SHOCK, Vol. 28, No. 1, pp. 15–23, 2007

Review Article

ROLE OF BACTERIAL ENDOTOXIN IN CHRONIC HEART FAILURE: THE GUT OF THE MATTER

Bambos M. Charalambous,* Robert C.M. Stephens,† Ian M. Feavers,‡ and H.E. Montgomery*

**University College London; †Institute of Child Health, University College London; and ‡Division of Bacteriology, National Institute of Biological Standards & Control, London, UK*

Received 4 Oct 2006; first review completed 8 Nov 2006; accepted in final form 8 Dec 2006

ABSTRACT—Proinflammatory cytokines are now thought to play a key role in the pathophysiology of chronic heart failure, driving both symptomatic presentation and disease progression. We propose that this proinflammatory state, in turn, may be sustained through a chronic release of enterically derived bacterial endotoxin. Human trials have indicated that bacterial decontamination of the gut with concomitant decrease in lipopolysaccharide (LPS) has a positive outcome on heart disease patients. Antiendotoxin antibodies may thus represent therapeutic agents in this setting. Previously, antiendotoxin antibodies were targeted to the inner hydrophobic lipid A moiety of endotoxin in an attempt to neutralize its toxicity. These antibodies failed because they lacked specificity and bound to LPS weakly. In contrast, our studies on antiendotoxin antibodies have revealed that antibodies targeted to the hydrophilic oligosaccharides of the endotoxin have the potential to bind specifically with high affinity. Development of immunotherapeutics that can reduce systemic LPS or other agents, such as bactericidal/permeability-increasing protein that can neutralize LPS and limit inflammation safely, will enable the role of LPS in chronic heart failure to be elucidated and may pave the way to develop a new generation of effective therapeutic agents that may be directed to the treatment of chronic heart failure.

KEYWORDS—Antibodies, hypoxia, inflammation, interleukins, nitric oxide, pathology, *E. coli*, endotoxin

INTRODUCTION

Chronic heart failure (CHF) patients with edema have elevated plasma concentrations of bacterial endotoxin (lipopolysaccharide [LPS], with significant activation of the immune system (1, 2) The LPS acts on systemic immune-competent cells to potently stimulate the production of proinflammatory cytokines (PICs) (3). Some PICs, such as tumor necrosis factor-α (TNF-α) and nitrous oxide (NO), are also cardiosuppressors, which may also exacerbate the course of the disease. Both LPS and the subsequent inflammatory response can alter the permeability of the gut, allowing more LPS to leak into the blood (4–7). This endogenous LPS further stimulates inflammatory responses and results in a positive feedback loop that may perpetuate the chronic inflammatory state and its associated depression of cardiac function. Although inflammation in heart disease is now well documented (8, 9), the role of LPS in heart disease is poorly delineated, and published data are often conflicting. In CHF patients, both desensitization to LPS with a concomitant decrease in the inflammatory response (10), and an increase in sensitivity to LPS with an increase in TNF-α production and a decrease in HLA-DR (a major histocompatibility complex, class II, cell surface receptor) expression in monocytes (11) have been observed. More recently, human studies suggest that enterically derived LPS drives inflammation: decontamination of the gut being associated with both a reduction in intestinal LPS and a decline in $CD14^+$ monocyte levels (12). In addition, selective digestive decontamination of the gut before cardiopulmonary bypass reduced the level of LPS and PICs (13) and improved postoperative outcome (14).

In this review, we put forward the argument that LPS is an underestimated and important contributor to the pathophysiology of CHF. In addition, we propose that agents that can either neutralize or decrease systemic LPS and lower the chronic inflammation observed in CHF patients would limit progression of heart disease and have a positive impact on patient mortality.

HEART FAILURE AND THE SYSTEMIC INFLAMMATORY RESPONSE

Low-grade systemic inflammation is a feature of both acute and chronic heart failure (15), with elevation in circulating PIC levels (16), such as TNF-α (17), interleukin (IL)-6 (15), and IL-1 (18), being associated with worsening of symptoms, hospital readmission, and even mortality (19). Increasing evidence indicates that this association may be causal. The PICs suppress myocardial contractility (20–25), a process in which cytokine-stimulated generation of local NO through increased expression of inducible NO synthase (20, 26–28) may play a key role. This leads to a depression of excitation-contraction coupling (29) and thus maximum extent and peak velocity of cardiomyocyte shortening (21)—effects due, at least in part, to alterations in mitochondrial respiration (21, 26, 30–36). Additional associated reductions in muscle resting membrane potential and sodium-potassium gradient (37) and mitochondrial density (37), impaired cellular substrate metabolism (37), a rise in the expression of matrix metalloproteinases and a fall in expression of their inhibitors

Address reprint requests to Bambos M. Charalambous, Royal Free & University College Medical School, Rowland Hill St, London NW3 2PF, UK. E-mail: b.charalambous@medsch.ucl.ac.uk.

DOI: 10.1097/shk.0b013e318033ebc5

(27), the potentiation of leukocyte adhesion (38), tissue hypoxia and cell death through apoptosis or necrosis (39), and a failure of adrenergic responsiveness (40, 41) contribute to such cardiac impairment.

In this regard, TNF-α and IL-6 may be especially potent. The TNF-α is produced directly by the failing heart (42–44) and systemically in response to other PICs. Elevated circulating levels of TNF-α are correlated to the severity of heart disease (45–48). The TNF-α is cardiodepressant through a number of pathways: (1) sphingosine release and suppression of the calcium transient (49, 50); (2) inhibition of the phosphoinositide pathway and of pyruvate dehydrogenase activity that reduces mitochondrial function (51); and (3) enhancing peroxynitrite production and the levels of matrix metalloproteinase 2 while reducing tissue inhibitor of matrix metalloproteinase 4 in the heart (52). The TNF-α may also engender peripheral tissue decompensation, stimulating inducible NO synthase, and thus NO, skeletal muscle apoptosis, and muscle wasting through the ATP-dependent ubiquitin-protease pathway that degrades proteins (53). Elevated levels of TNF-α result in upregulation of two TNF-α receptors (TNFRs), TNFR-1 and TNFR-2, with the former predominating and thus a good predictor of short-term (54) and long-term (19) prognosis in CHF patients (55). The plasma levels of both these receptors are also correlated with the degree of impairment of systemic ventricular function (56). The TNF-α is thus implicated in β-receptor uncoupling from adenylate cyclase, cardiomyocyte apoptosis, cardiac dysfunction, and systemic effects, including endothelial dysfunction, reduced skeletal muscle blood flow, and the development of anorexia and cachexia (57–59). Elevated TNF-α levels are thus inversely related to functional capacity and peak oxygen consumption (60).

Meanwhile, the PIC IL-6 is induced by diverse inflammatory stimuli and their associated hormonal and cytokine responses (16, 61, 62). Produced by activated leukocytes, fibroblasts, endothelial cells (63), and adipose tissue (64), it is the only cytokine that stimulates the synthesis of all the acute-phase proteins (63, 65–67) and thus has pleiotropic actions on cardiac function, inflammatory cell recruitment, lipid metabolism, and endothelial function (68). Interleukin 6 also causes cardiac adrenergic refractoriness (40, 41) and cardiomyocyte apoptosis (69), and depresses myocardial function (25, 41). Indeed, IL-6 is viewed by some as the most potent cytokine depressor of myocardial function of all (22). Circulating IL-6 levels are elevated in asymptomatic (70) and symptomatic (71, 72) CHF and correlate with impaired functional class, poorer left ventricular function, an increase in length of hospital stay (42, 73–75), and mortality (19, 55, 76). Levels of IL-6 and LPS have been shown to correlate with the severity of heart disease in adults (56). Several recent studies have also shown that levels of IL-6 are correlated invariably to the severity of heart disease (45–48).

THERAPY TARGETED TO INFLAMMATION

Chronic heart failure is accompanied by an elevation in levels of PICs and an inadequate parallel elevation in anti-inflammatory mediators. This imbalance of cytokines has been implicated in the development and progression of CHF, and in the last decade, attempts have been made to modulate this dysregulation (8). Except for one large mortality/morbidity study (77), with a subsequent substudy (78), all studies of immunomodulatory therapy in CHF have used small numbers of patients and have yielded inconclusive results (79, 80). Meanwhile, recent studies of i.v. immunoglobulin, thalidomide, and pentoxifylline highlight the potential benefits of immunomodulation in CHF patients and emphasize the need for larger, placebo-controlled mortality studies of immunomodulatory therapies in CHF (9).

LIPOPOLYSACCHARIDE AND INFLAMMATION

Thus, PIC levels are elevated in both acute (81) and chronic heart failure (19, 82) and may be causative in disease and symptom progression. Although the driving factors, which chronically provoke such synthesis, have yet to be fully understood, a growing corpus of work implicates LPS that may be derived from the gut in this capacity. Gram-negative bacteria can comprise around 10^9 of the 10^{12} total bacteria colonizing the healthy gastrointestinal tract (83). The LPS is the major glycolipid constituent of their outer membranes. The gastrointestinal tract thus contains sufficient LPS (~200– 300 mg) to kill the host many times over as indicated from the lethal range of nanogram per kilogram in the rabbit model (84, 85). The LPS potently induces the expression of PICs (86, 87), including TNF-α (88) and IL-6 (89–93), partly through the activation of the nuclear transcription factor nuclear factor (NF)-κB (94). The stimulation of NF-κB by both LPS and TNF-α results in a positive feedback loop of PIC generation (Fig. 1). This results in a spiraling cycle of inflammation, cardiodepression, ischemia, and damage of the villi, causing leakage of LPS, and then more inflammation (Fig. 1). This vicious cycle is exacerbated by LPS or gram-negative bacteria that can act directly on the intestine to increase its permeability and promote further leakage of LPS (4–7).

Recently, the toll-like receptor (TLR) (and TLR-4, in particular) has been implicated as a key component of the innate response in the heart (95). The TLRs are a family of receptors that recognize molecular patterns associated with pathogens, and several exogenous and endogenous ligands have been identified, including LPS (96, 97), fibrinogen (98), hyaluronan, fibronectin, and minimally modified low-density lipoprotein (99) and heat shock protein 60 (100). Ligand binding leads to the activation of several kinases and NF-κB. Enhanced monocyte and macrophage expression of costimulatory molecules, including B7-1 and B7-2, and PICs, including IL-1β, IL-6, IL-12, and TNF-α, have been demonstrated as downstream effects of TLR activation (101, 102). In this way, such ligands may initiate a powerful immune response even in the absence of infection.

Although the importance of TLRs in innate immune responses to microbes is well established, their role in heart disease processes is not well understood. An enhanced *in vitro* response of monocytes to LPS has been demonstrated in patients with recurrent unstable angina (103) and a role for

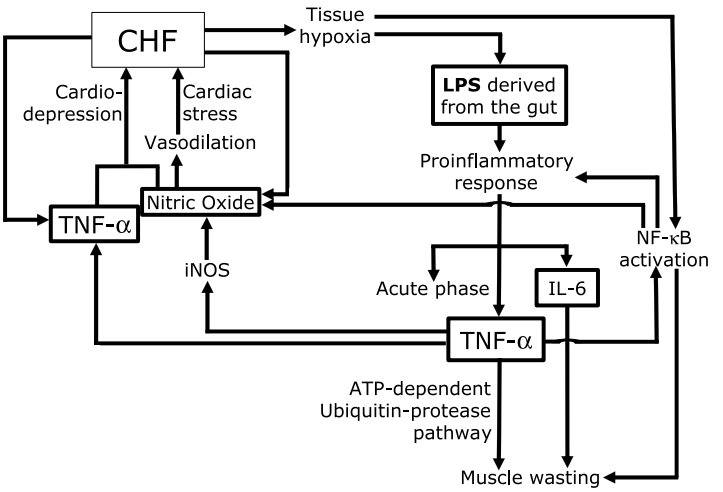

FIG. 1. **Proposed role of bacterial LPS in CHF.** iNOS indicates inducible NO synthase.

TLR-4 in outward arterial remodeling (104). It has also recently been shown that there is an expansion of circulating TLR-4–positive monocytes in patients with acute coronary syndrome (105). We therefore reason that TLR-4 and LPS may have an important role in the pathophysiology of heart disease.

THE ROLE OF MONOCYTES IN INFLAMMATION

Chemokines have a critical role in basal and inflammatory leukocyte trafficking, and their main targets are cells derived from bone marrow (106, 107). In addition to recruitment of blood cells, chemokines also induce responses beyond the immune system. For example, there is activation of endothelial cells that can result in angiogenic or angiostatic effects (108) and various responses in smooth muscle cells, fibroblasts, neurons, and epithelial cells. Produced in response to a proinflammatory stimulus, the chemokine CCL2/monocyte chemoattractant protein 1 recruits monocytes locally and induces them to leave the bloodstream and enter the surrounding tissue, becoming tissue macrophages. Other mediators such as complement, tissue growth factor-β, free radicals, and other CC chemokines may also have a role in regulating monocyte infiltration. The CC chemokines or β chemokines have two adjacent cysteines near the amino terminus of the protein and bind to CC chemokine receptors, of which 10 have been discovered to date, designated CCR1 to CCR10. These receptors are expressed on the surface of different cell types, allowing their specific attraction by the chemokines. The CC chemokines induce the migration of monocytes and other cell types, such as natural killer cells and dendritic cells. There are two principle subsets of human monocytes, the $CD14^{+}/CD16^{-}$ and $CD14^{lo}/CD16^{+}$, which raises the possibility that different chemokine profiles elicited by the inflammatory response may recruit distinct subsets of monocytes in heart disease (109).

Stimulated monocytes and macrophages, T cells, and mast cells synthesize a variety of PICs that include IL-1β, IL-6, and TNF-α. Cytokines upregulate endothelial cell adhesion molecules, recruit leukocytes, and induce smooth muscle cell migration and proliferation (110). Cytokines act systemically to initiate the acute-phase response, upregulating proteins involved in inflammation and hemostasis and resulting in a proinflammatory and prothrombotic state. Expression of tissue factor by inflammatory cells potently induces thrombus formation upon plaque rupture, leading to acute coronary syndromes. Inflammatory biomarkers, including C-reactive protein, complement proteins, IL-6, and white blood cell count predict the development of acute coronary syndromes. The C-reactive protein has been widely studied and consistently predicts future acute coronary syndrome events.

HOW DOES LPS TRIGGER INFLAMMATION?

An understanding of the structure of LPS and the molecular pathways involved in the triggering of inflammation may further aid in the development of anti-LPS therapeutics to study LPS in CHF patients and to develop effective immunotherapeutics to limit progression of CHF. The LPS binding protein (LBP) and CD14 play key roles in promoting innate immunity to gram-negative bacteria by transferring LPS to the signaling receptor complex, MD-2/TLR-4 (111, 112). In the absence of plasma, LPS binds poorly to

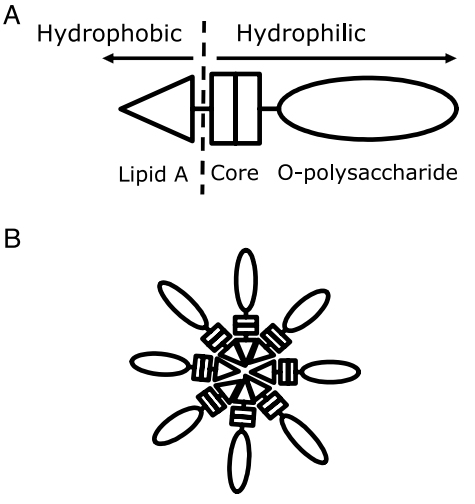

FIG. 2. **The LPS structure and formation of aggregates.**

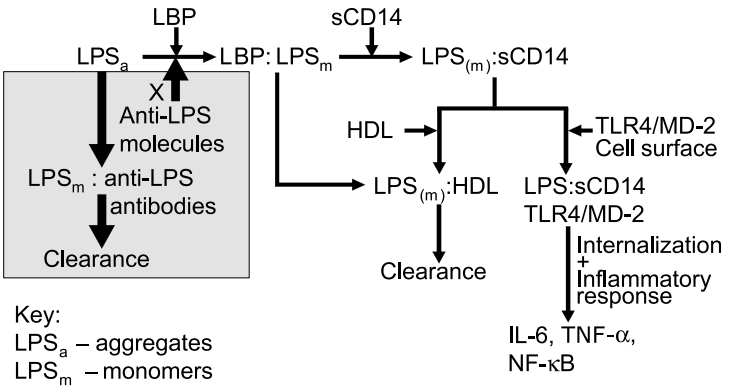

FIG. 3. **Disaggregation of LPS micelles through binding of LBP.** HDL indicates high-density lipoprotein.

leukocytes and only provokes a response at very high concentrations (113, 114). This is because LPS is an amphipathic molecule that forms aggregates in aqueous buffers with the lipid A on the inside and unable to bind to cells and trigger inflammation (Fig. 2) (115–117).

In plasma, two mutually exclusive proteins interact with LPS and modulate its biological activity. One of these, the LBP disassociates a single molecule of LPS from the aggregated LPS (Fig. 3). This LPS:LBP complex then interacts with the CD14 protein, possibly in combination with albumin (118, 119), TLR-4 (96, 101, 121), and MD-2 (122) proteins to initiate the inflammatory response (123, 124) (Fig. 3). The LPS:LBP complex also transfers LPS monomers to high-density lipoprotein particles and clearance of plasma LPS via the liver (125, 126) (Fig. 3). The other, bactericidal/permeability-increasing protein (BPI) also interacts with LPS aggregates, but unlike LBP, it stabilizes LPS aggregates and blocks the binding of LBP (127), and averts the inflammatory response mediated by LPS (128, 129).

LIPOPOLYSACCHARIDE IN HEART FAILURE

In CHF, there is reduced cardiac output that decreases the flow of blood to the tissues (130). The gut is particularly affected as it has a high demand for oxygen (up to 20% of the whole body's requirement) and thus readily becomes hypoxic (131). The CHF-associated gut mucosal edema further compromises gut function (1). The architecture of the gut mucosal microvasculature exposes the tip of the villus to the highest risk of ischemia in low flow states (132, 133). This causes necrosis and apoptosis of the epithelial cells at the tip of the villi (134, 135). The integrity of the mucosal epithelium is compromised and intestinal barrier dysfunction (136) ultimately allows translocation of endotoxin and gut bacteria (137–139). Even surgical anesthesia can cause mild ischemia of the gut and translocation of LPS (140). The possibility that LPS triggers inflammation and cytokine production in heart failure was first proposed by Anker et al. (2). Since then, elevated levels of LPS in CHF have been reported in many studies (1, 56, 88, 141). The amount of LPS in the circulation is sufficient to cause increased levels of PICs and the symptoms observed in CHF patients (56, 141). It has also been shown, at least in children, that the severity of the clinical outcome increases with higher levels of plasma LPS (142). Significant myocardial depression has been demonstrated in experimental human endotoxemia (143) by i.v. infusion with 4 ng/kg body weight of reference endotoxin from *Escherichia coli* 0113 (144). This dose of the reference endotoxin is a safe and well-recognized method of modeling the cardiovascular manifestations of sepsis and septic shock in healthy human volunteers (145).

Evidence for the role of endogenous LPS derived from the gut in inflammation has been obtained from a pilot study where patients with severe CHF had bacterial decontamination of the gut. This reduced intestinal LPS and decreased the inflammatory state (12). Decontamination of the gut before cardiopulmonary bypass was found to be associated with reduced levels of LPS and PICs (13), a finding that has been associated to an improved outcome when used in the postoperative period (14).

INTERVENTION THERAPY AGAINST LPS

Because we propose that endogenous LPS may be an important factor responsible for the underlying chronic inflammation seen in CHF, immunotherapeutic intervention strategies targeted to LPS may be beneficial to CHF patients. However, such strategies have only been applied to the treatment of sepsis, where the administration of antibodies, or passive immunization, to reduce levels of LPS gave variable and disappointing findings (146–149). An analysis of these trials revealed that many of them lacked detailed follow-up assessments of serum antibody and LPS levels to establish the sufficiency of antibodies administered and their ability to reduce plasma LPS levels (148). Thus, a benefit may have been achieved if sufficient anti-LPS antibodies had been administered—a conclusion supported by the findings that endotoxemic patients had a poorer prognostic outcome when their anti-LPS antibodies were depleted before subtoxic levels of plasma LPS were attained (150–154).

Other factors that would influence the efficacy of the antibody therapy are the target site, or epitope, and strength of binding. The epitopes of the monoclonal antibodies (mAbs) E5 (Xoma, Berkley, Calif) and HA-1 A (Centocor, Malvern, Pa) used in these trials were in the lipid A, as the concept was to neutralize the toxicity of LPS by blocking the binding of lipid A to cells (155). Unbelievably, subsequent *in vitro* analyses of mAbs E5 and HA-1A revealed that they exhibited

weak binding to LPS (156), neutralized LPS poorly (157, 158), bound nonspecifically to hydrophobic ligands, such as lipoproteins and cardiolipin (159), and to a variety of human B-cell and erythrocyte proteins (160, 161), and were toxic in a canine model of septic shock (162).

In contrast, anti-LPS antibodies with epitopes in the hydrophilic outer domain of the LPS have the potential to have very high affinities that are in the nanomolar range. For example, the antimeningococcal LPS mAb 9-2-L379 that targets the hydrophilic domain has a binding affinity, with a dissociation constant of 7.5 nM (163). The binding affinity was accurately determined by us using real-time kinetic analysis with a resonant mirror biosensor (164). Thus, we propose that the lack of demonstrable effect of the anti-LPS strategies to date represents inappropriate immunotherapeutics with poorly designed preclinical evaluation.

Alternatively, various other substances (some of which are licensed for use in humans) have been shown to neutralize or limit the inflammatory effects of LPS, for example, BPI or synthetic peptides derived from BPI (165–168) can be tested in animal models to establish an association between LPS and the heart. If such as association is established in the animal model, clinical trials on heart patients could be initiated because BPI is licensed for human use.

CAN NEW ANTI-LPS IMMUNOTHERAPEUTICS BE SUCCESSFUL?

The lessons that can be learned from the trials of anti-LPS immunotherapeutics to treat sepsis are that antibodies with poor binding affinity and insufficient specificity are unlikely to be effective. In addition, trials should be carefully designed to determine their efficacy and to establish whether sufficient levels of antibody were administered to reduce levels of systemic LPS. We postulate that high-affinity and specific antibodies that target LPS could be developed if their epitope lies in the immunogenic hydrophilic portion of LPS (169, 170) as opposed to the hydrophobic lipid A moiety used in the past. This proposal is supported by a human trial of active immunization against LPS sepsis, where a reduction in mortality was achieved with human antiserum raised by vaccinating with the core region of *E. coli* LPS (171). Most of the polyclonal antibody population would have been against the core hydrophilic sugars of the LPS with only a smaller subset against the less immunogenic lipid A moiety. A subsequent study in mice and rabbit infection models by Kirkland and Ziegler (172) showed that a mAb to an oligosaccharide determinant of LPS from *E. coli* 0111:B4 could protect from gram-negative infection. Active immunization of mice with the core of LPS from four gram-negative bacterial strains that colonize the gut: *E. coli* K12, *E. coli* R1, *Pseudomonas aeruginosa* PAC608, and *Bacteroides fragilis* showed protection against a lethal challenge of *E. coli* O18 LPS (173). Vaccines have also been developed against *E. coli* J5 (174), *Shigella sonnei* and *Shigella flexneri* 2a (175, 176), *Salmonella typhimurium* (177), and *Vibrio cholerae* (178), as well as to other human pathogens such as *P. aeruginosa* (179), *Pasteurella multocida* (180), *Brucella melitensis* (181), and *Francisella tularensis* (182). The efficacy of a single mAb targeted to a hydrophilic oligosaccharide protection of the LPS has yet to be confirmed in human trials.

Although these vaccination studies indicate that effective anti-LPS antibodies can be generated, the use of LPS as a vaccine component may be problematic because it is poorly immunogenic and as little as 4 ng/kg body mass can be toxic. The LPS may also mimic human antigens to camouflage the bacterium from host defenses and thus has the potential to raise autoimmune responses (183, 184). In an alternative strategy toward the development of safer anti-LPS vaccines, we have used peptide mimics of LPS. These were identified by direct interaction with a functional high-affinity mAb (163) with known specificity and whose epitope is within the core region of LPS that is accessible in the intact organism and does not include the toxic lipid A (185, 186).

To date, these anti-LPS immunotherapeutic strategies have only been tested against sepsis—with levels of systemic LPS that are much higher than in the inflammatory state observed in heart disease. Before such studies are extended to CHF patients, anti-LPS antibodies need to be developed that bind to LPS specifically and with high affinity. Using these immunotherapeutics to reduce systemic LPS will then allow the role of LPS in heart disease to be established in a suitable animal model, for example, in rabbits, as they are the only rodents with LPS sensitivities similar to humans. The potential of anti-LPS immunotherapeutics that can clear LPS rapidly and safely from the circulation can be determined. Subsequently, they may then need to be refined and licensed for human clinical trials. For example, if these antibodies were produced in an animal, they could be humanized by replacement with a human antibody constant (Fc) domain (187).

CONCLUDING REMARKS

Chronic heart failure afflicts millions of people worldwide. Despite modern pharmacotherapy, mortality remains high: 40% die within a year of diagnosis, and a similar percentage of those worst affected annually thereafter. Similarly, associated morbidity is also high: annually, CHF accounts for 2% of all hospital inpatient days and 5% of all emergency medical admissions to hospital. Hospital admissions due to heart failure are projected to rise by 50% during the next 25 years mainly caused by the aging of the population. This does not include the rising tide of obesity in developed countries that is an important risk factor of heart disease. The health care costs per patient increase from 8 to 30 times in cases of severe disease compared with those with mild symptoms. Additional social and financial burdens to patient, carers, family, and state are likely to be even greater.

Many processes are activated in CHF but the causal role for LPS has yet to be established, and no effective anti-LPS intervention therapy for any disease has been developed, despite evidence to show that it will be beneficial and is an intensive research in this area. Preliminary studies on gut decontamination that reduces systemic LPS are beginning to reveal tantalizing evidence for a role of LPS in CHF. We propose that the development of safe anti-LPS immunotherapeutics that

can reduce LPS and inflammation is within our grasp and that these will pave the way to elucidate the role of LPS in the pathophysiology of heart disease. These immunotherapeutics coupled with the potential that agents such as BPI (that can limit LPS-mediated inflammation) may have on heart disease will provide new technological platforms for intervention studies that can limit the progression of heart disease and reduce mortality.

REFERENCES

1. Niebauer J, Volk HD, Kemp M, et al.: Endotoxin and immune activation in chronic heart failure: a prospective cohort study. *Lancet* 353:1838–1842, 1999.
2. Anker SD, Egerer KR, Volk HD, et al.: Elevated soluble CD14 receptors and altered cytokines in chronic heart failure. *Am J Cardiol* 79:1426–1430, 1997.
3. Yang RB, Mark MR, Gray A, et al.: Toll-like receptor-2 mediates lipopolysaccharide-induced cellular signalling. *Nature* 395:284–288, 1998.
4. O'Dwyer ST, Michie HR, Ziegler TR, et al.: A single dose of endotoxin increases intestinal permeability in healthy humans. *Arch Surg* 123: 1459–1464, 1988.
5. Salzman AL, Wang H, Wollert PS, et al.: Endotoxin-induced ileal mucosal hyperpermeability in pigs: role of tissue acidosis. *Am J Physiol* 266: G633–G646, 1994.
6. Ding J, Magnotti LJ, Huang Q, et al.: Hypoxia combined with *Escherichia coli* produces irreversible gut mucosal injury characterized by increased intestinal cytokine production and DNA degradation. *Shock* 16:189–195, 2001.
7. Lobo SM, De Backer D, Sun Q, et al.: Gut mucosal damage during endotoxic shock is due to mechanisms other than gut ischemia. *J Appl Physiol* 95: 2047–2054, 2003.
8. Aukrust P, Yndestad A, Ueland T, et al.: Anti-inflammatory trials in chronic heart failure. *Heart Fail Monit* 5:2–9, 2006.
9. Aukrust P, Yndestad A, Ueland T, et al.: The role of intravenous immunoglobulin in the treatment of chronic heart failure. *Int J Cardiol* 112:40–45, 2006.
10. Sharma R, Bolger AP, Rauchhaus M, et al.: Cellular endotoxin desensitization in patients with severe chronic heart failure. *Eur J Heart Fail* 7:865–868, 2005.
11. Kruger S, Kunz D, Graf J, et al.: Endotoxin hypersensitivity in chronic heart failure. *Int J Cardiol* 115:159–163, 2007.
12. Conraads VM, Jorens PG, De Clerck LS, et al.: Selective intestinal decontamination in advanced chronic heart failure: a pilot trial. *Eur J Heart Fail* 6:483–491, 2004.
13. Martinez-Pellus AE, Merino P, Bru M, et al.: Endogenous endotoxemia of intestinal origin during cardiopulmonary bypass. Role of type of flow and protective effect of selective digestive decontamination. *Intensive Care Med* 23:1251–1257, 1997.
14. Fox MA, Peterson S, Fabri BM, et al.: Selective decontamination of the digestive tract in cardiac surgical patients. *Crit Care Med* 19:1486–1490, 1991.
15. Anker SD, von Haehling S: Inflammatory mediators in chronic heart failure: an overview. *Heart* 90:464–470, 2004.
16. Gabay C, Kushner I: Acute-phase proteins and other systemic responses to inflammation. *N Engl J Med* 340:448–454, 1999.
17. Levine B, Kalman J, Mayer L, et al.: Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 323:236–241, 1990.
18. Blum A, Sclarovsky S, Rehavia E, et al.: Levels of T-lymphocyte subpopulations, interleukin-1 beta, and soluble interleukin-2 receptor in acute myocardial infarction. *Am Heart J* 127:1226–1230, 1994.
19. Rauchhaus M, Doehner W, Francis DP, et al.: Plasma cytokine parameters and mortality in patients with chronic heart failure. *Circulation* 102: 3060–3067, 2000.
20. Kumar A, Haery C, Parrillo JE: Myocardial dysfunction in septic shock. *Crit Care Clin* 16:251–287, 2000.
21. Kumar A, Brar R, Wang P, et al.: Role of nitric oxide and cGMP in human septic serum–induced depression of cardiac myocyte contractility. *Am J Physiol* 276:R265–R276, 1999.
22. Nakanishi K, Takeda S, Terajima K, et al.: Myocardial dysfunction associated with proinflammatory cytokines after esophageal resection. *Anesth Analg* 91:270–275, 2000.
23. Cain BS, Meldrum DR, Dinarello CA, et al.: Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit Care Med* 27:1309–1318, 1999.
24. Kraut EJ, Chen S, Hubbard NE, et al.: Tumor necrosis factor depresses myocardial contractility in endotoxemic swine. *J Trauma* 46:900–906, 1999.
25. Parker MM: Pathophysiology of cardiovascular dysfunction in septic shock. *New Horiz* 6:130–138, 1998.
26. Oddis CV, Finkel MS: Cytokine-stimulated nitric oxide production inhibits mitochondrial activity in cardiac myocytes. *Biochem Biophys Res Commun* 213:1002–1009, 1995.
27. Mayers I, Hurst T, Puttagunta L, et al.: Cardiac surgery increases the activity of matrix metalloproteinases and nitric oxide synthase in human hearts. *J Thorac Cardiovasc Surg* 122:746–752, 2001.
28. Kumar A, Kumar A, Parrillo JE: Cytokines and septic myocardial depression: nitric oxide versus sphingosine/ceramide? *Crit Care Med* 27:1391–1393, 1999.
29. Haque R, Kan H, Finkel MS: Effects of cytokines and nitric oxide on myocardial E-C coupling. *Basic Res Cardiol* 93(suppl 1):86–94, 1998.
30. Laycock SK, Vogel T, Forfia PR, et al.: Role of nitric oxide in the control of renal oxygen consumption and the regulation of chemical work in the kidney. *Circ Res* 82:1263–1271, 1998.
31. Loke KE, Laycock SK, Mital S, et al.: Nitric oxide modulates mitochondrial respiration in failing human heart. *Circulation* 100:1291–1297, 1999.
32. Loke KE, McConnell PI, Tuzman JM, et al.: Endogenous endothelial nitric oxide synthase–derived nitric oxide is a physiological regulator of myocardial oxygen consumption. *Circ Res* 84:840–845, 1999.
33. Shen W, Wolin MS, Hintze TH: Defective endogenous nitric oxide–mediated modulation of cellular respiration in canine skeletal muscle after the development of heart failure. *J Heart Lung Transplant* 16:1026–1034, 1997.
34. Wolin MS, Hintze TH, Shen W, et al.: Involvement of reactive oxygen and nitrogen species in signalling mechanisms that control tissue respiration in muscle. *Biochem Soc Trans* 25:934–939, 1997.
35. Zhang X, Xie YW, Nasjletti A, et al.: ACE inhibitors promote nitric oxide accumulation to modulate myocardial oxygen consumption. *Circulation* 95:176–182, 1997.
36. Zhao G, Bernstein RD, Hintze TH: Nitric oxide and oxygen utilization: exercise, heart failure and diabetes. *Coron Artery Dis* 10:315–320, 1999.
37. Wagenmakers AJ: Muscle function in critically ill patients. *Clin Nutr* 20: 451–454, 2001.
38. Rosenbloom AJ, Pinsky MR, Bryant JL, et al.: Leukocyte activation in the peripheral blood of patients with cirrhosis of the liver and SIRS. Correlation with serum interleukin-6 levels and organ dysfunction. *JAMA* 274:58–65, 1995.
39. Marshall JC: Inflammation, coagulopathy, and the pathogenesis of multiple organ dysfunction syndrome. *Crit Care Med* 29:S99–S106, 2001.
40. Finkel MS, Oddis CV, Jacob TD, et al.: Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 257:387–389, 1992.
41. Oddis CV, Finkel MS: Cytokines and nitric oxide synthase inhibitor as mediators of adrenergic refractoriness in cardiac myocytes. *Eur J Pharmacol* 320:167–174, 1997.
42. Torre-Amione G, Kapadia S, Benedict C, et al.: Proinflammatory cytokine levels in patients with depressed left ventricular ejection fraction: a report from the Studies of Left Ventricular Dysfunction (SOLVD). *J Am Coll Cardiol* 27:1201–1206, 1996.
43. Habib FM, Springall DR, Davies GJ, et al.: Tumour necrosis factor and inducible nitric oxide synthase in dilated cardiomyopathy. *Lancet* 347: 1151–1155, 1996.
44. Kapadia SR, Oral H, Lee J, et al.: Hemodynamic regulation of tumor necrosis factor-alpha gene and protein expression in adult feline myocardium. *Circ Res* 81:187–195, 1997.
45. Valgimigli M, Merli E, Malagutti P, et al.: Hydroxyl radical generation, levels of tumor necrosis factor-alpha, and progression to heart failure after acute myocardial infarction. *J Am Coll Cardiol* 43:2000–2008, 2004.
46. Mizia-Stec K, Gasior Z, Zahorska-Markiewicz B, et al.: Serum tumour necrosis factor-alpha, interleukin-2 and interleukin-10 activation in stable angina and acute coronary syndromes. *Coron Artery Dis* 14:431–438, 2003.
47. Jolda-Mydlowska B, Salomon P: Cytokines and remodelling of the heart in patients with congestive heart failure. *Pol Arch Med Wewn* 109:23–33, 2003.
48. Geppert A, Steiner A, Zorn G, et al.: Multiple organ failure in patients with cardiogenic shock is associated with high plasma levels of interleukin-6. *Crit Care Med* 30:1987–1994, 2002.
49. Oral H, Dorn GW, Mann DL: Sphingosine mediates the immediate negative inotropic effects of tumor necrosis factor-alpha in the adult mammalian cardiac myocyte. *J Biol Chem* 272:4836–4842, 1997.
50. Favory R, Lancel S, Marchetti P, et al.: Endotoxin-induced myocardial dysfunction: evidence for a role of sphingosine production. *Crit Care Med* 32:495–501, 2004.

51. Muller-Werdan U, Engelmann H, Werdan K: Cardiodepression by tumor necrosis factor-alpha. *Eur Cytokine Netw* 9:689–691, 1998.
52. Gao CQ, Sawicki G, Suarez-Pinzon WL, et al.: Matrix metalloproteinase-2 mediates cytokine-induced myocardial contractile dysfunction. *Cardiovasc Res* 57:426–433, 2003.
53. Adams V, Jiang H, Yu J, et al.: Apoptosis in skeletal myocytes of patients with chronic heart failure is associated with exercise intolerance. *J Am Coll Cardiol* 33:959–965, 1999.
54. Ferrari R, Bachetti T, Confortini R, et al.: Tumor necrosis factor soluble receptors in patients with various degrees of congestive heart failure. *Circulation* 92:1479–1486, 1995.
55. Deswal A, Petersen NJ, Feldman AM, et al.: Cytokines and cytokine receptors in advanced heart failure: an analysis of the cytokine database from the Vesnarinone trial (VEST). *Circulation* 103:2055–2059, 2001.
56. Sharma R, Bolger AP, Li W, et al.: Elevated circulating levels of inflammatory cytokines and bacterial endotoxin in adults with congenital heart disease. *Am J Cardiol* 92:188–193, 2003.
57. Mann DL: Inflammatory mediators and the failing heart: past, present, and the foreseeable future. *Circ Res* 91:988–998, 2002.
58. Bolger AP, Anker SD: Tumour necrosis factor in chronic heart failure: a peripheral view on pathogenesis, clinical manifestations and therapeutic implications. *Drugs* 60:1245–1257, 2000.
59. von Haehling S, Jankowska EA, Anker SD: Tumour necrosis factor-alpha and the failing heart-pathophysiology and therapeutic implications. *Basic Res Cardiol* 99:18–28, 2004.
60. Cicoira M, Bolger AP, Doehner W, et al.: High tumour necrosis factor-alpha levels are associated with exercise intolerance and neurohormonal activation in chronic heart failure patients. *Cytokine* 15:80–86, 2001.
61. Nieman DC: Immune response to heavy exertion. *J Appl Physiol* 82: 1385–1394, 1997.
62. Jansky L, Pospisilova D, Honzova S, et al.: Immune system of cold-exposed and cold-adapted humans. *Eur J Appl Physiol Occup Physiol* 72:445–450, 1996.
63. Heinrich PC, Castell JV, Andus T: Interleukin-6 and the acute phase response. *Biochem J* 265:621–636, 1990.
64. Mohamed-Ali V, Flower L, Sethi J, et al.: beta-Adrenergic regulation of IL-6 release from adipose tissue: in vivo and in vitro studies. *J Clin Endocrinol Metab* 86:5864–5869, 2001.
65. Castell JV, Gomez-Lechon MJ, David M, et al.: Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. *FEBS Lett* 242:237–239, 1989.
66. Castell JV, Gomez-Lechon MJ, David M, et al.: Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology* 12:1179–1186, 1990.
67. Dalmon J, Laurent M, Courtois G: The human beta fibrinogen promoter contains a hepatocyte nuclear factor 1–dependent interleukin-6–responsive element. *Mol Cell Biol* 13:1183–1193, 1993.
68. Kishimoto T: The biology of interleukin-6. *Blood* 74:1–10, 1989.
69. Wollert KC, Drexler H: The role of interleukin-6 in the failing heart. *Heart Fail Rev* 6:95–103, 2001.
70. Raymond RJ, Dehmer GJ, Theoharides TC, et al.: Elevated interleukin-6 levels in patients with asymptomatic left ventricular systolic dysfunction. *Am Heart J* 141:435–438, 2001.
71. Munger MA, Johnson B, Amber IJ, et al.: Circulating concentrations of proinflammatory cytokines in mild or moderate heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 77:723–727, 1996.
72. Aukrust P, Ueland T, Lien E, et al.: Cytokine network in congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. *Am J Cardiol* 83:376–382, 1999.
73. Carlstedt F, Lind L, Lindahl B: Proinflammatory cytokines, measured in a mixed population on arrival in the emergency department, are related to mortality and severity of disease. *J Intern Med* 242:361–365, 1997.
74. Roig E, Orus J, Pare C, et al.: Serum interleukin-6 in congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am J Cardiol* 82:688–690, 1998. A8.
75. Tsutamoto T, Hisanaga T, Wada A, et al.: Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. *J Am Coll Cardiol* 31: 391–398, 1998.
76. Orus J, Roig E, Perez-Villa F, et al.: Prognostic value of serum cytokines in patients with congestive heart failure. *J Heart Lung Transplant* 19:419–425, 2000.
77. Hjalmarson A, Fagerberg B: MERIT-HF mortality and morbidity data. *Basic Res Cardiol* 95(suppl 1):I98–I103, 2000.
78. Gullestad L, Ueland T, Brunsvig A, et al.: Effect of metoprolol on cytokine levels in chronic heart failure-a substudy in the Metoprolol Controlled-Release Randomised Intervention Trial in Heart Failure (MERIT-HF). *Am Heart J* 141:418–421, 2001.
79. Gullestad L, Kjekshus J, Damas JK, et al.: Agents targeting inflammation in heart failure. *Expert Opin Investig Drugs* 14:557–566, 2005.
80. Gullestad L, Aukrust P: Review of trials in chronic heart failure showing broad-spectrum anti-inflammatory approaches. *Am J Cardiol* 95:17C–23C, 2005.
81. Brunkhorst FM: Endotoxins in chronic heart failure. *Lancet* 354:599–600, 1999.
82. Rauchhaus M, Koloczek V, Volk H, et al.: Inflammatory cytokines and the possible immunological role for lipoproteins in chronic heart failure. *Int J Cardiol* 76:125–133, 2000.
83. Wilson M: *Microbial Inhabitants of Humans; Their Ecology and Role in Health and Disease*. Cambridge, NY: Cambridge University Press, 2005.
84. Simon GL, Gorbach SL: The human intestinal microflora. *Dig Dis Sci* 31:147S–162S, 1986.
85. Simon GL, Gorbach SL: Intestinal flora in health and disease. *Gastroenterology* 86:174–193, 1984.
86. Moran AP: Structure-bioactivity relationships of bacterial endotoxins. *J Toxicol Toxin Rev* 14:47–83, 1994.
87. Rietschel ET, Brade L, Holst O, et al.: Molecular structure of bacterial endotoxin in relation to bioactivity. In: Nowotny A, Spitzer JJ, Ziegler EJ (eds.): *Endotoxin Research Series, Vol 1*. Amsterdam, The Netherlands: Elsevier Science Publishers B.V., pp 15–32, 1990.
88. Genth-Zotz S, von Haehling S, Bolger AP, et al.: Pathophysiologic quantities of endotoxin-induced tumor necrosis factor-alpha release in whole blood from patients with chronic heart failure. *Am J Cardiol* 90:1226–1230, 2002.
89. van Deventer SJ, Buller HR, ten Cate JW, et al.: Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. *Blood* 76:2520–2526, 1990.
90. Le Contel C, Vinit MA, Parant FJ, et al.: Differential priming for endotoxin-induced circulating cytokine production by tumor necrosis factor-alpha and interleukin 1 beta. *Cytokine* 2:375–380, 1990.
91. Cicco NA, Lindemann A, Content J, et al.: Inducible production of interleukin-6 by human polymorphonuclear neutrophils: role of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha. *Blood* 75:2049–2052, 1990.
92. Bailly S, Ferrua B, Fay M, et al.: Differential regulation of IL 6, IL 1 A, IL 1 beta and TNF alpha production in LPS-stimulated human monocytes: role of cyclic AMP. *Cytokine* 2:205–210, 1990.
93. Andersson U, Matsuda T: Human interleukin 6 and tumor necrosis factor alpha production studied at a single-cell level. *Eur J Immunol* 19:1157–1160, 1989.
94. Libermann TA, Baltimore D: Activation of interleukin-6 gene expression through the NF-kappa B transcription factor. *Mol Cell Biol* 10:2327–2334, 1990.
95. Oyama J, Blais C Jr, Liu X, et al.: Reduced myocardial ischemia-reperfusion injury in toll-like receptor 4–deficient mice. *Circulation* 109:784–789, 2004.
96. Poltorak A, He X, Smirnova I, et al.: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282:2085–2088, 1998.
97. Lien E, Means TK, Heine H, et al.: Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 105: 497–504, 2000.
98. Smiley ST, King JA, Hancock WW: Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 167:2887–2894, 2001.
99. Miller YI, Viriyakosol S, Binder CJ, et al.: Minimally modified LDL binds to CD14, induces macrophage spreading via TLR4/MD-2, and inhibits phagocytosis of apoptotic cells. *J Biol Chem* 278:1561–1568, 2003.
100. Ohashi K, Burkart V, Flohe S, et al.: Cutting edge: heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 164:558–561, 2000.
101. Medzhitov R, Preston-Hurlburt P, Janeway CA Jr: A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 388:394–397, 1997.
102. Schnare M, Barton GM, Holt AC, et al.: Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2:947–950, 2001.
103. Liuzzo G, Angiolillo DJ, Buffon A, et al.: Enhanced response of blood monocytes to in vitro lipopolysaccharide-challenge in patients with recurrent unstable angina. *Circulation* 103:2236–2241, 2001.
104. Hollestelle SC, De Vries MR, Van Keulen JK, et al.: Toll-like receptor 4 is involved in outward arterial remodeling. *Circulation* 109:393–398, 2004.
105. Methe H, Kim JO, Kofler S, et al.: Expansion of circulating Toll-like receptor 4–positive monocytes in patients with acute coronary syndrome. *Circulation* 111:2654–2661, 2005.

106. Gerard C, Rollins BJ: Chemokines and disease. *Nat Immunol* 2:108–115, 2001.
107. Moser B, Loetscher P: Lymphocyte traffic control by chemokines. *Nat Immunol* 2:123–128, 2001.
108. Strieter RM, Polverini PJ, Arenberg DA, et al.: Role of C-X-C chemokines as regulators of angiogenesis in lung cancer. *J Leukoc Biol* 57:752–762, 1995.
109. Frangogiannis NG: Targeting the inflammatory response in healing myocardial infarcts. *Curr Med Chem* 13:1877–1893, 2006.
110. Carter AM: Inflammation, thrombosis and acute coronary syndromes. *Diab Vasc Dis Res* 2:113–121, 2005.
111. Kitchens RL, Thompson PA: Modulatory effects of sCD14 and LBP on LPS-host cell interactions. *J Endotoxin Res* 11:225–229, 2005.
112. Heumann D, Roger T: Initial responses to endotoxins and Gram-negative bacteria. *Clin Chim Acta* 323:59–72, 2002.
113. Wright SD: CD14 and innate recognition of bacteria. *J Immunol* 155:6–8, 1995.
114. Ulevitch RJ, Tobias PS: Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 13:437–457, 1995.
115. Gioannini TL, Zhang D, Teghanemt A, et al.: An essential role for albumin in the interaction of endotoxin with lipopolysaccharide-binding protein and sCD14 and resultant cell activation. *J Biol Chem* 277:47818–47825, 2002.
116. Shands JW Jr, Chun PW: The dispersion of gram-negative lipopolysaccharide by deoxycholate. Subunit molecular weight. *J Biol Chem* 255: 1221–1226, 1980.
117. Brogden KA, Phillips M: The ultrastructural morphology of endotoxins and lipopolysaccharides. *Electron Microsc Rev* 1:261–278, 1988.
118. Wright SD, Ramos RA, Tobias PS, et al.: CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 249:1431–1433, 1990.
119. Hailman E, Lichenstein HS, Wurfel MM, et al.: Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* 179:269–277, 1994.
120. Deleted in proof.
121. Takeuchi O, Hoshino K, Kawai T, et al.: Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 11:443–451, 1999.
122. Shimazu R, Akashi S, Ogata H, et al.: MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med* 189: 1777–1782, 1999.
123. Tobias PS, Soldau K, Ulevitch RJ: Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *J Exp Med* 164:777–793, 1986.
124. Triantafilou M, Triantafilou K: Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol* 23:301–304, 2002.
125. Wurfel MM, Kunitake ST, Lichenstein H, et al.: Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J Exp Med* 180:1025–1035, 1994.
126. Wurfel MM, Hailman E, Wright SD: Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. *J Exp Med* 181:1743–1754, 1995.
127. Gazzano-Santoro H, Meszaros K, Birr C, et al.: Competition between rBPI23, a recombinant fragment of bactericidal/permeability-increasing protein, and lipopolysaccharide (LPS)–binding protein for binding to LPS and gram-negative bacteria. *Infect Immun* 62:1185–1191, 1994.
128. Elsbach P, Weiss J: The bactericidal/permeability-increasing protein (BPI), a potent element in host-defense against gram-negative bacteria and lipopolysaccharide. *Immunobiology* 187:417–429, 1993.
129. Tobias PS, Soldau K, Iovine NM, et al.: Lipopolysaccharide (LPS)-binding proteins BPI and LBP form different types of complexes with LPS. *J Biol Chem* 272:18682–18685, 1997.
130. Cohn JN: The management of chronic heart failure. *N Engl J Med* 335:490–498, 1996.
131. Grum CM: Tissue oxygenation in low flow states and during hypoxemia. *Crit Care Med* 21:44–49, 1993.
132. David H, Siems WG, Ellermann J: Ultrastructure and biochemistry of ischemic damages of small intestinal epithelial cells. *Exp Toxicol Pathol* 44:325–335, 1992.
133. Parks DA, Granger DN: Contributions of ischemia and reperfusion to mucosal lesion formation. *Am J Physiol* 250:G749–G753, 1986.
134. Noda T, Iwakiri R, Fujimoto K, et al.: Programmed cell death induced by ischemia-reperfusion in rat intestinal mucosa. *Am J Physiol* 274:G270–G276, 1998.
135. Ikeda H, Suzuki Y, Suzuki M, et al.: Apoptosis is a major mode of cell death caused by ischaemia and ischaemia/reperfusion injury to the rat intestinal epithelium. *Gut* 42:530–537, 1998.
136. Chiu CJ, McArdle AH, Brown R, et al.: Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 101:478–483, 1970.
137. Brunkhorst FM, Clark AL, Forycki ZF, et al.: Pyrexia, procalcitonin, immune activation and survival in cardiogenic shock: the potential importance of bacterial translocation. *Int J Cardiol* 72:3–10, 1999.
138. Lemaire LC, van Wagensveld BA, van Gulik TM, et al.: Bacterial translocation to the thoracic duct in a setting of ischemia, partial resection and reperfusion of the porcine liver. *Dig Surg* 16:222–228, 1999.
139. Dowdall JF, Winter DC, Bouchier-Hayes DJ: Inosine modulates gut barrier dysfunction and end organ damage in a model of ischemia-reperfusion injury. *J Surg Res* 108:61–68, 2002.
140. Bolke E, Jehle PM, Graf M, et al.: Inflammatory response during abdominal and thyroid surgery: a prospective clinical trial on mediator release. *Shock* 16:334–339, 2001.
141. Peschel T, Schonauer M, Thiele H, et al.: Invasive assessment of bacterial endotoxin and inflammatory cytokines in patients with acute heart failure. *Eur J Heart Fail* 5:609–614, 2003.
142. Lequier LL, Nikaidoh H, Leonard SR, et al.: Preoperative and postoperative endotoxemia in children with congenital heart disease. *Chest* 117:1706–1712, 2000.
143. Kumar A, Bunnell E, Lynn M, et al.: Experimental human endotoxemia is associated with depression of load-independent contractility indices: prevention by the lipid a analogue E5531. *Chest* 126:860–867, 2004.
144. Elin RJ, Wolff SM, McAdam KP, et al.: Properties of reference *Escherichia coli* endotoxin and its phthalylated derivative in humans. *J Infect Dis* 144: 329–336, 1981.
145. Suffredini AF, Fromm RE, Parker MM, et al.: The cardiovascular response of normal humans to the administration of endotoxin. *N Engl J Med* 321: 280–287, 1989.
146. Greenman RL, Schein RM, Martin MA, et al.: A controlled clinical trial of E5 murine monoclonal IgM antibody to endotoxin in the treatment of gram-negative sepsis. The XOMA Sepsis Study Group. *JAMA* 266:1097–1102, 1991.
147. Bone RC: Gram-negative sepsis: a dilemma of modern medicine. *Clin Microbiol Rev* 6:57–68, 1993.
148. Cross AS, Opal SM, Bhattacharjee AK, et al.: Immunotherapy of sepsis: flawed concept or faulty implementation? *Vaccine* 17(suppl 2):13–21, 1999.
149. Norrby-Teglund A, Ihendyane N, Darenberg J: Intravenous immunoglobulin adjunctive therapy in sepsis, with special emphasis on severe invasive group A streptococcal infections. *Scand J Infect Dis* 35:683–689, 2003.
150. Goldie AS, Fearon KC, Ross JA, et al.: Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. The Sepsis Intervention Group. *JAMA* 274:172–177, 1995.
151. Wakefield CH, Barclay GR, Fearon KC, et al.: Proinflammatory mediator activity, endogenous antagonists and the systemic inflammatory response in intra-abdominal sepsis. Scottish Sepsis Intervention Group. *Br J Surg* 85:818–825, 1998.
152. Strutz F, Heller G, Krasemann K, et al.: Relationship of antibodies to endotoxin core to mortality in medical patients with sepsis syndrome. *Intensive Care Med* 25:435–444, 1999.
153. Bennett-Guerrero E, Panah MH, Barclay GR, et al.: Decreased endotoxin immunity is associated with greater mortality and/or prolonged hospitalization after surgery. *Anesthesiology* 94:992–998, 2001.
154. Rothenburger M, Soeparwata R, Deng MC, et al.: The impact of anti-endotoxin core antibodies on endotoxin and cytokine release and ventilation time after cardiac surgery. *J Am Coll Cardiol* 38:124–130, 2001.
155. Mashimo J, Mizutani T, Mita A, et al.: Neutralization of Shwartzman-inducing activity by antibodies recognizing the Re core or lipid A structures of lipopolysaccharide from *Salmonella minnesota* R595 and *Pseudomonas vesicularis* JCM1477. *Microbiol Immunol* 35:423–434, 1991.
156. Warren HS, Amato SF, Fitting C, et al.: Assessment of ability of murine and human anti-lipid A monoclonal antibodies to bind and neutralize lipopolysaccharide. *J Exp Med* 177:89–97, 1993.
157. Baumgartner JD, Heumann D, Gerain J, et al.: Association between protective efficacy of anti-lipopolysaccharide (LPS) antibodies and suppression of LPS-induced tumor necrosis factor alpha and interleukin 6. Comparison of O side chain-specific antibodies with core LPS antibodies. *J Exp Med* 171:889–896, 1990.
158. Marra MN, Thornton MB, Snable JL, et al.: Endotoxin-binding and -neutralizing properties of recombinant bactericidal/permeability-increasing protein and monoclonal antibodies HA-1A and E5. *Crit Care Med* 22: 559–565, 1994.
159. Baumgartner JD, Heumann D, Glauser MP: The HA-1A monoclonal antibody for gram-negative sepsis. *N Engl J Med* 325:281, 1991. [Letter to editor].
160. Bhat NM, Bieber MM, Chapman CJ, et al.: Human antilipid A monoclonal antibodies bind to human B cells and the i antigen on cord red blood cells. *J Immunol* 151:5011–5021, 1993.
161. Helmerhorst EJ, Maaskant JJ, Appelmelk BJ: Anti-lipid A monoclonal

antibody centoxin (HA-1A) binds to a wide variety of hydrophobic ligands. *Infect Immun* 66:870–873, 1998.
162. Quezado ZM, Natanson C, Alling DW, et al.: A controlled trial of HA-1A in a canine model of gram-negative septic shock. *JAMA* 269:2221–2227, 1993.
163. Charalambous BM, Evans J, Feavers IM, et al.: Comparative analysis of two meningococcal immunotyping monoclonal antibodies by resonant mirror biosensor and antibody gene sequencing. *Clin Diagn Lab Immunol* 6:838–843, 1999.
164. Suker J, Charalambous BM: Applications of optical biosensor techniques to the characterisation of PortA antibody binding kinetics. In: Pollock DA, Maiden MCJ (eds.): *Methods in Molecular Medicine: Meningococcal Vaccines Methods and Protocols*. Totowa, NJ: Humana Press, pp 129–143, 2002.
165. Jiang Z, Hong Z, Guo W, et al.: A synthetic peptide derived from bactericidal/permeability-increasing protein neutralizes endotoxin in vitro and in vivo. *Int Immunopharmacol* 4:527–537, 2004.
166. Dankesreiter S, Hoess A, Schneider-Mergener J, et al.: Synthetic endotoxin-binding peptides block endotoxin-triggered TNF-alpha production by macrophages in vitro and in vivo and prevent endotoxin-mediated toxic shock. *J Immunol* 164:4804–4811, 2000.
167. Ciornei CD, Egesten A, Engstrom M, et al.: Bactericidal/permeability-increasing protein inhibits endotoxin-induced vascular nitric oxide synthesis. *Acta Anaesthesiol Scand*46:1111–1118, 2002.
168. Levy O: Therapeutic potential of the bactericidal/permeability-increasing protein. *Expert Opin Investig Drugs* 11:159–167, 2002.
169. Estabrook M, Baker CJ, Griffiss JM: The immune response of children to meningococcal lipooligosaccharides during disseminated disease is directed towards primarily against two monoclonal antibody–defined epitopes. *J Infect Dis* 167:966–970, 1993.
170. Griffiss JM, Brandt BL, Broud DD, et al.: Immune response of infants and children to disseminated infections with *Neisseria meningitidis*. *J Infect Dis* 150:71–79, 1984.
171. Ziegler EJ, McCutchan JA, Fierer J, et al.: Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 307:1225–1230, 1982.
172. Kirkland TN, Ziegler EJ: An immunoprotective monoclonal antibody to lipopolysaccharide. *J Immunol* 132:2590–2592, 1984.
173. Bennett-Guerrero E, McIntosh TJ, Barclay GR, et al.: Preparation and preclinical evaluation of a novel liposomal complete-core lipopolysaccharide vaccine. *Infect Immun* 68:6202–6208, 2000.
174. Cross AS, Opal SM, Palardy JE, et al.: Phase I study of detoxified *Escherichia coli* J5 lipopolysaccharide (J5dLPS)/group B meningococcal outer membrane protein (OMP) complex vaccine in human subjects. *Vaccine* 21:4576–4587, 2003.
175. Passwell JH, Ashkenazi S, Harlev E, et al.: Safety and immunogenicity of *Shigella sonnei*-CRM9 and *Shigella flexneri* type 2a-rEPAsucc conjugate vaccines in one- to four-year-old children. *Pediatr Infect Dis J* 22:701–706, 2003.
176. Fries LF, Montemarano AD, Mallett CP, et al.: Safety and immunogenicity of a proteosome-*Shigella flexneri* 2a lipopolysaccharide vaccine administered intranasally to healthy adults. *Infect Immun* 69:4545–4553, 2001.
177. Kang HY, Curtiss R III: Immune responses dependent on antigen location in recombinant attenuated *Salmonella typhimurium* vaccines following oral immunization. *FEMS Immunol Med Microbiol* 37:99–104, 2003.
178. Eko FO, Mayr UB, Attridge SR, et al.: Characterization and immunogenicity of *Vibrio cholerae* ghosts expressing toxin-coregulated pili. *J Biotechnol* 83: 115–123, 2000.
179. Pier GB: Promises and pitfalls of *Pseudomonas aeruginosa* lipopolysaccharide as a vaccine antigen. *Carbohydr Res* 338:2549–2556, 2003.
180. Ryu HI, Kim CJ: Immunologic reactivity of a lipopolysaccharide-protein complex of type A *Pasteurella multocida* in mice. *J Vet Sci* 1: 87–95, 2000.
181. Estein SM, Cassataro J, Vizcaino N, et al.: The recombinant Omp31 from *Brucella melitensis* alone or associated with rough lipopolysaccharide induces protection against *Brucella ovis* infection in BALB/c mice. *Microbes Infect* 5:85–93, 2003.
182. Conlan JW, Shen H, Webb A, et al.: Mice vaccinated with the O-antigen of *Francisella tularensis* LVS lipopolysaccharide conjugated to bovine serum albumin develop varying degrees of protective immunity against systemic or aerosol challenge with virulent type A and type B strains of the pathogen. *Vaccine* 20:3465–3471, 2002.
183. Mandrell RE, McLaughlin R, Aba Kwaik Y, et al.: Lipooligosaccharides (LOS) of some *Haemophilus* species mimic human glycosphingolipids, and some LOS are sialylated. *Infect Immun* 60:1322–1328, 1992.
184. Moran AP, Prendergast MM, Appelmelk BJ: Molecular mimicry of host structures by bacterial lipopolysaccharides and its contribution to disease. *FEMS Immunol Med Microbiol* 16:105–115, 1996.
185. Charalambous BM, Feavers IM: Mimotope vaccines. *J Med Microbiol* 50: 937–939, 2001.
186. Brett PJ, Tiwana H, Feavers IM, et al.: Characterisation of oligopeptides that cross-react with carbohydrate specific antibodies by real-time kinetic, in-solution competition ELISA and immunological analyses. *J Biol Chem* 277: 20468–20476, 2002.
187. Winter G, Milstein C: Man-made antibodies. *Nature* 349:293–299, 1991.