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Review

Experimental human endotoxemia as a model of systemic inflammation

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ABSTRACT

Systemic inflammation plays a pivotal role in a multitude of conditions, including sepsis, trauma, major surgery and burns. However, comprehensive analysis of the pathophysiology underlying this systemic inflammatory response is greatly complicated by variations in the immune response observed in critically ill patients, which is a result of inter-individual differences in comorbidity, comedication, source of infection, causative pathogen, and onset of the inflammatory response. During experimental human endotoxemia, human subjects are challenged with purified endotoxin (lipopolysaccharide) intravenously which induces a short-lived, well-tolerated and controlled systemic inflammatory response, similar to that observed during sepsis. The human endotoxemia model can be conducted in a highly standardized and reproducible manner, using a carefully selected homogenous study population. As such, the experimental human endotoxemia model does not share the aforementioned clinical limitations and enables us to investigate both the mechanisms of systemic inflammation, as well as to evaluate novel (pharmacological) interventions in humans in vivo. The present review provides a detailed overview of the various designs, organ-specific changes, and strengths and limitations of the experimental human endotoxemia model, with the main focus on its use as a translational model for sepsis research.

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1. Introduction

Systemic inflammation plays a pivotal role in a multitude of conditions, including sepsis, trauma, major surgery, and burns [1]. For many years, efforts have been undertaken to unravel the complex etiology of the systemic inflammatory response. Unfortunately, comprehensive analysis of the pathophysiology underlying this response is greatly complicated by variation in the immune responses observed in critically ill patients, which are a result of inter-individual differences in comorbidity, comedication, source of infection, causative pathogen, and onset of the inflammatory response. The heterogeneity between patients impedes evaluation of pathophysiological mechanisms and hampers accurate comparison of (pharmacological) interventions. As a consequence, large numbers of patients need to be included in clinical trials to demonstrate intervention efficacy. And strikingly, even when these numbers were met, many of the positive results found in preclinical (animal) studies of systemic inflammation could not be reproduced in expensive (phase III) clinical trials [2,3]. Therefore, an intermediate step is highly warranted to improve translation of preclinical animal data to sepsis patients, which will likely prevent more disappointing results, and may allocate resources more efficiently.

The experimental human endotoxemia model can be used to overcome the aforementioned constraints of translating preclinical results into clinical practice. During experimental human endotoxemia, volunteers are challenged with purified endotoxin (lipopolysaccharide [LPS]) derived from the Gram-negative bacterium Escherichia coli. An intravenous challenge with LPS induces a short-lived, well-tolerated, and controlled systemic inflammatory response, mimicking the initial inflammatory response observed in septic patients [4]. Importantly, these challenges can be conducted in a highly standardized and reproducible manner in a carefully selected (homogeneous) study population. To this end, experimental human endotoxemia does not share the aforementioned limitations of clinical trials [4,5] and therefore is an example of translational research that facilitates us to investigate the mechanisms of systemic inflammation and to evaluate novel (pharmacological) interventions in humans in vivo [5].

The aim of the present review is to provide a detailed overview of the various designs, organ-specific changes, and strengths and limitations of the experimental human endotoxemia model, with the main focus on its use as a translational model for sepsis research.

2. The human endotoxemia model

For over a century, the experimental human endotoxemia model has been successfully applied using LPS in different types, dosages, and forms of administration [1,4,5]. The models used are safe, well-tolerated, and bear no known long-term health risks to the participants. The most frequently described method is by intravenous administration of LPS, although other forms have also been described, e.g., intratracheal administration [6]. The cornerstone of the endotoxemia model is the interaction of LPS with the Toll Like receptor (TLR)-4, an interaction which also constitutes the first step in the inflammatory cascade in for example Gram-negative sepsis [4,7]. Intravenous LPS administration elicits a transient and controlled systemic inflammatory response, clinically characterized by an increase in core temperature of approximately 1.5–2 °C, flu-like symptoms (such as headache, chills, fatigue, myalgia, backache, and nausea) during 2–4 h, as well as hemodynamic alterations (tachycardia, tachypnea, and decrease in blood pressure).

2.1. Experimental setup

After approval of the local ethics committee and written informed consent, all subjects are thoroughly screened prior to inclusion (using medical history, physical examination, laboratory tests, and a 12-leads electrocardiogram). The procedure and general requirements for conducting a LPS challenge are displayed in Fig. 1.

2.2. Dose regimens

Currently, the only commercially available LPS for use in humans is the GMP-grade E. Coli Type O113 (Lot no. 94332) produced by List Biological Laboratories in Campbell, USA. The use of different doses have been well described in literature, varying from extremely low dosages of 0.1–0.3 ng/kg (only causing minor cytokine peaks with the absence of clinical symptoms), to a maximum used dose of 4 ng/kg (resulting in a profound systemic inflammatory response accompanied by the aforementioned clinical symptoms) [4,8,9]. The magnitude of the inflammatory response after LPS is highly dose-dependent and therefore, higher dosage regimens are mainly used as an in vivo translational model for sepsis, while the lower dosage ranges are increasingly applied to model a state of low grade inflammation, which is observed in e.g. diabetes and metabolic syndrome [9,10]. While most studies use the traditional single bolus administration of LPS, a recent study proposed a novel method consisting of a bolus administration of 1 ng/kg followed by a continuous administration of 1 ng/kg/h for 3 h [1]. This continuous model resulted in a more prolonged inflammatory response compared to bolus LPS administration, exemplified by higher plasma cytokine concentrations and circulating leukocytes, and an increased duration of fever and clinical symptoms, while no safety issues were reported. Therefore, the continuous model may better resemble the ongoing inflammation seen during sepsis, and may also provide a larger (and clinically more relevant) time window to examine the effects of immunomodulatory interventions.

2.3. Repeated experimental human endotoxemia

The immune response in septic patients is highly variable and can comprise both a hyperinflammatory as well as an immunosuppressed phenotype. The latter state, also known as sepsis-induced immunoparalysis, is increasingly recognized as the overriding immune dysfunction in sepsis, rendering patients vulnerable to secondary infections and impairing sepsis outcome. However, the identification of immunoparalyzed patients remains difficult, this is why few clinical studies have investigated immunostimulatory therapies. Interestingly, LPS administration results in a phenomenon called ‘endotoxin tolerance’, which is characterized by severely attenuated cytokine responses and less pronounced changes in clinical symptoms upon a second challenge, compared
to the first LPS challenge [11,12]. Endotoxin tolerance bears many hallmarks of (sepsis-induced) immunoparalysis, such as a decreased ex vivo cytokine production of LPS-stimulated monocytes and a decreased HLA-DR expression on monocytes [11]. To this end, the repeated human endotoxemia model can be used to model sepsis-induced immunoparalysis, allowing us to investigate immunostimulatory therapies in humans in vivo, without the aforementioned clinical constraints [11]. Please note that the similarities between endotoxin tolerance and immunoparalysis are mainly observed in the innate immune response, as LPS administration does not strongly affect the adaptive immune response.

3. Experimental human endotoxemia-induced organ-specific effects

The intravenous administration of LPS, especially within the high dose ranges (2–4 ng/kg), exerts many organ-specific effects. Since the model is conducted in a highly standardized and reproducible manner, it offers an unique opportunity to investigate the pathophysiology underlying these changes and to evaluate new (organ-specific) interventions [9]. In the following section, we complement previous reviews on experimental endotoxemia [4,5,8] and focus on the effects of experimental human endotoxemia on important organ systems, and recite the most important interventions concerning each organ system. An overview of the LPS-induced organ-specific effects is displayed in Fig. 2.

3.1. The immune system

Upon administration, LPS monomers bind to TLR-4-MD2 receptor-domains expressed on antigen presenting cells, which leads to an activated TLR-4 dimeric receptor complex. Mediated by adaptor protein MYD88, several intracellular protein-complex-pathways are subsequently activated, resulting in the activation of the transcription factor NF-κB. Binding of NF-κB to its target genes triggers the synthesis of RNA-sequences which are translated to various pro- and anti-inflammatory cytokines. Plasma cytokine levels upon LPS administration have a distinct, dose-dependent and highly reproducible time course [1,7,8]. Tumor necrosis factor (TNF-α) is the first to peak (90–120 min) and is believed to be the primary mediator leading to the LPS-induced systemic inflammatory cascade. TNF-α is closely followed by peak concentrations of IL-1β, IL-6, but also of the anti-inflammatory cytokine IL-10, all of which have maximum plasma concentrations at approximately 3 h following LPS administration. Within 6–8 h after the LPS challenge, all plasma cytokines levels return to baseline values [1].
The inflammatory cytokine responses observed during experimental human endotoxemia, as well as a multitude of interventions targeted against it have been extensively studied. A study investigating gender-based differences during experimental endotoxemia, found significantly higher peak TNF-α concentrations in females, while no difference in the anti-inflammatory IL-10 cytokine response was observed [13]. Arguably, this more pronounced pro-inflammatory response in females could partially explain both the lower incidence, and the better outcome of sepsis in female patients. Prednisolone, a well-established anti-inflammatory therapeutic, was found to reduce the release of pro-inflammatory cytokines TNF-α and IL-6 during endotoxemia in a dose-dependent manner, while it enhanced the release of the anti-inflammatory cytokine IL-10 [14]. These findings might explain the high susceptibility to infection in patients often observed after prolonged use of corticosteroids.

Using repeated human endotoxemia as a model for sepsis-induced immunoparalysis, several immunostimulatory agents have been investigated for their effect on preventing or restoring the suppressed immune function. Of interest, while *ex vivo* endotoxin tolerance (*ex vivo* re-stimulation of leukocytes with LPS) resolves quickly, *in vivo* endotoxin tolerance (*in vivo* re-challenge with LPS) persists for at least 2 weeks [15]. Based on the poor correlation between *ex vivo* and *in vivo* responses, clearly *ex vivo* endotoxin tolerance kinetics do not accurately reflect the *in vivo* innate immune response, further emphasizing the need for an *in vivo* translational model of endotoxin tolerance. Indeed, in a recent double-blind randomized controlled trial it was shown that treatment with IFN-γ during repeated experimental endotoxemia could partially reverse endotoxin tolerance [11]. These *in vivo* results further support the hypothesis that immunostimulatory agents could be a promising treatment option to reverse sepsis-induced immunoparalysis.

### 3.2. Hematology

Sequestration of immune cells results in neutrophilia, and a mono- and lymphocytopenia, with lowest counts at 2 and 4 h following intravenous administration of LPS, respectively [1]. Interestingly, a recent study using deuterium labeling to monitor immune cells, reported that the repopulation of monocytes is achieved by the early release of classical monocytes from the bone marrow, and not by return of monocytes from the marginating pool (monocytes that do not circulate as they adhere to the endothelium) [16]. Another study investigating the function of different neutrophil subsets during experimental endotoxemia found a down-regulation of neutrophil receptors necessary for chemotaxis, microbial recognition and killing [17]. Moreover, a moderately
decreased interaction with opsonised Staphylococcus epidermidis bacteria was found [17]. Intriguingly, these suppressed neutrophil phenotypes were also associated with a marked upregulation of the capacity to produce reactive oxygen species (ROS), which are essential for neutrophil bactericidal activity. These apparently contradictory findings may be explained by priming and activation of already circulating neutrophils by LPS leading to the enhanced ROS production on one hand, while LPS also induces the release of refractory, immature neutrophils with lower antimicrobial functionality from the bone marrow.

3.3. The cardiovascular system

Endotoxin-induced systemic inflammation has profound effects on the heart and vascular system. In the first hours following endotoxin administration, a hyperdynamic cardiovascular state is present, characterized by decreased systemic vascular resistance, lower arterial blood pressure and blunted responsiveness to sympathetic vasoconstrictors, as well as increased cardiac output and heart rate [1,18–20]. Myocardial contractility parameters (including left ventricular ejection fraction) typically show a biphasic response, with an initial increase, but later decrease (even though the hyperdynamic state persists for 6–12 h) [18,19,21]. Experimental human endotoxemia therefore represents (at least to a certain extent) cardiovascular manifestations of sepsis, and can be used to study the cardiovascular effects of interventions. For example, an antagonist against the toxic lipid A component of LPS (E5531) inhibited the early hyperdynamic cardiovascular response, as well as the late myocardial depression [21] and recombinant human activated protein C (APC) prevented the decrease in mean arterial blood pressure (MAP) during human endotoxemia [22].

3.4. The microcirculation and vascular endothelial permeability

Extensive microcirculatory alterations are present during sepsis, resulting in a mismatch of oxygen supply and demand due to shunting [23]. In addition, tissue edema develops due to vascular hyperpermeability combined with the need for fluid resuscitation. This contributes to development of for instance acute respiratory distress syndrome (ARDS) and acute kidney injury (AKI) [24]. It is well known that LPS administration can cause profound vascular leakage in animals [25], and indeed, plasma obtained during human endotoxemia causes vascular leakage in vitro [26]. In contrast, an increased capillary leakage could not be detected in humans in vivo, indicating that the experimental human endotoxia model is not suitable to study microvascular permeability [27]. Prolonged duration and increased severity of the inflammatory response observed in animal models of endotoxemia, as a consequence of higher dose administration of LPS (approx. 1000 times higher) likely explain this discrepancy. Note that there is one report that describes the case of a laboratory worker self-administering an endotoxin. This resulted in sepsis and ARDS [28].

3.5. The kidneys

The development of AKI is common in sepsis and associated with increased mortality [36]. AKI is thought to arise through a complex mechanism of immune-mediated microvascular and tubular dysfunction [37]. Similar to sepsis, several urinary tubular damage markers are also increased following experimental human endotoxemia. For example, glutathione-S-transferase-A1 (GSTA1-1), a marker of proximal tubule damage, was increased in urine and correlated with increased nitric oxide (NO) metabolite excretion [38]. Interestingly, co-administration of the inducible nitric oxide synthase (iNOS) inhibitor aminoguanidine during human endotoxemia reduced upregulation of iNOS mRNA, urinary NO metabolites and urinary GSTA1–1, suggesting a role of iNOS and NO in renal proximal tubule damage [38]. More recently, significant increases of urinary B2MG and KIM–1, markers indicating (subtle) proximal tubular damage were reported. In contrast, many other markers, among which serum creatinine, were not affected [35].

3.6. Pulmonary system

In addition to intravenous administration, LPS has also been administered intrapulmonary to induce a local inflammatory response in the bronchoalveolar space [6], which bears features of acute lung injury and/or pneumonia [6]. In this pulmonary LPS model, intravenous co-administration of activated protein C (APC) resulted in both anti-coagulatory and anti-inflammatory effects in the bronchoalveolar space [6,39], whereas intrapulmonary APC administration led to a pro-coagulatory and pro-inflammatory phenotype [40]. Moreover, changes in respiratory capacity and pulmonary gas exchange were recently investigated following both systemic and pulmonary endotoxin administration [41]. Both models decreased forced expiratory volume (FEV1) and forced vital capacity (FVC), although this effect was more pronounced after intravenous LPS administration. Furthermore, the alveolar-arterial oxygen gradient increased after intravenous, but not after intrapulmonary LPS administration [41].

3.7. Respiratory muscle weakness

Systemic inflammation is thought to contribute to respiratory muscle weakness in mechanically ventilated patients, which may lead to weaning problems [42]. A recent study investigated whether LPS affects diaphragm function, and if it could act as a model of respiratory muscle weakness. In contrast to animal studies and findings in critically ill patients, in vivo diaphragm contractility was not impaired, but rather augmented during experimental human endotoxemia, possibly related to the release of stress hormones [43].

3.8. Coagulation

Experimental human endotoxemia induces platelet activation and consumption, prolonged APTT and an increased INR [44,45]. In addition, increased fibrinolysis and fibrinogen consumption is reported, which is followed by downregulation of fibrinolysis. Primary hemostasis is reduced, whereas secondary hemostasis is enhanced [45]. Different interventions that act on coagulation and/
or fibrinolysis have been examined using the human endotoxemia model. Colistin, which electrostatically interacts with LPS [46], blunted endothelial activation and the fibrinolytic response, while activation of the coagulation system was not severely affected. This suggests that colistin may exert other effects besides its antimicrobial activity in patients with Gram-negative sepsis [46]. In a study with APC (also mentioned earlier), no effects were observed on fibrinolysis, coagulation or inflammation during human endotoxemia [22]. Interestingly, the human endotoxemia model is not necessarily limited to application in healthy subjects. Factor V Leiden (FVL) mutations (causing hypercoagulability) were initially thought to worsen clinical outcome in patients with disseminated intravascular coagulation in sepsis, although clinical trials and animal data later indicated the opposite. Therefore, a study was conducted to explore the mechanism of action of the FVL mutation during human endotoxemia [47]. Interestingly, males with a heterozygous FVL mutation showed an enhanced fibrinolytic response to endotoxin, possibly due to higher levels of soluble fibrin acting as cofactor in tissue plasminogen-induced plasminogen activation. This may therefore result in better clearance of fibrin deposits, a reduction of fibrinogen levels and generation of 'anticoagulant' fibrinogen degradation products.

### 3.9. The gastrointestinal tract

During sepsis, the permeability of the intestinal barrier increases, allowing translocation of bacteria and luminal contents (e.g. pancreatic enzymes) [36]. Thirty years ago, the increased intestinal permeability following endotoxin administration was first reported, when a study found urinary secretion of the (normally) non-metabolizable sugars lactulose and mannitol after oral ingestion [48]. Results were confirmed in another study using polyethylene glycol, indicating that inflammation-induced paracellular permeability and not ischemia-mediated enterocyte damage was responsible for the increased intestinal permeability [49]. Interestingly, increased permeability seems to be limited to the small intestine [50].

The gut microbiota is known to have an important interaction with gut-barrier function and modulation of the immune system during sepsis [51]. In addition, sepsis and/or antibiotics influence gut-microbiota composition. Nevertheless, antibiotic-induced alterations in microbiota composition did not influence inflammation during experimental human endotoxemia, even though gut microbiota diversity was decreased by broad-spectrum antibiotics [52]. Moreover, no endotoxemia-induced increase in liver damage marker GSTA1-1 was observed, indicating that the human endotoxemia model is probably too mild to cause inflammation-induced liver injury [53].

### 4. Limitations of the human endotoxemia model

To date, the experimental human endotoxemia is the only in vivo model of systemic inflammation. However, several important factors may limit its clinical relevance and extrapolation to clinical research in sepsis. A summary of the main differences between experimental human endotoxemia and sepsis is displayed in Table 1, a summary of the main similarities is displayed in Table 2. The differences listed in Table 1, as well as those mentioned earlier for the different organ systems, underline that while some aspects of experimental endotoxemia are highly comparable to sepsis, others are markedly different (e.g. capillary leakage, liver injury, and an adaptive immune response). Considering the complex pathophysiology of systemic inflammation, experimental endotoxemia should therefore never be envisioned as an alternative or

#### Table 1

<table>
<thead>
<tr>
<th>Differences between experimental human endotoxemia and sepsis.</th>
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<tbody>
<tr>
<td><strong>Experimental human endotoxemia</strong></td>
</tr>
<tr>
<td>Subject characteristics</td>
</tr>
<tr>
<td>- (Mostly) young adults</td>
</tr>
<tr>
<td>- (Mostly) healthy</td>
</tr>
<tr>
<td>- (Mostly) males</td>
</tr>
<tr>
<td>Inflammatory cascade</td>
</tr>
<tr>
<td>- Highly standardized (known time of onset)</td>
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<tr>
<td>- LPS-induced</td>
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<tr>
<td>- TLR-4 mediated</td>
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<tr>
<td>- Predominantly innate immune response</td>
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<tr>
<td>Cytokines</td>
</tr>
<tr>
<td>- Short elevation of plasma cytokines (hours)</td>
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<tr>
<td>Cardiovascular clinical features</td>
</tr>
<tr>
<td>- Short ‘hyperdynamic response’ with limited decrease in blood pressure</td>
</tr>
<tr>
<td>- No capillary leak/shock symptoms</td>
</tr>
<tr>
<td>- Transient flu-like symptoms</td>
</tr>
<tr>
<td>- Well-tolerated</td>
</tr>
<tr>
<td>- Safe</td>
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<tr>
<td>- No long-term effects</td>
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</tbody>
</table>

4. Future improvements on the model

Research performed during the past decade has also focused on ways to further improve the comparability between experimental human endotoxemia and sepsis. As mentioned earlier, in a recently introduced continuous model of (high dose) LPS administration, bolus administration was followed by continuous LPS infusion during three hours [1]. This lead to more sustained and higher plasma cytokine concentrations, more circulating leukocytes, and an increased duration of fever and clinical symptoms compared to LPS bolus injection models. The continuous LPS infusion model may therefore better resemble the state of ongoing inflammation as observed during sepsis. Future research should concentrate on further optimizing LPS dosage regimens, possibly through even longer durations of continuous LPS administration.

5. Conclusion

Experimental human endotoxemia is a translational model of systemic inflammation in humans in vivo and has been successfully performed in thousands of healthy volunteers. It has proven to be safe, well-tolerated and without any known long-term health risks for the participating subjects. Unlike the heterogeneous inflammatory response observed in sepsis patients, the inflammatory responses observed during experimental endotoxemia are mono-causative (TLR-4 driven), highly standardized, and reproducible, greatly facilitating the possibilities for between-subject and intervention-based comparisons. The overlap in inflammatory response, clinical parameters, and organ-specific changes emphasize that experimental human endotoxemia may serve as a model for sepsis. However, the model also has limitations, implying that direct comparisons between experimental endotoxemia and clinical causes of systemic inflammation should always be made with caution. Nevertheless, the multitude of studies performed on the observed cytokine responses, the different inflammation-induced organ dysfunctions, as well as therapeutic interventions directed against these have been indispensable in advancing our understanding of the complex pathophysiology of systemic inflammation and sepsis. Therefore, the experimental human endotoxemia model should be used to facilitate the translation of preclinical work to the sepsis population.

Conflicts of interest and source of funding

The authors declare no financial or ethical conflicts of interest.

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