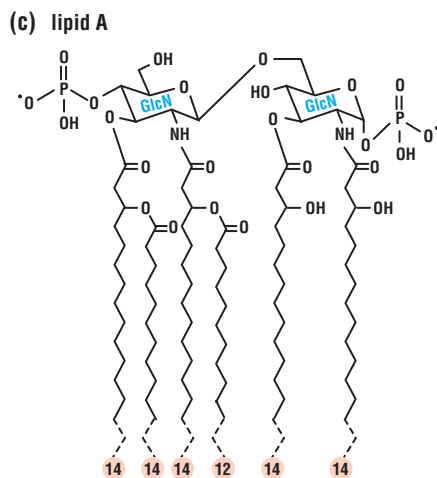
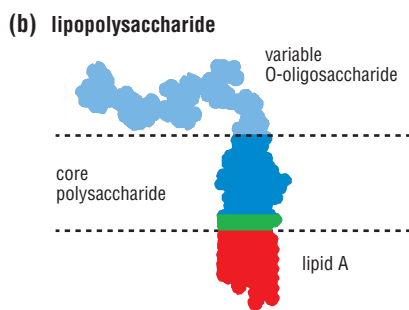
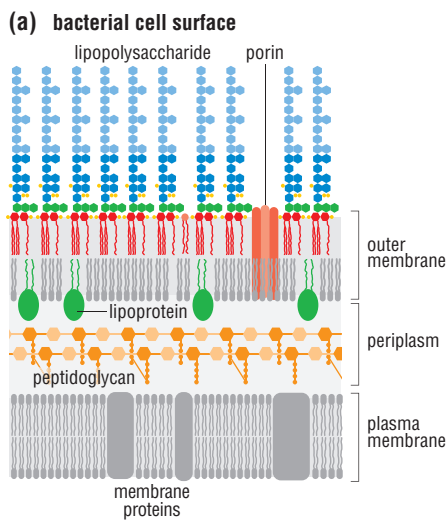


## 9-3 Sepsis Syndrome: Bacterial Endotoxin



**Figure 9-6 Structure of LPS** (a) Simplified diagram of Gram-negative bacterial cell surface showing LPS and some other major features. Approximately  $3 \times 10^6$  LPS molecules decorate 75% of the surface of Gram-negative bacteria. (b) The LPS molecule consists of an outer O-specific oligosaccharide that is highly variable among different species, a conserved inner core, and a lipid—lipid A—that forms part of the bacterial outer membrane. (c) Lipid A is a phosphoglycolipid consisting of a core hexosamine disaccharide with ester- and amide-linked acylated fatty acid tails arranged in either asymmetric or symmetric arrays that anchor the structure in the membrane. The asymmetric structure of *E. coli* lipid A is shown here. The numbers refer to the number of carbon atoms in the tail.

### Sepsis syndrome is a systemic response to invasive pathogens

Sepsis syndrome, or sepsis, is an adverse systemic response to infection that includes fever, rapid heartbeat and respiration, low blood pressure and organ dysfunction associated with compromised circulation. Approximately 250,000–750,000 cases of sepsis occur annually in the United States of America, with mortality ranging from 20% to 50% overall and as high as 90% when shock develops. Sepsis can occur through infection with Gram-positive bacteria and even fungi and viruses, or as a consequence of secreted toxins, which we discuss in the next section. However, the sepsis syndrome occurs commonly in response to lipopolysaccharide (LPS) from Gram-negative bacteria, which will be illustrated here.

### Lipopolysaccharide recognition occurs via the innate immune system

LPS is a major constituent of Gram-negative bacterial cell walls (see section 3-0) and is essential for membrane integrity. The portion of LPS that causes shock is the innermost and most highly conserved phosphoglycolipid, lipid A (Figure 9-6), which acts by potently inducing inflammatory responses that are life-threatening when systemic (see below), and is known as bacterial **endotoxin**. Multicellular organisms from horseshoe crabs and fruit flies to humans have evolved proteins specialized for the recognition of LPS. These proteins are found both on the surface of phagocytic cells and as soluble proteins in blood.

LPS is removed by macrophages through scavenger receptors (for example, SR-A) that are highly expressed in the liver and are thus positioned to remove LPS from portal blood draining the intestines, and by neutrophils through the primary granule protein, bactericidal permeability-increasing protein (BPI), which is toxic to Gram-negative bacteria (see section 3-9). The homologous LPS-binding protein, LBP, transfers LPS to membrane-bound or soluble CD14, enabling interactions with Toll-like receptors (TLRs) on the phagocyte membrane (see section 3-10), and to high-density lipoprotein (HDL) particles for removal. In mice and humans the LPS receptor includes CD14, an LPS-interacting moiety, TLR4, the signal transducing element, and MD-2, a small extracellular protein tightly bound to TLR4 (see Figure 3-34). Mice deficient in any of the LPS receptor components are more susceptible to Gram-negative bacterial infection but, at the same time, are less susceptible to the sepsis syndrome.

### Sepsis results from the activation of lipopolysaccharide-responsive cells in the bloodstream

TLRs have a lethal function in the septic shock syndrome. The physiological function of signaling through phagocyte TLRs is to induce the release of the cytokines TNF, IL-1, IL-6, IL-8 and IL-12 and trigger the inflammatory response, which is critical to containing bacterial infection in the tissues. However, if infection disseminates in the blood, the widespread activation of phagocytes in the bloodstream is catastrophic.

Humans injected with purified LPS develop a cytokine cascade in the serum (Figure 9-7). The early cytokine response (TNF, IL-6 and IL-8) coincides with the onset of fever and the activation of blood neutrophils, monocytes and lymphocytes. A subsequent increase in the numbers of circulating neutrophils, or **neutrophilia**, is driven by effects of colony stimulating factors, such as G-CSF, whereas the decreased numbers of circulating lymphocytes and monocytes, designated **lymphopenia** and **monocytopenia**, is sustained by their activation-induced exit and retention in peripheral sites. This is followed by a pituitary response and a regulatory or antiinflammatory response (see section 3-15).

#### Definitions

**endotoxin:** (of bacteria) a non-secreted toxin inherent in the cell membrane and that induces strong innate inflammatory responses that can be fatal when systemic.

**LD<sub>50</sub>:** the dose at which 50% of treated individuals die.

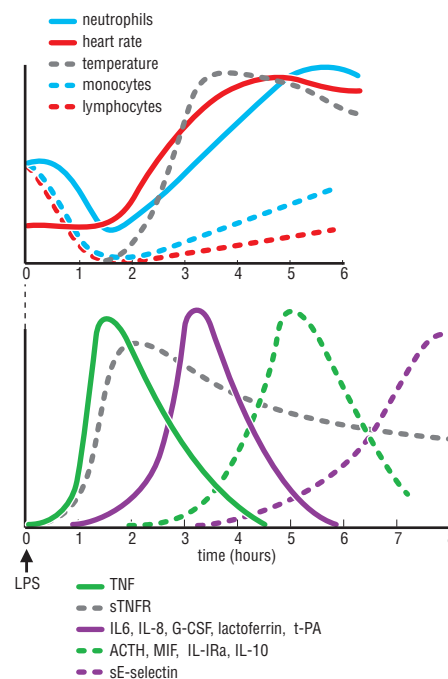
**lymphopenia:** a decrease in the numbers of circulating lymphocytes.

**monocytopenia:** a decrease in the numbers of circulating monocytes.

**neutrophilia:** a rise in the number of circulating neutrophils.

**protein C:** a vitamin K-dependent plasma serine protease that is synthesized in liver and activated by a complex of thrombin bound to thrombomodulin on membranes and that then cleaves coagulant factors VIIIa and Va, inactivating them.

**Figure 9-7 Time course of sepsis** The clinical manifestations of sepsis are shown above the successive waves of the serum cytokine cascade. (Cytokine concentrations are not shown to scale.) In humans injected with purified LPS, TNF rises almost immediately and peaks at 1.5 h; the sharp decline of TNF may be due to modulation by its soluble receptor sTNFR. A second wave of cytokines that peaks at 3 h activates the acute-phase response in the liver and the systemic pituitary response (via IL-6 and IL-1) and the activation and chemotaxis of neutrophils (via IL-6, IL-8 and G-CSF). Neutrophil activation results in the release of lactoferrin from neutrophil secondary granules; the activation of endothelial procoagulants is shown by the rise of tissue plasminogen activator (t-PA). Pituitary-derived adrenocorticotropic hormone (ACTH) and migration inhibition factor (MIF) peak at 5 h and coincide with peak levels of the regulatory cytokines IL-1Ra and IL-10 that counteract the release or activity of inflammatory cytokines. Diffuse endothelial activation is shown by the appearance of soluble E-selectin that peaks at about 8 h and remains elevated for several days.



## Inflammation leads to widespread endothelial cell activation and organ dysfunction

Cytokine production in the bloodstream results in widespread endothelial cell activation, with expression of adhesion molecules, activation of the coagulation cascade and the production of chemokines and cytokines by the endothelial cells themselves, with consequent amplification of the inflammatory cascade. The adhesion and activation of circulating neutrophils at the endothelium results in both oxidative and elastase-mediated damage, resulting in the loss of vascular integrity and failure to maintain adequate blood pressure. TNF and IL-1 also depress myocardial function directly. Refractory shock, with leakage of edema fluid, and the failure of organs with large capillary beds, such as the lung and kidney, leads to death.

Levels of circulating TNF, IL-6, IL-1 and LPS are directly correlated with the probability of death in humans with sepsis. Despite this, anti-LPS and anti-TNF antibodies, soluble TNF receptors, IL-1Ra and corticosteroids have all failed to alter the outcome of septic shock. Greater success has been achieved with activated **protein C**, an antithrombic, antiinflammatory serine protease activated by thrombin and consumed during sepsis. Levels of activated protein C and antithrombin III are inversely correlated with the probability of death from sepsis, and replacement of activated protein C can reduce the relative risk of death during severe sepsis by almost 20%.

## Animal models have helped clarify mechanisms of sepsis

Two widely used models are commonly referred to as the high-dose and low-dose models (Figure 9-8). High-dose LPS challenge involves injection of mice intraperitoneally or intravenously with doses typically between 25 and 100 µg per animal. The LD<sub>50</sub> approximates 150 µg, with death occurring in about 35 hours. Mortality is due to proinflammatory cytokines and widespread endothelial cell injury. Mice with defects in neutrophil adhesion or activation demonstrate a higher LD<sub>50</sub> in this assay. The low-dose LPS model relies on concurrent administration (from 1 h before to 4 h after) of D-galactosamine, a potent and specific inhibitor of hepatic macromolecular synthesis. Mice given 300 mg galactosamine/kg (typically 20 mg) have an LD<sub>50</sub> of 0.5 ng LPS, with death occurring in about 7 h. In the low-dose model, death is due to massive hepatic necrosis in response to LPS by a process dependent upon TNF and IFN-γ. Mice deficient in the production or recognition of these cytokines demonstrate a higher LD<sub>50</sub> in the low-dose model. Mice deficient in the clearance or recognition of LPS itself demonstrate an altered LD<sub>50</sub> in both models.

### Susceptibility to LPS Toxicity in Gene Knockout Mice

Defect	Protein	High LPS	Low LPS/D-Gal
LPS recognition	CD14	decreased	decreased
	LBP	no change	decreased
	TLR4	decreased	decreased
	MD-2	decreased	decreased
	MyD88	decreased	decreased
phagocyte function	SR-A	enhanced	not done
	Hck/Fgr	decreased	no change
	ICAM-1	decreased	no change
	L-selectin	decreased	not done
	GM-CSF	decreased	not done
inflammation	TNFR1	no change	decreased
	TNFR2	no change	no change
	IL-1Ra	enhanced	not done
	IL-1β	no change	no change
	IFN-γR	decreased	decreased
	caspase 1	decreased	not done

**Figure 9-8 Table of susceptibility to LPS toxicity in gene knock-out mice** The proteins encoded by the deleted genes are listed. SR-A is scavenger receptor A; Hck and Fgr are Src-family kinases with an essential role in integrin-mediated migration of neutrophils out of the bloodstream. D-Gal: D-galactosamine.

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