize host responses to the microbial world—to respond appropriately to a threat while tolerating the presence

of a symbiont. As these mechanisms are becoming understood, it is apparent that they must challenge a model of host-microbial interactions that casts the bacterium as an enemy to be ruthlessly eliminated, rather than as an essential component of the environment whose excess or deficiency can disadvantage the host. In other words, they argue for an endocrine, rather than a toxin, model in the understanding of host-microbial interactions.

For example, recent work has shown that interactions between TLRs in the gut epithelium and the commensal flora of the gut actually serve to protect the epithelium from injury by a toxin such as dextran sulphate sodium and that, in the absence



**Figure 5.** Survival among wild-type C3H H3N mice and C3H HeJ mice, which have a point mutation in the extracellular domain of Toll-like receptor 4 that prevents binding of lipopolysaccharide (A). In contrast to C3H HeN mice, C3H HeJ mice were resistant to the lethality of endotoxin challenge but died after challenge with inoculum of *Salmonella enterica* that was readily cleared by wild-type mice. Data are from Vazquez-Torres et al. [94]. B, Clearance of fungi after experimental challenge with *Candida* species among C3H H3N and C3H HeJ mice. Data are from Netea et al. [96]. \* $P < .05$ .

of recognition of the commensal flora by host TLRs, injury is enhanced [97]. Of importance, when gut epithelial cells are rendered hypoxic, commensal organisms are able to evoke an inflammatory response [98]. Although our focus has been on endotoxin and its interactions with the innate immune system, the intricacies of normal host-microbial interactions are reflected in a number of striking but perhaps counterintuitive observations. Neutrophil-mediated inflammation is terminated through the programmed cell death, or apoptosis, of the neutrophil, a process that not only removes potentially injurious host immune cells but also initiates a program of counterinflammation and tissue repair, activated through the phagocytosis of the apoptotic cell [99]. Phagocytosis of bacteria [100] or fungi [101] induces neutrophil apoptosis. In an in vivo model of neutrophil-mediated lung injury evoked by intestinal ischemia-reperfusion, Sookhai et al. [102] showed that administration of killed *E. coli* into the lung could reduce pulmonary neutrophilia and improve survival. Ironically, the challenge in regulating inflammation in sepsis may prove to be not simply the presence of uncontrolled infection but also the absence of an infectious trigger to facilitate the resolution of inflammation.

Sepsis is the host response to infection. An endocrine model of this response has important implications as we evaluate therapeutic strategies that target the underlying biological processes. It may, for example, be desirable not to ablate the cytokine response but rather to target it to an optimal level to contend with the particular challenge. Moreover, neutralization of exogenous triggers of this process may not always be desirable. If the ability to respond to endotoxin impairs survival in certain models of infection, then endotoxin neutralization in sepsis may harm certain patients: In a large phase 3 trial of an antiendotoxin antibody, patients with gram-positive infection who received the antibody actually experienced a higher mortality rate that approached statistical significance [103]. Furthermore, several randomized trials of antibiotic therapy for nosocomial infection in the intensive care unit have shown that a reduction in antibiotic exposure is associated with an improved clinical outcome [104, 105].

Randomized trials have an important impact in establishing optimal approaches to contemporary management of diseases. However, the questions evaluated through such trials are shaped and constrained by conceptual models of the disease being studied, and it is appropriate, from time to time, to reevaluate the model in light of an accumulating body of scientific data. Such a reevaluation in sepsis promises to be both timely and rewarding.

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