

Human Endotoxemia as a Model of Systemic Inflammation

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Abstract: Systemic inflammation is a pathogenetic component in a vast number of acute and chronic diseases such as sepsis, trauma, type 2 diabetes, atherosclerosis, and Alzheimer's disease, all of which are associated with a substantial morbidity and mortality. However, the molecular mechanisms and physiological significance of the systemic inflammatory response are still not fully understood. The human endotoxin model, an *in vivo* model of systemic inflammation in which lipopolysaccharide is injected or infused intravenously in healthy volunteers, may be helpful in unravelling these issues. The present review addresses the basic changes that occur in this model. The activation of inflammatory cascades as well as organ-specific haemodynamic and functional changes after lipopolysaccharide are described, and the limitations of human-experimental models for the study of clinical disease are discussed. Finally, we outline the ethical considerations that apply to the use of human endotoxin model.

Keywords: Cytokine, diabetes, endotoxin, inflammation, LPS, interleukin-6, sepsis, tumor necrosis factor- α .

BACKGROUND

Systemic inflammation is a pathogenetic component in a vast number of acute and chronic diseases. Sepsis, defined as the acute systemic inflammatory response to infection, is the most common cause of death in intensive care units with a case fatality ratio of 29% [1], which corresponds to approximately 200,000 annual deaths or 10% of all deaths in the United States [2]. Even in the absence of infection, systemic inflammation contributes the pathophysiology of critical illness, i.e. in the context of major trauma, burns, pancreatitis, and after major surgery [3]. Moreover, inflammation has been invoked as a major pathogenetic factor in a number of chronic diseases such as type 2 diabetes (T2D) [4-6], atherosclerotic diseases (e.g., cerebrovascular and ischaemic heart disease [7-10]), and Alzheimer's dementia (AD) [11,12]. These conditions affect millions of people worldwide, are major causes of death in both low-, middle- and high-income countries [13,14], and thus place a substantial burden upon the health care systems. Hence, a better understanding of the underlying molecular and pathophysiological mechanisms in acute as well as in chronic inflammation is urgently warranted, as this may lead to optimized prevention and treatment of these disorders.

Studies of pathophysiological changes during acute inflammatory disorders are potentially confounded by the absence of a well-defined onset time of inflammation, as well as by considerable delays from the presumed initiation of inflammation until the study is conducted. In conditions characterized by chronic low-grade inflammation, not only differences in the duration of disease but also the presence of comorbidity may blunt and disturb any causality and thus bias conclusions. Animal studies are useful for investigating early pathophysiological changes as well as the development of organ failure during systemic inflammation; however, these studies are potentially confounded by major inter-species differences in the sensitivity and immune response to various types of inflammatory stimuli [15-18]. Therefore, human *in vivo* models of systemic inflammation may be useful for studying the molecular and physiological effects of inflammation and its significance in acute and chronic diseases. The present review describes the use of such a highly standardized human-experimental model, the human endotoxin model.

INFLAMMATION IN HUMAN DISEASE

Inflammation is initiated by tissue damage or by the presence of pathogen-associated molecular patterns (PAMPs) on invading

microorganisms, i.e. lipopolysaccharide (LPS; also referred to as endotoxin), peptidoglycans, and CpG nucleic acids. PAMPs bind to pattern-recognition receptors (PRRs) on host cells. Of the latter, Toll-like receptors (TLRs) [19,20], a family of transmembrane receptors that are expressed on the surface of monocytes and macrophages, dendritic cells, intestinal epithelial and endothelial cells, sense extracellular PAMPs. In contrast, NOD-like receptors and RIG-like receptors are intracellular PRRs, which recognize invading microorganisms in the cytoplasm of the host cell [21,22].

Binding of ligands to TLRs initiates downstream intracellular signaling pathways that ultimately result in the activation of the nuclear transcription factor, nuclear factor κ B (NF- κ B) [23-26], which in turn stimulates the transcription of genes coding for pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukin (IL)-1 β [25]. These cytokines activate an assortment of inflammatory cascades including the complement system, the coagulation system and the production of NO, all of which participate in eliminating invading microorganisms. TNF- α and IL-1 β stimulate the expression of adhesion molecules on endothelial cells, thus facilitating leukocyte migration, and further mediate important features of the acute phase response, such as the induction of fever [27]. In addition, stimulation of the innate immune system and of inflammatory mediators increase tissue perfusion and vascular permeability and enhance cell migration, all of which may facilitate the final eradication of the micro-organism [22,28].

Likewise, activation of NOD-like receptors and RIG-like helicases induces pro-inflammatory cytokines [21]. Interestingly, not only intracellular PAMPs, but also danger-associated molecular patterns (DAMPs) in molecules and cellular products arising from acute non-infectious cellular stress or cell damage, activate some of these intracellular receptors [21,29]. This indicates that some inflammatory pathways may be activated both by infection as well as by non-infectious conditions associated with cellular damage such as trauma, burns or major surgery.

The pro-inflammatory cytokines subsequently stimulate a second wave of cytokines. By acting on the hypothalamo-pituitary-adrenal (HPA) axis [30,31], IL-1 β and IL-6 induce the production of cortisol, which provides a negative feed-back loop by inhibiting cytokine gene expression [32]. IL-6 stimulates the production of anti-inflammatory cytokines [33], such as IL-10 and IL-1 receptor antagonist (IL-1ra) [34], which binds to IL-1 receptors in competition with IL-1. Additionally, IL-6 triggers the shedding of soluble TNF- α receptors (sTNFRs) [33]. These receptors also have an anti-inflammatory effect, as they bind TNF- α , thus attenuating the effects of this cytokine [35,36]. The anti-inflammatory phase is central for the resolution of the immune response, and the balance between pro- and anti-inflammatory activity may be crucial for the final outcome [37]. Also, in the setting of local infection or tissue

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injury, a well-adapted anti-inflammatory response may contain local inflammation and prevent it from evolving into systemic inflammation.

Systemic inflammation may be classified as acute or chronic. Acute systemic inflammation such as in sepsis, trauma, burns, and surgery is characterized by rapid and immense (up to 100-fold) increases in plasma-levels of pro-inflammatory cytokines [38], as well as by considerable increases in most other immunological mediators including acute phase reactants [39]. On the contrary, chronic low-grade inflammation is characterized by a modest but sustained increase in cytokines and acute phase reactants, usually of two to three fold [40], and may be a key player in the pathogenesis of most chronic non-communicable diseases such as T2D, atherosclerosis and AD.

Human models of systemic inflammation have been developed with the purpose of mimicking the changes in inflammatory mediators encountered in acute as well as chronic inflammatory disease, but in a controlled, standardised experimental setting.

HUMAN MODELS OF SYSTEMIC INFLAMMATION

The most widespread model of systemic inflammation is probably the human endotoxin model, in which purified LPS (endotoxin) from *Escherichia coli* or other Gram-negative bacteria is administered intravenously to healthy volunteers. Alternatively, the infusion of recombinant human TNF- α or IL-6 has been applied in order to explore the mechanisms whereby these specific cytokines influence metabolism during high- and low-grade inflammation. In this review, however, emphasis will be on the endotoxin model.

The Human Endotoxin Model

Administration of bacterial pyrogens to humans was first described in the 1890s, when cancer patients were treated with a

mixture of bacteria in order to reduce tumor growth [41]. This effect was later shown to be due to the induction of TNF- α [42]. As late as the 1990s, endotoxin treatment was administered for the treatment of patients with advanced cancer [43]. Perhaps the first time that LPS was administered with another aim than direct treatment was in 1955, where purified *Pseudomonas* LPS was injected to patients with agammaglobulinemia and healthy controls in order to disclose the nature of the immunological deficit of the former group. Since then, a large number of human studies have been conducted using this model, predominantly with *E. coli* LPS because of the high reproducibility of effects. Less often *Salmonella abortus equi* LPS has been used.

Endotoxin refers to the complex of LPS in the outer leaflet of the outer membrane of most Gram-negative bacteria (Fig. 1), associated with variable amounts of phosphate and protein [44,45]; conversely, the term LPS refers to the purified lipopolysaccharide moiety. Despite the inaccuracy of the term, experimental "endotoxemia" refers to the intravenous injection of LPS; the terms LPS and endotoxin are used almost synonymously in the literature as well as throughout this review. LPS consists of three covalently linked regions; lipid A, a core oligosaccharide, and an O-side chain [46,47]. The O-side chain and the core polysaccharides are both immunogenic, the parts against which the infected host produces antibodies, and are able to produce some of the effects of native LPS. However, the toxicity of LPS resides within lipid A [48,49]; purified lipid A most likely retains all of the effects of native LPS, *in vivo* and *in vitro* [46].

Intravenous administration of purified standard reference LPS to healthy volunteers induces an acute systemic inflammatory response, which, at least partially, mimics the inflammatory response of early sepsis as well as other acute inflammatory conditions. For the classical studies of sepsis pathophysiology, a bolus injection of higher doses of *E. coli* LPS (2-4 ng/kg body weight (BW)) has been used. Although the inflammatory response in non-Gram-negative

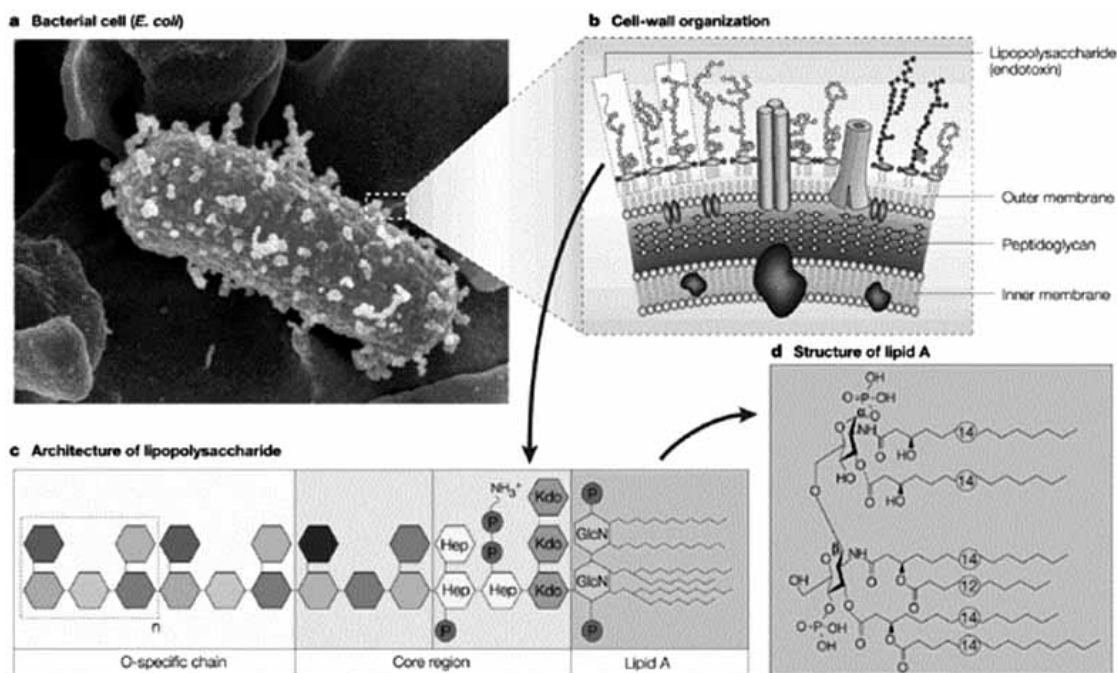


Fig. (1). A Gram-negative bacterium. Electron micrograph of *Escherichia coli* (a), together with a schematic representation of the location of lipopolysaccharide (LPS; endotoxin) in the bacterial cell wall (b) and the architecture of LPS (c). Also shown is the primary structure of the toxic centre of LPS, the lipid A component (d).

GlcN, D-glucosamine; Hep, L-glycero-D-manno-heptose; IM, inner membrane; Kdo, 2-keto-3-deoxy-octulosonic acid; OM, outer membrane; P, phosphate; PG, peptidoglycan.

Modified from Beutler and Rietschel, *Nat. Rev. Immunol.*, 2003, 2, 169-176 [153].

sepsis is mediated by other bacterial components than LPS (e.g. lipoteichoic acid in the Gram-positive bacterial wall), the intracellular signal transduction mechanisms are largely the same as in Gram-negative sepsis. Hence, knowledge regarding the pathophysiology of Gram-negative sepsis is probably relevant to sepsis in general, irrespective of microbial etiology. Most likely, this also applies to other conditions characterized by acute systemic inflammation such as trauma, burns and major surgery.

Lately, our laboratory has implemented low-dose intravenous bolus injection [50], as well as continuous infusion [51], of *E. coli* LPS (0.06-0.2 ng/kg BW) with the aim of studying low-grade inflammation. The administration of these very low doses of LPS in an experimental setting induces 2-10 fold increases in plasma cytokines which resembles the levels reported in chronic low-grade inflammatory conditions [52].

After injection, LPS can be measured in plasma within a few minutes and is more or less cleared from this compartment during the following 15 minutes [53]. From plasma, most LPS is transported to the liver for metabolic degradation, whereas a minor amount is metabolized in the spleen, lungs, kidneys and adrenal glands [54]. Over the following days, LPS is excreted mainly in the feces and to a lesser extent in the urine and pulmonary secretions [55]. "Endotoxemia" is, in general, used for the clinical and biochemical findings that occur after the intravenous administration of LPS, even after this substance in itself has been cleared from the bloodstream.

Infusion of LPS or recombinant human cytokines is, at best, an insufficient model of systemic inflammation, let alone of sepsis.

The doses needed in humans to mimic severe sepsis with regard to organ dysfunction are unsafe and ethically unacceptable. Moreover, the use of healthy subjects as well as of LPS rather than living Gram-negative bacteria is inadequate for reproducing the complex effects of severe sepsis, trauma or burns in patients with significant pre-existing chronic diseases and/or organ dysfunction. Moreover, the highly dynamic changes in cytokine levels observed after LPS injection [56] differ substantially from the more sustained levels reported in critically ill patients [57]. In agreement with this, animal studies show that LPS injection carries the same mortality rates as sepsis elicited by experimental ligation and puncture of the cecum in mice [58]; however, both kinetics and the magnitude of the following cytokine production vary significantly between the two methods. With regard to chronic low-grade inflammation, this phenomenon is probably mimicked only partially with low-dose endotoxin administration to humans; the intravascular levels of cytokines after LPS injection may easily exceed those observed in patients with T2D, but do so on an acute rather than on a chronic basis, thus obviating the possibility of evaluating the effects of more sustained elevations in cytokine levels on organ function. Finally, neutrophilia is a common finding after low-dose endotoxin administration [59]; although slightly elevated neutrophil counts have been reported in type 2 diabetes [60] as well as in chronic kidney disease [61] this may not be a universal finding with chronic inflammation.

Thus, the human endotoxin model carries a risk of inappropriate extrapolation from experimental findings to the clinical setting. However, with these limitations, the endotoxin model as well as the titrated infusion of recombinant cytokines described below render

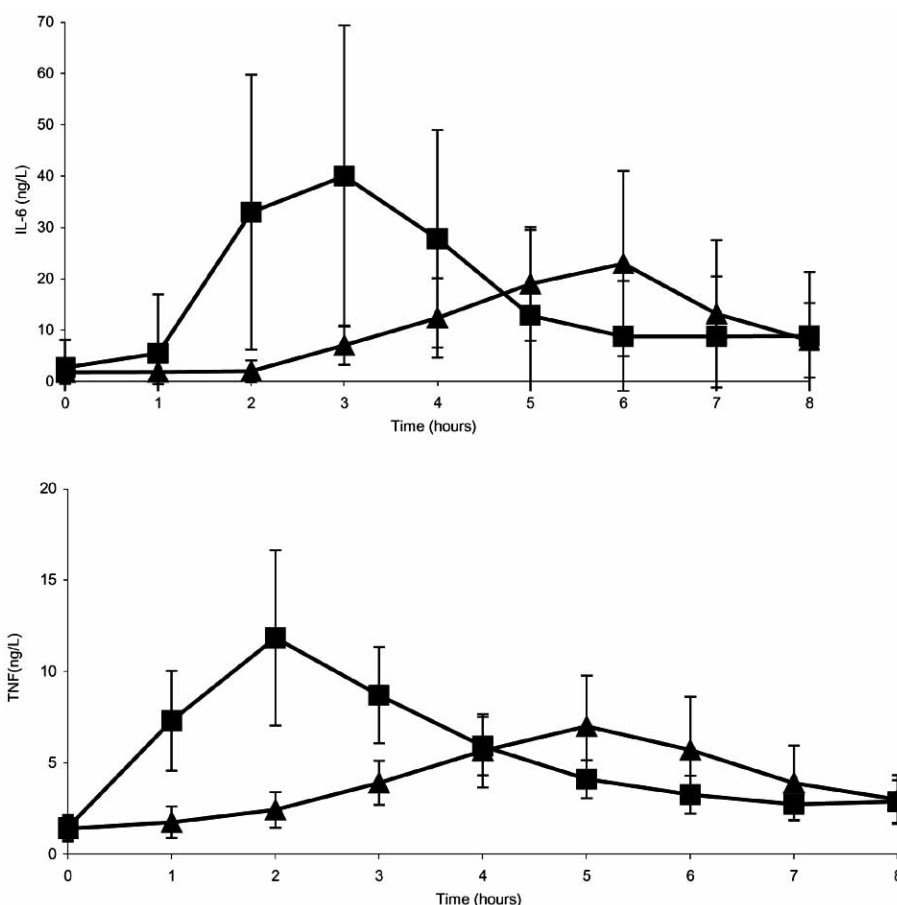


Fig. (2). Plasma TNF- α and plasma IL-6 after *E. coli* endotoxin (0.3 ng/kg), given either as an IV bolus injection (■) or a 4-h IV infusion (▲) in young healthy volunteers ($n = 10$).

Modified from Taudorf *et al. Clin. Vaccine Immunol.*, 2007, 14(3), 250-255 [51].

an opportunity to study immunological, metabolic, humoral and physiological changes due to significant increases in inflammatory mediators.

Infusion of Recombinant Human Cytokines

Given that plasma levels of TNF- α and IL-6 are elevated in diseases such as sepsis, atherosclerosis, T2D, and the metabolic syndrome [62-67], intravenous injection or infusion of either cytokine is relevant in order to study the specific pathophysiological roles of these cytokines during systemic inflammation.

During infusion of recombinant cytokines such as TNF- α and IL-6, steady state is reached within approximately 30 minutes. As in the endotoxin model, doses can be titrated in order to reach plasma concentrations similar to the condition that is modelled (i.e., high- or low-grade inflammation).

INFLAMMATORY CASCADES AND IMMUNE CELLS IN HUMAN-EXPERIMENTAL SYSTEMIC INFLAMMATION

A variety of cascades are activated during experimental systemic inflammation in humans.

Cytokines

During acute systemic inflammation or after induction of experimental endotoxemia, LPS-induced production of pro-inflammatory cytokines (TNF- α and IL-1 β), chemokines, and other inflammatory mediators aid in mounting the innate immune response. The time course of the cytokine changes after an intravenous bolus injection of *E. coli* LPS is highly uniform and reproducible; applying a continuous LPS infusion instead of a bolus injection changes the profile over time (Fig. 2) [51]. Thus, the endotoxin model may be useful for studying cytokine kinetics and interplays early in the inflammatory response. Within the first hour after LPS administration, TNF- α , soluble TNF- α receptor (sTNFr), IFN- γ , and IL-6 appear in plasma. Of these, TNF- α shows a monophasic peak after 90 minutes, whereas IL-6 and IFN- γ peak after 120 minutes [32,53,56,68-71]. IL-1 β peaks sometime after TNF- α ,

but before IL-6 [72]; however, most studies have been unable to detect this cytokine after *in vivo* exposure to endotoxin [53,69,70,73]. The anti-inflammatory response emerges somewhat later (Fig. 3), with IL-10 reaching a maximum after approximately 3 hours [69] and IL-1ra peaking at 4 hours after exposure to endotoxin [56]. Increases in chemokines such as IL-8, monocyte chemoattractant protein (MCP)-1, and neutrophil attractant protein (NAP)-1 can also be detected subsequent to the administration of LPS [74-76].

Cytokine and chemokine dynamics after endotoxin are dose-dependent. Thus, higher doses of *E. coli* LPS, such as 2-4 ng/kg BW, cause an almost supraphysiological (up to 2000-fold) increase in TNF- α [70,77]. Doses in the range of 0.3-1 ng/kg BW result in a 3-100 fold rise [51], which resembles the concentrations during human sepsis more accurately [78-82]. To create a model of low-grade inflammation with a 2-3 fold increase in TNF and IL-6, as little as 0.06-0.1 ng/kg BW may be administered [52]. The qualitative changes, as stated previously, are very uniform and reproducible, but the magnitude of the cytokine response to endotoxin is highly individual. Genetic polymorphisms [83,84], comorbidity, age [56] and gender [69] may all play a role for variations in the elicited response.

Arachidonic Acid Metabolites

The eicosanoid hormones are derived from polyunsaturated fatty acids and play a central role in inflammation. Arachidonic acid, a 20:4 fatty acid, is the major precursor of several classes of signalling molecules, e.g. prostaglandins and thromboxanes that are synthesized *via* the cyclooxygenase system, and the leukotrienes and lipoxins that are produced through the action of lipoxygenases [37,85]. Upon stimulation with LPS, macrophages and monocytes produce prostaglandin PGE₂ [86], hepatic Kupffer cells secrete PGD₂ [87], and macrophages release thromboxane A₂ and PGI₂ [88]. Neutrophils synthesize leukotrienes and thromboxane A₂ [89], whereas endothelial cells produce PGI₂ [90]. Apart from the direct LPS stimulus, the production of arachidonic acid metabolites is affected by the downstream cytokines secreted in response to LPS administration [91]. The mediators serve as inflammatory stimuli

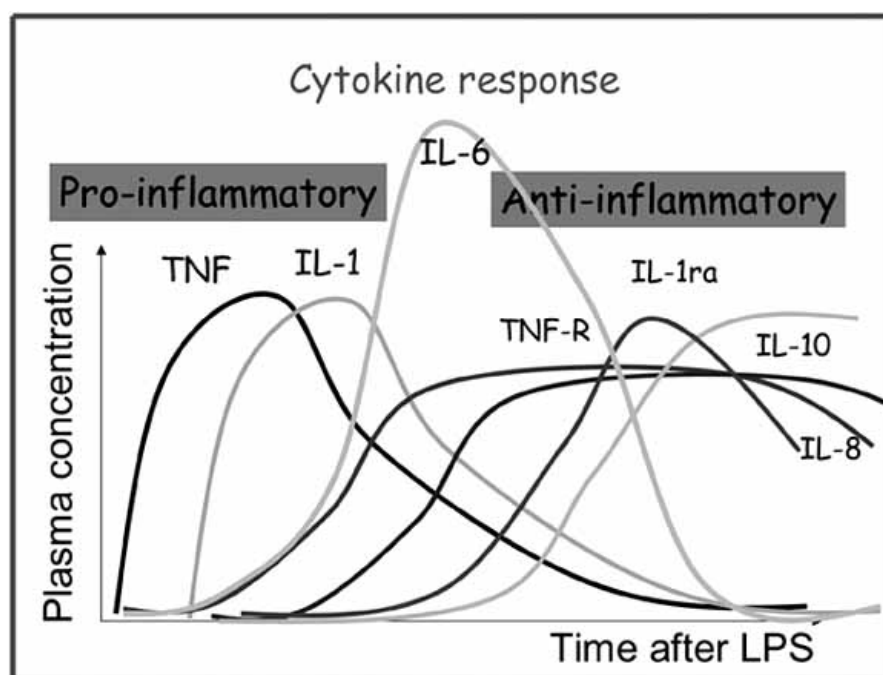


Fig. (3). The time course of plasma cytokine levels after an IV bolus injection of *E. coli*. Peak concentrations are shown on an arbitrary scale. The initial release of pro-inflammatory cytokines is followed by a rise in IL-6 and subsequently by the anti-inflammatory cytokines.

for immune cells [92], affect vasomotor tone [93] and capillary permeability [94], are involved in platelet aggregation [95], and play a major role in mediating the fever response [96] during the inflammatory response. They are therefore usually considered substantial contributors to the clinical presentation of systemic inflammation. Administration of ibuprofen, a cyclooxygenase inhibitor, greatly attenuates the symptoms associated with endotoxin administrations in human volunteers, but did not affect plasma levels of TNF- α [97]; however, to our knowledge the arachidonic acid system has not been further addressed in human models of systemic inflammation.

The NO System

Nitric oxide is an ubiquitous physiological mediator, which is essential to the regulation of e.g. vasomotor tone, thrombogenesis, and neural plasticity [98]. NO is generated by the conversion of arginine to citrulline by nitric oxide synthase (NOS), which is found in an endothelial (or constitutive) (eNOS), an inducible (iNOS), and a neural (nNOS) form in the human body [99]. Endotoxemia may upregulate inducible NO-synthase in the vasculature [100] as well as in the renal tubular cells [101].

Complement System

Although activation of the complement system in Gram-negative infection is regarded as secondary to the presence of LPS in Gram-negative infection [102], this has not been observed in human volunteers after an intravenous bolus of *E. coli* LPS; only an upregulation of complement receptors on the surface of neutrophils has been reported [53,103]. This suggests that continuous exposure to endotoxin, such as is associated with the presence of live bacteria in Gram-negative infection, may be necessary to evoke complement activation *in vivo*, even though neutrophils in themselves are primed to react to complement components by a much shorter exposure to LPS, at least *ex vivo*.

Coagulation System

The presence of LPS and proinflammatory cytokines changes the endothelial cells from being mainly anticoagulant to a procoagulant profile with an upregulation of tissue factor [104] and a simultaneous downregulation of thrombomodulin [105]. Tissue factor, which is also upregulated on monocytes [106], subsequently activates the coagulation pathway, thereby inducing the formation of fibrin clots. Endogenous fibrinolysis is reduced as a result of the release of plasminogen activator inhibitor (PAI)-1 from platelets and endothelial cells, stimulated by proinflammatory cytokines and thrombin [107]. PAI-1 potently inhibits tissue plasminogen activator (t-PA) and thus ultimately counteracts the lysis of fibrin clots.

Following administration of 2-4 ng/kg BW of *E. coli* LPS, an early activation of the fibrinolytic system occurs, which is then followed by a later and more prolonged activation of the coagulation. Thus, t-PA and von Willebrand Factor antigen have been shown to increase after 60 to 90 minutes and to peak after 180 to 240 minutes at levels of two to six fold above baseline [53,108]. PAI levels were observed to increase after 150 minutes and to peak at approximately 240 minutes [53,109]. With regard to anticoagulant proteins, an endotoxin challenge in humans had no or only limited effect on the levels of total, free and bound protein S, and on the levels of total C4 binding protein (C4BP), C4BP β and C4BP β -[110]. Thus, the inflammatory response evoked by the injection of LPS appears to promote activation of the coagulation cascade without inducing major changes in the expression of anticoagulant proteins.

To our knowledge, no studies to date have studied the coagulation system after the administration of recombinant human cytokines.

Immune Cells

Administration of *E. coli* LPS to humans causes an initial decline in neutrophils during the first 15 to 30 minutes, which is then followed by an increase to levels above baseline within the first 90 minutes [53]. The initial drop in circulating neutrophils is probably explained by an increased cell margination along the endothelium due to both LPS and a cytokine-mediated upregulation of vascular adhesion factors [111]. Neutrophils reach maximum concentrations of three to four times above baseline values approximately 4-6 hours after exposure to endotoxin and slowly normalize within the following 12-24 hours [15,51,77,112,113]. The observed neutrocytosis may be a result of an endotoxin-mediated generation of IL-1 β , TNF- α and colony stimulating factors (CSF), the latter of which increases the production and accelerates the maturation of myeloid precursors within the bone marrow. Additionally, animal studies indicate that certain chemotactic factors such as IL-8 and leukotriene B₄ cause leukocytosis by promoting mobilization of neutrophils from bone marrow stores [114]; a similar mechanism may cause the observed changes during endotoxemia in humans.

LPS changes the function of neutrophils by priming them for enhanced responses to other stimuli. For instance, the administration of *E. coli* LPS causes neutrophils to express complement receptors even without activating the complement components themselves [103].

Monocytopenia and lymphopenia after the administration of *E. coli* endotoxin are observed with a nadir at 1½ hours [56] and 4 hours [15,115,116], respectively; the monocyte counts normalize within 6 to 8 hours and lymphocytes after 8-12 hours [56,68,117]. Although both apoptosis and redistribution of lymphocytes to the spleen and the peripheral lymphoid tissue have been hypothesized [117], the cause of this lymphopenia remains obscure.

The changes in circulating neutrophil, lymphocyte and monocyte numbers during intravenous infusion of recombinant TNF- α resembles the changes seen after an bolus injection of *E. coli* LPS [118], indicating that the effect of LPS in experimental endotoxemia is mediated, at least in part, through this cytokine. In both experimental settings, a dose-response effect has been observed [77,118].

Endothelial cells play an essential role, both in the pathophysiology of acute inflammatory conditions and in low-grade inflammation [67, 119]. It is difficult to study the function of endothelial cells *in vivo* in humans; nevertheless, the presence of vWF and t-PA after the administration of endotoxin indicates an endothelial cell activation [53].

Humoral Responses

In general, studies of ACTH, cortisol and growth hormone during systemic inflammation have been performed either to study these hormones as mediators of the stress-response or for elucidating inflammation-induced changes in glucose metabolism. Endotoxin administration and TNF- α or IL-6 infusions all activate the hypothalamo-pituitary-adrenal (HPA) axis, as shown by increases in plasma levels of adrenocorticotropic hormone (ACTH) and cortisol [31,34,51,70,77,118,120,121]; the degree of HPA activation appears to depend both on the type and the dose of endotoxin administered.

Cholinergic signalling is also involved in anti-inflammatory reactions [122], but has so far only been studied in the endotoxin model and not following cytokine infusion. The vagal nerve, when stimulated, releases acetylcholine (ACh), which inhibits the activation of macrophages and the production of pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α . The hydrolysis and inactivation of ACh increases with age, which is associated with increased levels of cytokines. *E. coli* LPS injection resulted in an

increase in ACh esterase activity and thus in ACh hydrolysis which was associated with an enhanced proinflammatory response in young volunteers [123].

SYSTEMIC AND ORGAN-RELATED CHANGES IN HUMAN MODELS OF SYSTEMIC INFLAMMATION

Systemic Features

Within 1 hour after the intravenous administration of LPS to humans, volunteers experience varying degrees of flu-like symptoms, e.g. chills, headache, myalgias and arthralgias, nausea and photophobia. A large inter-individual variation is seen with regard to the reported discomfort. In general, the presence of symptoms is a dose-dependent phenomenon, and volunteers receiving injection of low doses of LPS are usually asymptomatic despite consistent increase in plasma cytokines [59]. The administration of *S. abortus equi* LPS differs from *E. coli* LPS in that it results in fewer subjective symptoms [124]. Most subjects will experience an attenuation of symptoms within 2 to 6 hours in parallel with the fading of the pro-inflammatory response. The most reproducible clinical findings among subjects include an increase in core temperature and a tachycardia. The body temperature shows a dose-dependent increase of around 2°C. Most likely it results from a complex interaction between endogenous pyrogens such as TNF- α , IL-6 and prostaglandins induced by LPS [125]; the fever is probably the major cause of the observed increase in heart rate.

Infusion of recombinant TNF- α results in fever, pituitary and stress-hormone release, as well as an acute phase response, all of which are similar to those seen in healthy subjects after being exposed to experimental endotoxemia [118]. Infusion of IL-6 results in similar symptoms, which are dose-dependent [126].

Organ-Related Changes

CNS

There is an association between systemic low-grade inflammation and the age-related decline in cognitive function [12,40,127,128]; consequently, models of experimental systemic inflammation have been applied to study this relationship. Furthermore, these models have been applied to investigate the underlying pathophysiological mechanisms in sepsis-associated encephalopathy, a reduction in consciousness that is frequently observed in patients with sepsis [129]. Accordingly, sleepiness and amnesia have been observed after exposure to 2 ng/kg BW *E. coli* LPS [53], and the injection of 0.8 ng/kg BW *S. abortus equi* LPS resulting in TNF- α levels of around 100 pg/ml had negative effects on memory function and increased symptoms of anxiety and depression [124]. Interestingly, administration of a low dose 0.1 ng/kg BW *E. coli* LPS, which causes only small increases in TNF- α to around 5 pg/ml, affected memory function positively [130].

Global cerebral blood flow and oxidative metabolism have been measured in two studies of high-dose *E. coli* endotoxemia in healthy volunteers. In one study, 4 ng/kg BW was administered; no change neither in cerebral blood flow nor in cerebral oxidative metabolism were observed when measured on an hourly basis after injection [131]. In contrast, a reduction in cerebral blood flow occurring 90 minutes after administration of 2 ng/kg BW has been reported, occurring simultaneously with the peak in plasma levels of TNF- α . This decline, which was not associated with a reduction in cerebral oxygen metabolism, was sufficiently explained by a hyperventilatory response with concomitant hypocapnia. Despite a marked increase in plasma levels of TNF- α and IL-6, no cerebral exchange of these cytokines to or from the blood was observed, suggesting that the brain does not contribute to the systemic inflammatory response in this experimental model of early sepsis [129].

Cardiovascular Function

A hyperdynamic state, with decreases in arterial pressure that are associated with severe acute systemic inflammation can, at least partially, be reproduced by the administration of higher doses (2-4 ng/kg BW) of *E. coli* LPS [132]. Mean arterial pressure decreases with a nadir occurring 4-6 hours after injection [53,69,133] and normalizes hereafter. Administration of low LPS doses (0.06-0.3 ng/kg BW) does not trigger changes in blood pressure, although more invasive haemodynamic investigations with the aim of measuring cardiac output, to our knowledge, have not been conducted with these doses. Increases in heart rate at up to 20 beats per minute within the first 4 hours are quite consistently observed, both after low and high doses of endotoxin. No severe cardiovascular complications resulting from endotoxin administration to healthy subjects have been reported.

Pulmonary Function

Administration of 4 ng/kg BW *E. coli* LPS results in an increase in respiratory rate, a decreased inspiratory time and widened alveolar-arterial oxygen tension gradient; these responses are probably mediated by the cyclooxygenase pathway, since ibuprofen blunts these effects of LPS [134]. Also, high LPS doses have been reported to cause dyspnea as well as increases in fractional inspiratory time, minute ventilation and mean inspiratory coefficient [134]. Studies using local instillation of endotoxin into the lungs have been performed and provide information concerning whole lung inflammation and factors that initiate, amplify and resolve local lung inflammation. This may be of relevance during e.g. asthma and dust-related occupational lung diseases, where endotoxin may contribute to the pathogenesis [135].

Renal Function

A bolus injection of 20 IU/kg BW *E. coli* endotoxin (corresponding to 4 ng/kg BW) caused a significant increase in renal plasma flow up to 7 hours after the administration [136]. Apart from the investigation of inflammation-induced blood flow alterations, LPS has also been used for the study of the upregulation of renal iNOS and the effect of NO on tubular integrity during acute inflammation. The injection of *E. coli* LPS (2 ng/kg BW) resulted in a 34-fold increase in iNOS mRNA in cells isolated from the urine of healthy volunteers. This corresponds well to the 40-fold increase observed in sepsis patients, who were also included in the study [101]. An increase in urinary Glutathione-S-transferase-A1, a marker of proximal tubular damage, was also found.

Gastrointestinal Function

Increased intestinal permeability may be seen after endotoxin administration in healthy subjects. Accordingly, excretion of orally administered lactulose, a marker of gut barrier function, was increased twofold during the 12-hour period after an *E. coli* endotoxin challenge [137]. The human endotoxin model has also been applied to explore the effects of total parental nutrition on gut permeability [138]. Subjects fed on total parental nutrition show a significantly higher efflux of lactate, suggesting that prolonged bowel rest may increase bowel permeability to either microorganisms or LPS, thus enhancing the effects of the administered endotoxin. The increased barrier permeability may be a feature of the upper rather than the lower gastrointestinal tract, as the colorectal permeability appears to be unchanged after an intravenous bolus injection of endotoxin at 2 ng/kg BW [139].

The impact of LPS administration on liver function in humans has been studied with regard to changes in the function of the cytochrome P450 system. The administration of LPS (4 ng/kg BW) was found to reduce cytochrome P450-dependent drug clearance [140,141]. No change in liver alanine aminotransferases, alkaline phosphatase or bilirubin was observed. Since the decline in drug clearance coincided with the peak in plasma TNF- α , this cytokine may be involved in these changes. Indeed, animal studies have

shown that administration of pro-inflammatory cytokines are capable of inducing similar changes in cytochrome P450 activity as those seen after LPS administration [142-144].

The administration of an LPS dose corresponding to 4 ng/kg BW to human volunteers was followed by a hepatic efflux (calculated as the hepatic blood flow multiplied by the veno-arterial concentration difference) of TNF- α [145]; suggesting a role for the local splanchnic production of cytokines in eliciting and maintaining the overall systemic inflammatory response.

Metabolism and Inflammation

Fong *et al.* performed a high dose *E. coli* LPS study with a thorough examination of the splanchnic and peripheral tissue metabolic responses to acute inflammation [145]. These authors observed a progressive hyperglycemia from 2 hours after the injection, which persisted until the end of the study at 6 hours; this was associated with an increased splanchnic efflux of glucose. Hypoaminoacidaemia with increases in the splanchnic amino acid uptake was observed, and the arterial level of free fatty acids (FFA) increased along with a triglyceride efflux from the splanchnic bed. These changes in substrate metabolism were not followed by hormonal (insulin, glucagon, adrenaline, cortisol) changes that were sufficient to explain the alterations. However, a net efflux of TNF- α from the splanchnic organs suggests that these tissues are a major site for production of this cytokine, and that locally released TNF- α after exposure to LPS may affect the tissue in a paracrine fashion. These findings also indicate that the splanchnic bed may play an important role in changes in substrate metabolism during acute inflammation.

Increase in circulating FFAs have also been demonstrated in a low-dose *E. coli* LPS (0.2 ng/kg BW) study [59]. Interestingly, the endotoxin-induced increases were suppressed during both a hyperglycemic and a hyperinsulinemic euglycemic clamp, suggesting that high levels of insulin may decrease circulating FFA. Since FFA has potentially deleterious effects, including generation of free oxygen radicals [146,147], activation of NF- κ B [148], and insulin resistance [149], the beneficial effects of insulin administration to critically ill patients [150] may at least in part be caused by a reduction in FFAs.

Studies employing infusion of recombinant cytokines have shown that TNF- α , but not IL-6, induces insulin resistance as indicated by a decline in whole-body insulin-mediated glucose uptake [126,151].

ETHICAL CONSIDERATIONS

New knowledge gained from experimental studies involving people who are sick or at risk of illness may be of benefit for society as well as the individuals participating in these studies. In contrast, studies involving healthy human volunteers imply a risk without a direct benefit for the subjects themselves. The Belmont report stated that "interests other than those of the subject may on some occasions be sufficient by themselves to justify the risks involved in the research, so long as the subjects' rights have been protected". Indeed, human volunteers have been used for more than a century to study transmission mechanisms and natural history of infectious diseases such as yellow fever, malaria, hepatitis and the common cold [152]. In the case of human models of systemic inflammation, these experiments have also been used for many years worldwide. To our knowledge, there have been no serious or permanent adverse effects to either the administration of LPS or the infusion of recombinant human cytokines in such human-experimental setups. The models provide important mechanistic insights and information regarding inflammatory conditions that are major healthcare problems worldwide, including aspects that are hard to study during the natural course of disease, in animal models, or in cell lines. There-

fore, in a setup where ethical approval by the relevant authorities is followed up with proper information, a thorough physical examination of the subjects in order to disclose any medical conditions that could turn out to be a hazard when eliciting systemic inflammation, and careful monitoring of subjects by experienced staff, we believe that it is ethically acceptable to use such models in human volunteers.

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